Anxiety and the neurobiology of uncertain threat anticipation

Juyoen Hur[1*]
Jason F. Smith[2*]
Kathryn A. DeYoung[2,3,4*]
Allegra S. Anderson[7]
Jinyi Kuang[8]
Hyung Cho Kim[2,5]
Rachael M. Tillman[2]
Manuel Kuhn[2]
Andrew S. Fox[9,10]
Alexander J. Shackman[2,5,6]

[1]Department of Psychology, Yonsei University, Seoul 03722, Republic of Korea. Departments of [2]Psychology, and [3]Family Science; [4]Center for Healthy Families; [5]Neuroscience and Cognitive Science Program; and [6]Maryland Neuroimaging Center, University of Maryland, College Park, MD 20742 USA. [7]Department of Psychological Sciences, Vanderbilt University, Nashville, TN 37240 USA. [8]Penn Social Norm Group, University of Pennsylvania, PA 19104 USA. [9]Department of Psychology and [10]California National Primate Research Center, University of California, Davis, CA 95616 USA

* contributed equally

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Address Correspondence to:
Juyoen Hur (juh614@gmail.com)[1] or Alexander J. Shackman (shackman@umd.edu)[2,5,6]

ABSTRACT (245/250)

When extreme, anxiety—a state of distress and arousal prototypically evoked by uncertain danger—can become debilitating. Uncertain anticipation is a shared feature of situations that elicit signs of anxiety across disorders, species, and assays. Despite the profound significance of anxiety for human health and wellbeing, the neurobiology of uncertain threat anticipation remains remarkably unsettled. Leveraging a paradigm adapted from animal research and optimized for functional MRI, we examined the neural circuits engaged during the anticipation of temporally uncertain and certain threat in 99 individuals. Results revealed that uncertain and certain threat are anatomically co-localized in the neocortex and extended amygdala (EA). Comparison of the two threat conditions demonstrated that this core network can be fractionated, with fronto-cortical regions showing relatively stronger engagement during the anticipation of uncertain threat, and the EA showing the reverse pattern. Although there is widespread agreement that the bed nucleus of the stria terminalis and dorsal amygdala—the two major subdivisions of the EA—play a critical role in orchestrating adaptive responses to potential danger, their precise contributions to human anxiety have remained contentious. Follow-up analyses demonstrated that these regions show statistically indistinguishable responses to uncertain and certain threat, indicating the need to reformulate prominent models of anxiety, including the National Institute of Mental Health's Research Domain Criteria. These observations provide a framework for conceptualizing anxiety and fear, for understanding the functional neuroanatomy of threat anticipation in humans, and for guiding the development of more effective intervention strategies for pathological anxiety.

INTRODUCTION

Anxiety is widely conceptualized as a state of distress, arousal, and vigilance that can be elicited by the anticipation of uncertain danger (1-5). Anxiety lies on a continuum and, when extreme, can be debilitating (6-8). As Daniel Defoe wrote in *Robinson Crusoe*, "*fear of danger is 10,000 times more terrifying than danger itself…and…the burden of anxiety greater, by much, than the evil which we are anxious about*" (9, p. 140). Anxiety disorders are the most common family of psychiatric illnesses and existing treatments are inconsistently effective (7, 10-14), underscoring the urgency of developing a clearer understanding of the neural systems engaged by uncertain threat anticipation.

Perturbation and recording studies in mice have begun to reveal the specific molecules and cellular ensembles that underlie defensive responses to uncertain threat (3, 15, 16), but the relevance of these discoveries to the complexities of human anxiety remains unclear. Humans and mice diverged ~75 MYA, leading to marked behavioral, genetic, and neurobiological differences between the two species (17). The role of neocortical regions that are especially well-developed in humans—including the midcingulate cortex (MCC), anterior insula (AI), and dorsolateral prefrontal cortex (dlPFC)—remains particularly opaque, reflecting equivocal or absent anatomical homologies and the use of disparate paradigms across species (18-24).

In human research, anxiety has received considerably less scientific attention than other aspects of emotion and motivation. The vast majority of human neuroimaging studies—including recent and on-going mega-studies[1]—have relied on static photographs of emotion-expressing faces or, less commonly, aversive images or Pavlovian threat cues. None of these approaches are suitable for understanding

[1] ABCD (25), Duke Neurogenetics (26), Human Connectome Project (27), IMAGEN (28), Philadelphia Neurodevelopmental Cohort (29), and UK Biobank (30).

sustained states of distress and arousal elicited by the anticipation of uncertain threat. Preliminary work using various kinds of uncertain threat tasks—including the unpredictable presentation of shocks, aversive images, screams, and faces—has begun to reveal the broad contours of the neural systems underlying human anxiety (3, 31-35). Yet progress has been slowed by paradigms that confound variation in threat certainty with differences in perception (e.g. anticipation vs. presentation of shocks; (34, 36-38)). These concerns are magnified in functional MRI (fMRI) studies, where samples are typically small, hampering reproducibility (n<30; 32, 39); technical limitations abound (e.g. regressor collinearity), making it difficult to disambiguate signals associated with the anticipation and presentation of threat; reinforcers are relatively mild, threatening validity; and reinforcer delivery is often inconsistent. For example, in one highly cited early study (38), the probability of shock delivery in the 'certain threat' condition was only 54%, thwarting decisive inferences (31, 32). In short—with the exception of a few intensively scrutinized regions (e.g. amygdala; 12)—we know remarkably little about the distributed brain circuitry underlying anxious states in humans.

The role of the central extended amygdala—a circuit encompassing the dorsal amygdala in the region of the central nucleus (Ce) and the bed nucleus of the stria terminalis (BST)—remains particularly contentious. Inspired by an earlier generation of gross perturbation studies in rodents (40), it is widely believed that these two regions are functionally dissociable, with the amygdala mediating phasic responses to clear-and-immediate danger and the BST mediating sustained responses to uncertain-or-remote danger (36, 37, 41, 42). This 'strict-segregation' hypothesis has even been enshrined in the National Institute of Mental Health's (NIMH) Research Domain Criteria (RDoC) framework (43). Yet a rapidly growing body of optogenetic, chemogenetic, and electrophysiological evidence gleaned from animal models suggest that defensive responses to uncertain threat are assembled by microcircuits

encompassing both regions (3, 12, 44-48). This work has motivated the competing hypothesis that the dorsal amygdala and BST are both important substrates for human anxiety (3, 31).

To address these fundamental questions, we combined whole-brain fMRI with a novel threat-anticipation task in a sample of 99 healthy adults. A multiband pulse sequence and advanced co-registration and spatial normalization techniques enhanced our ability to resolve smaller subcortical regions (**Supplementary Method**). Building on earlier neuroimaging work (37, 49), the Maryland Threat Countdown (MTC) paradigm is an fMRI-optimized version of temporally uncertain threat assays that have been validated using fear-potentiated startle and acute pharmacological manipulations in mice (50, 51), rats (52), and humans (53), maximizing its translational relevance. As shown in **Fig. 1**, the MTC paradigm takes the form of a 2 (*Valence:* Threat/Safety) × 2 (*Temporal Certainty:* Uncertain/Certain) randomized event-related design. On Certain Threat trials, subjects saw a descending stream of integers (e.g. 30, 29, 28...) for 18.75 s, a duration sufficient to enable the dissection of onset-evoked from sustained hemodynamic responses. To ensure robust emotion induction, this anticipatory epoch ('countdown') always culminated with the delivery of a multi-modal reinforcer, consisting of a noxious electric shock, unpleasant photograph (e.g. mutilated body), and thematically related audio clip (e.g. scream). Uncertain Threat trials were similar, but the integer stream was randomized and presented for an uncertain and variable duration (8.75-32.5 s; M=18.75 s). Thus, on Uncertain Threat trials, subjects knew that the threat was coming, but they did not know when it would occur. Safety trials were similar, but terminated with the delivery of benign stimuli (e.g. just-perceptible electrical stimulation). Comparison of the perceptually well-matched anticipatory epochs afforded a rigorous means of isolating neural circuits recruited during the anticipation of uncertain threat, unlike traditional fear conditioning or 'threat-of-shock' paradigms (32, 54).

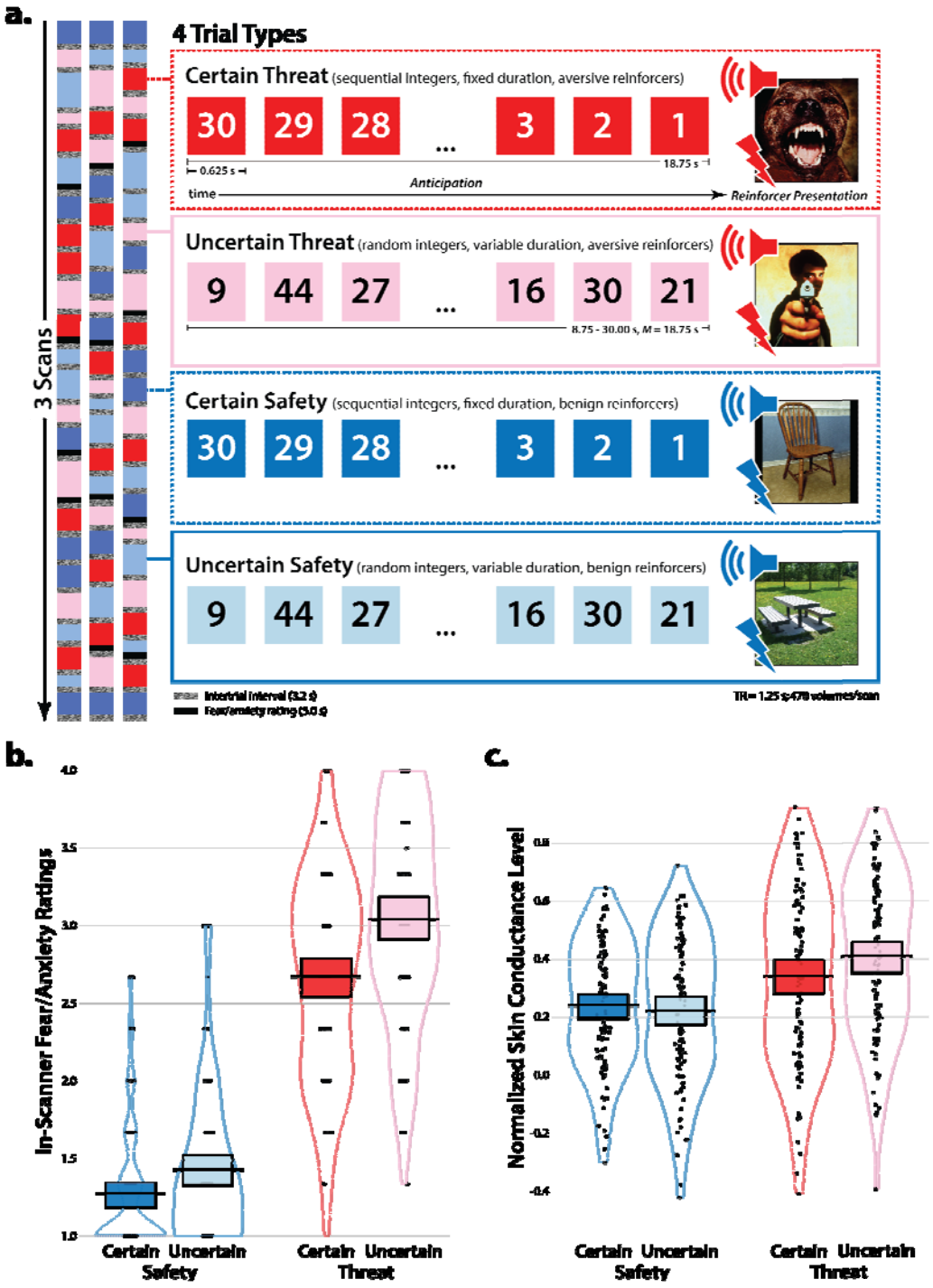


Figure 1. Maryland Threat Countdown (MTC) Paradigm. As shown schematically in panel ***a***, the MTC paradigm takes the form of a 2 (*Valence:* Threat/Safety) × 2 (*Temporal Certainty:* Uncertain/Certain) design. See the main text for a general description and **Supplement** for details. Subjects provided ratings of anticipatory fear/anxiety for each trial type during each scan. Skin conductance was continuously acquired during scanning. Simulations were used to optimize the detection and deconvolution of task-related hemodynamic signals (variance inflation factors <1.54). Central panels depict the structure of each trial type. Trial valence was continuously signaled during the anticipatory epoch by the background color of the display. Trial certainty was signaled by the nature of the integer stream. Certain trials always began with the presentation of 30. On Uncertain trials, integers were randomly drawn from a uniform distribution ranging from 1 to 45 to reinforce the belief that uncertain trials could be much longer than certain ones. To mitigate potential confusion and eliminate mnemonic demands, a lower-case 'c' or 'u' was presented at the lower edge of the display throughout the anticipatory epoch (not depicted). As shown in panels ***b*** and ***c***, threat anticipation robustly increased subjective symptoms (in-scanner ratings) and objective signs (skin conductance) of anxiety, and this was particularly evident when the timing of aversive stimulation was uncertain (Valence × Certainty, *p*s<.001; Uncertain Threat > Certain Threat, *p*s<.001). Panels ***b*** and ***c*** depict the data (*black points; individual participants*), density distribution (*bean plots*), Bayesian 95% highest density interval (HDI; *colored bands*), and mean (*black bars*) for each condition. HDIs permit population-generalizable visual inferences about mean differences and were estimated using 1,000 samples from a posterior Gaussian distribution. Abbreviations—TR, repetition time (i.e. the time required to collect a single volume of fMRI data).

RESULTS

Uncertain Threat anticipation elicits robust symptoms and signs of anxiety

As shown in **Fig. 1**, threat anticipation markedly increased subjective symptoms (in-scanner ratings) and objective signs (skin conductance) of anxiety, and this was particularly evident when the timing of aversive stimulation was *un*certain (Valence × Certainty, *ps*<.001; Uncertain Threat > Certain Threat, *ps*<.001; see **Supplementary Results**). These results confirm the validity of the MTC paradigm for understanding the neural circuits underpinning anxiety.

Uncertain Threat anticipation recruits a distributed network of subcortical and cortical regions

As detailed in the **Supplement**, a voxelwise GLM was used to identify brain regions recruited during the anticipation of temporally Uncertain Threat (Uncertain Threat > Uncertain Safety; FDR q<.05, whole-brain corrected). As shown in **Fig. 2**, this highlighted a widely distributed network of regions previously implicated in the expression and regulation of human fear and anxiety (3, 32, 54, 55), including the MCC; AI extending into the frontal operculum (FrO); dlPFC extending to the frontal pole (FP); brainstem encompassing the periaqueductal grey (PAG); basal forebrain in the region of the BST; and dorsal amygdala in the region of the central and medial nuclei. Heightened activity during the anticipation of Uncertain Threat was also evident in the orbitofrontal cortex, basal ganglia, hippocampus, and ventrolateral amygdala in the region of the lateral nucleus (**Supplementary Table 1**). Consistent with prior work (e.g. 49, 56), Uncertain Threat anticipation was also associated with *reduced* activity in a set of midline regions that resembled the default mode network (e.g. anterior rostral sulcus, postcentral gyrus, and precuneus), as well as the posterior insula and parahippocampal gyrus (**Supplementary Table 2**). Reduced activity was also observed in the most rostral tip of the amygdala, underscoring the functional heterogeneity of this complex structure (3, 38, 57-60).

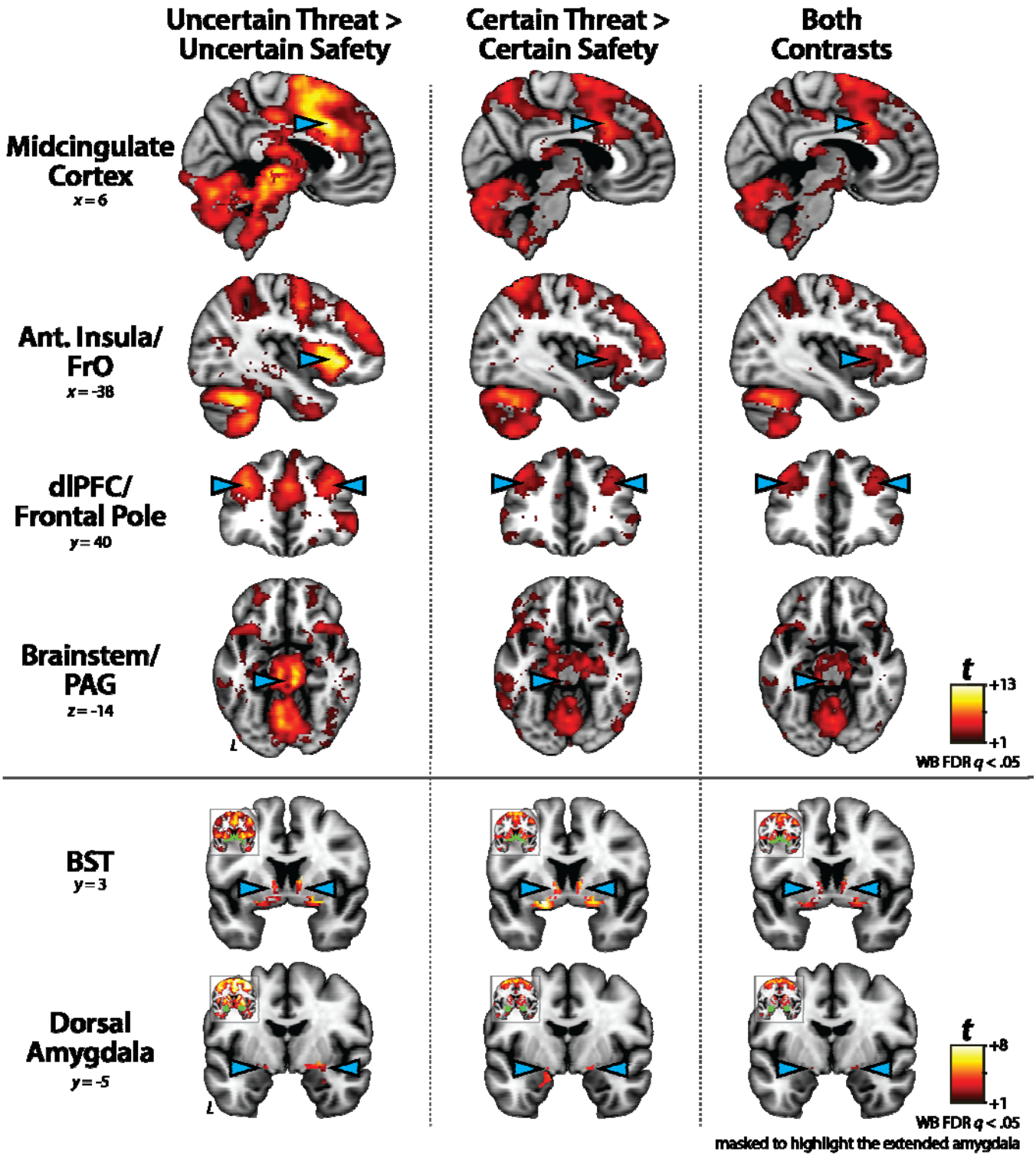
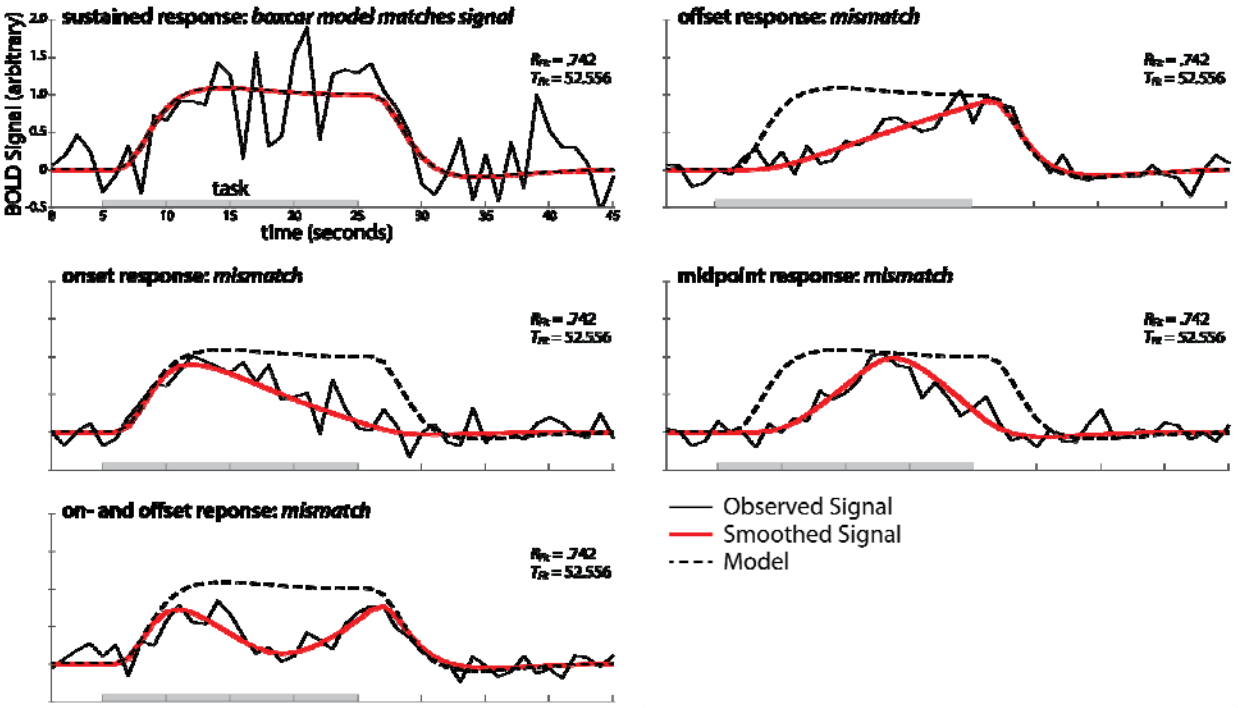


Figure 2. The anticipation of Uncertain and Certain Threat recruits broadly similar networks. Key regions (*cyan arrowheads*) showing significantly elevated activity during the anticipation of Uncertain Threat (*left column*) and Certain Threat (*center column*) compared to their respective control conditions. Voxels showing significantly increased activity in both contrasts are depicted in the *right column*. BST and dorsal amygdala images are masked to highlight significant voxels in extended amygdala (*green*). Coronal insets depict the thresholded statistical parametric maps without the additional mask. Taken together, these observations indicate that these regions are sensitive to both temporally certain and uncertain threat.. Abbreviations—Ant., anterior; BST, bed nucleus of the stria terminalis; dlPFC, dorsolateral prefrontal cortex; FrO, frontal operculum; L, left; PAG, periaqueductal grey; WB, whole-brain corrected.

Uncertain Threat anticipation elicits sustained hemodynamic activity

Anxiety is often conceptualized as a sustained state (1, 4, 5, 61), and it is tempting to interpret regions of enhanced activity (e.g. **Fig. 2**) through this lens. But do we actually see evidence of sustained responses during the anticipation of Uncertain Threat? Although a wide variety of other signals are physiologically plausible (e.g. 44, 62) (**Fig. S2**), the vast majority of fMRI studies never address this question; they instead assume the shape of the hemodynamic response and focus on estimates of response magnitude ('activation'). To address this ambiguity, we used a finite impulse response (FIR) approach to estimate responses elicited by the anticipation of Uncertain Threat and Uncertain Safety on a moment-by-moment (1.25 s) basis. Doing so revealed significantly sustained activity across key cortical (MCC, AI/FrO, dlPFC/FP) and subcortical (PAG, BST, dorsal amygdala) regions for the first time (Uncertain Threat > Uncertain Safety; 6.25-30 s; **Fig. 3** and **Supplementary Table 3**).



Supplementary Fig. S2. Interpretive ambiguities of canonical HRF modeling. The canonical approach to fMRI analysis models the amplitude of anticipatory activity (*solid black line*) under the assumption that it approximates a 'boxcar-like' square-wave shape (*dotted line;* convolution of a canonical HRF with task duration). In some cases, such as the upper-left panel, the hemodynamic signal and the model will match. But in others, it won't. Importantly, a variety of physiologically plausible hemodynamic responses can produce similarly strong and statistically significant results (T = 52.556 in this example), highlighting the importance of modeling the BOLD signal on a TR-by-TR basis.

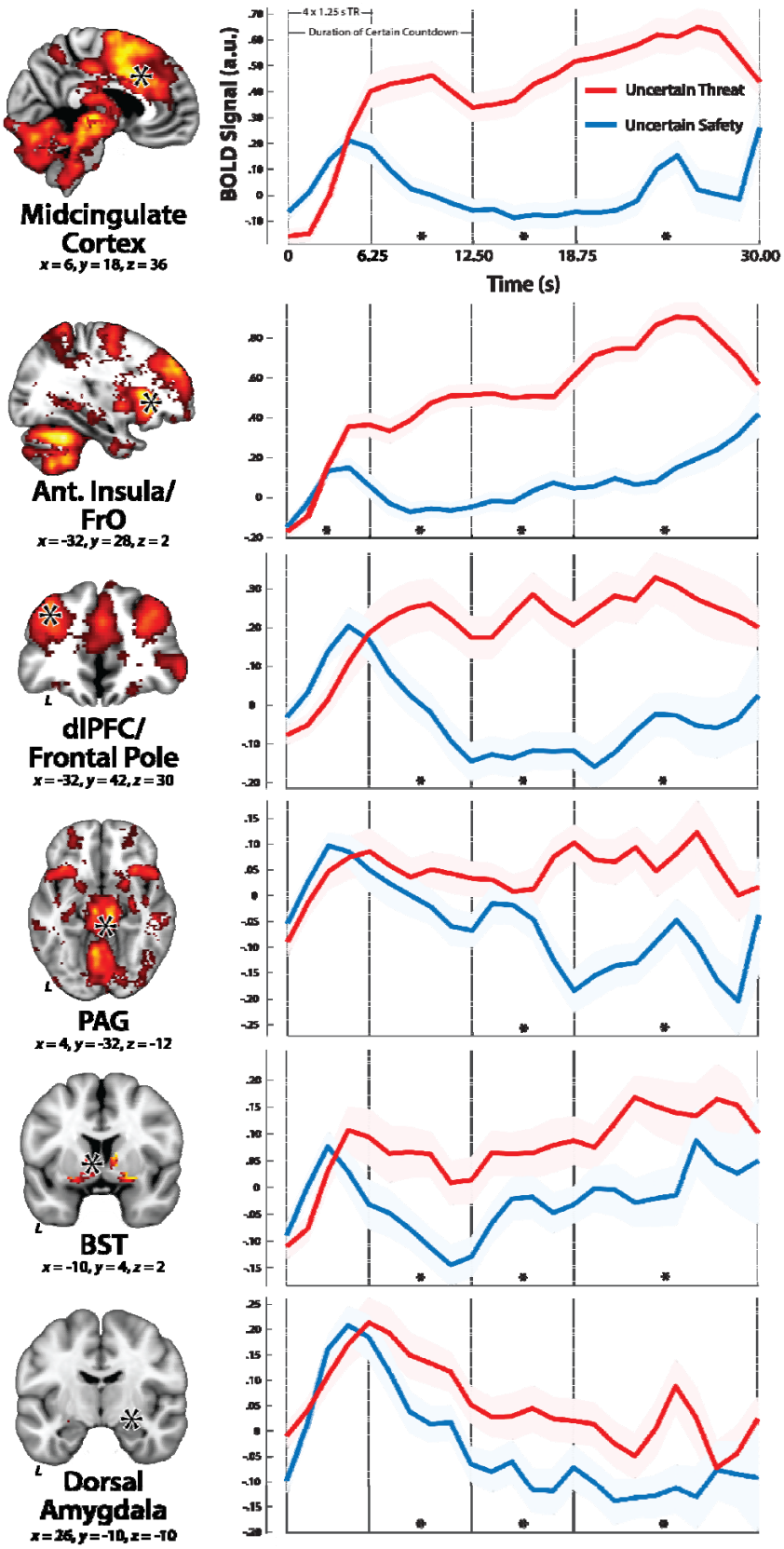


Figure 3. Regions sensitive to Uncertain Threat show sustained hemodynamic activity. Mean responses to the anticipatory epoch were estimated on a moment-by-moment (1.25 s) basis for Uncertain Threat (*red*) and Uncertain Safety (*blue*) trials, using data from the local maxima of key clusters (*black-and-white asterisks in the left panels*) identified using a canonical analytic approach. Given the temporal resolution and autocorrelation of the hemodynamic signal, data were averaged for 4 windows (TR-1 to TR-5, TR-6 to TR-10, TR-11 to TR-15, and TR-16 to TR-24), spanning a total of 24 measurements (30 s). Windows are indicated by broken vertical lines. Shaded envelopes depict the standard error of the mean. Significant differences are indicated by the black asterisks in the right panels (*p*s<.05; **Supplementary Table 3**). Abbreviations—Ant., anterior; BST, bed nucleus of the stria terminalis; dlPFC, dorsolateral prefrontal cortex; FrO, frontal operculum; L, left; PAG, periaqueductal grey; TR, repetition time (the time needed to acquire a single volume of fMRI data).

Certain Threat anticipation recruits an anatomically and functionally similar network

Having identified a distributed neural circuit sensitive to Uncertain Threat, we used a parallel approach to identify regions recruited during the anticipation of *Certain* Threat (Certain Threat > Certain Safety; FDR q<.05, whole-brain corrected). As shown in **Fig. 2**, results were similar to those found for Uncertain Threat (**Supplementary Tables 4-5**). In fact, a minimum conjunction analysis (Logical 'AND;' (63)) revealed voxelwise co-localization in every key cortical and subcortical region, including the BST and dorsal amygdala (**Fig. 2** and **Supplementary Table 6**). FIR results provided additional evidence of functional convergence across conditions, with all but one of these key regions—the PAG—showing sustained levels of heightened hemodynamic activity during the anticipation of Certain Threat (**Fig. S4; Supplementary Table 7**). Taken together, these results suggest that this network of subcortical and cortical regions is sensitive to multiple kinds of threat anticipation, both certain and uncertain.

The threat anticipation network can be fractionated into subdivisions

To determine whether regions recruited during threat anticipation are sensitive to temporal uncertainty, we directly compared the Uncertain and Certain Threat conditions (FDR q<.05, whole-brain corrected). This indicated that the threat anticipation network can be fractionated into subdivisions. As shown in **Fig. 4**, key cortical regions (MCC, AI/FrO, and dlPFC/FP) showed a further increase in activity during the anticipation of *Uncertain* Threat (**Supplementary Table 8**). In contrast, the BST and dorsal amygdala showed the reverse pattern, with relatively greater activity during the anticipation of *Certain* Threat (**Supplementary Table 9**). The PAG did not discriminate the two threat conditions.

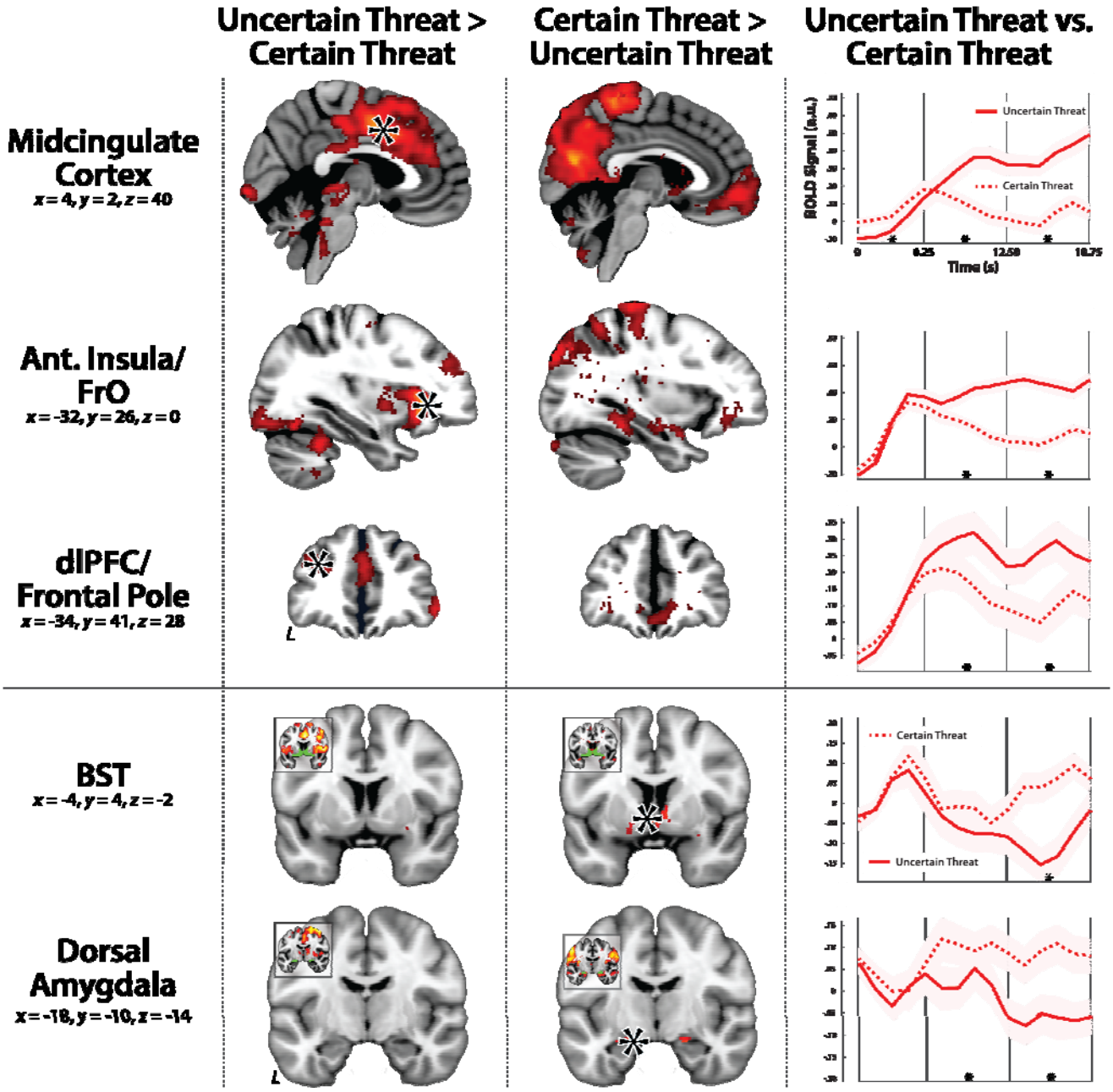


Figure 4. The threat anticipation network can be fractionated into subdivisions. The midcingulate cortex, anterior insula/frontal operculum, and dlPFC showed greater activity during the anticipation of Uncertain Threat (*left column*), whereas the BST and dorsal amygdala showed greater activity during the anticipation of Certain Threat (*center column*). Thresholds and other conventions are identical to **Fig. 3**. The r*ight column* depicts moment-by-moment (every TR; 1.25 s) hemodynamic responses during the anticipation of Uncertain Threat (*solid red line*) and Certain Threat (*broken red line*). Data were extracted from the local maxima of key clusters (*black-and-white asterisks in the left and center columns*) identified using a canonical HRF GLM approach. Given the temporal resolution and autocorrelation of the hemodynamic signal, data were averaged for 3 windows (TR-1 to TR-5, TR-6 to TR-10, and TR-11 to TR-15), spanning a total of 15 measurements (18.75 s). Windows are indicated by broken vertical lines. Shaded envelopes depict the standard error of the mean. Significant differences are indicated by the black asterisks in the right panels (*ps*<.05; **Supplementary Table 10**). Abbreviations—Ant., anterior; BST, bed nucleus of the stria terminalis; dlPFC, dorsolateral prefrontal cortex; FrO, frontal operculum; L, left; PAG, periaqueductal grey.

Uncertain and Certain Threat anticipation elicit statistically equivalent responses in the extended amygdala

Our results indicate that the BST and dorsal amygdala—the two major subdivisions of the EA—respond similarly to threat anticipation. Both regions show significantly elevated activity during threat anticipation, and this is evident whether or not the timing of aversive stimulation is uncertain (**Fig. 2**). Furthermore, both regions showed parallel increases in activity during the anticipation of Certain Threat (**Fig. 4**). Yet it remains possible that the BST and the amygdala exhibit subtler differences in threat sensitivity. To rigorously test this, we directly compared regional responses to the 3 threat contrasts shown in **Figs. 3 and 4** (equivalent to testing Region × Condition interactions; see the **Supplementary Method** for details). As shown in **Fig. 5**, mean differences were small to very-small and all non-significant (**Supplementary Table 11**). Likewise, the proportion of subjects showing numerically greater activity in either region never exceeded 55% (**Fig. 5**). Naturally, these results do not license claims of regional equivalence. While it is impossible to demonstrate that the true difference in regional activity is zero, the two one-sided tests (TOST) procedure provides a well-established and widely used statistical framework for testing whether mean differences (here, in regional activity) are small enough to be considered equivalent (64, 65). For present purposes, we considered differences smaller than a 'medium' standardized effect (Cohen's d_z=.35) statistically equivalent. Results revealed significant equivalence for each of the threat contrasts (*ps*=.001-.03; **Fig. 5** and **Supplementary Table 11**). Although these statistical results clearly do not demonstrate that the amygdala and the BST are functionally interchangeable, they do enable us to decisively reject strong claims of functional segregation (i.e. that the BST is sensitive to uncertain danger, whereas the amygdala is not).

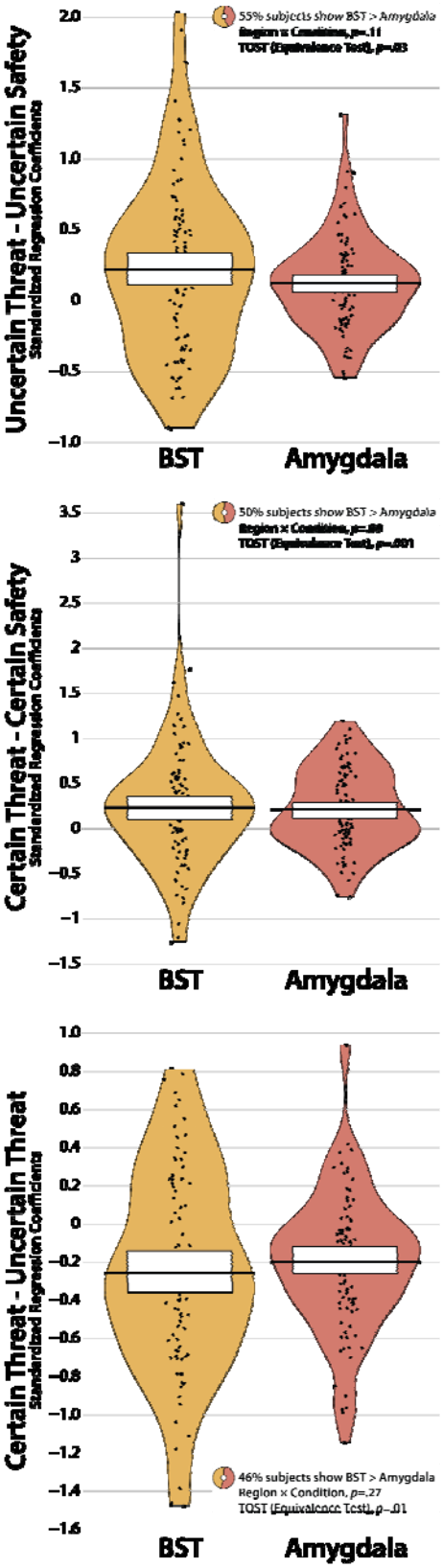
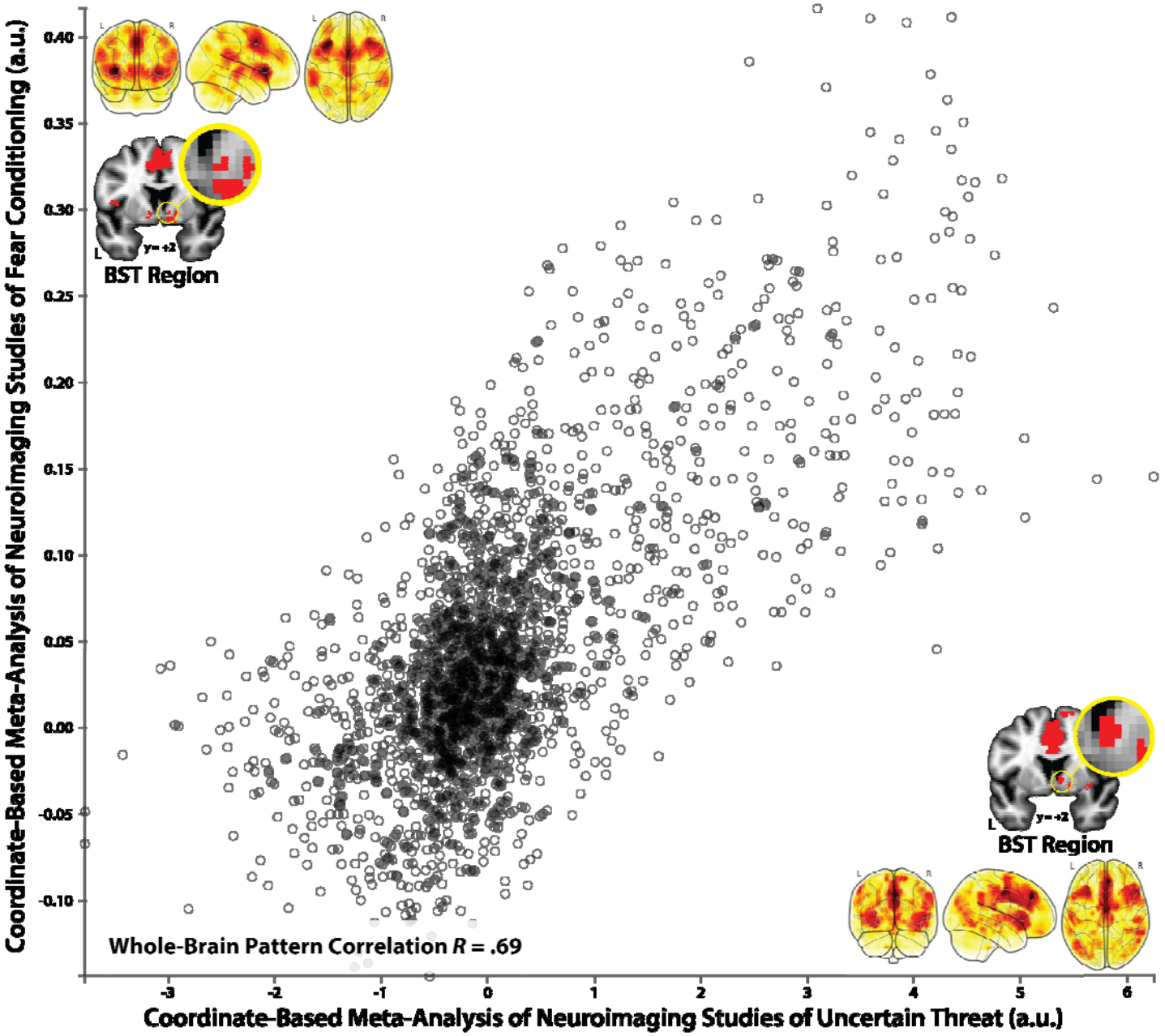


Figure. 5. The BST and dorsal amygdala show statistically equivalent responses during threat anticipation. Direct comparison of BST and amygdala responses for each threat contrast (see the **Supplementary Method** for details). While it is impossible to demonstrate that the true difference in regional hemodynamic activity is zero, the two one-sided tests (TOST) procedure provides a well-established and widely used statistical framework for testing whether mean differences in regional activity are small enough to be considered equivalent (64, 65). Results revealed significant equivalence for all contrasts. Figure depicts the data (*black points; individual participants*), density distribution (*bean plots*), Bayesian 95% highest density interval (HDI; *colored bands*), and mean (*black bars*) for each condition. HDIs permit population-generalizable visual inferences about mean differences and were estimated using 1,000 samples from a posterior Gaussian distribution. Inset ring plots indicate the percentage of subjects showing greater activity in the BST compared to the dorsal amygdala for each contrast.

DISCUSSION

Uncertain threat anticipation is the prototypical trigger of anxiety (2), a core theme that cuts across disorders, species, and assays, including novelty, darkness, and other so-called 'diffuse' threats. Despite the profound significance of anxiety for human health and wellbeing, the neural systems recruited by uncertain threat have remained incompletely understood. Leveraging a translationally relevant paradigm optimized for fMRI signal decomposition (**Fig. 1**), our results reveal that the anticipation of temporally uncertain aversive stimulation recruits a distributed network of fronto-cortical (MCC, AI/FrO, and dlPFC/FP) and subcortical (PAG, BST, and dorsal amygdala) regions (**Fig. 2**), mirroring robust changes in subjective emotional experience and objective autonomic physiology (**Fig. 1**). Using a FIR approach, close inspection of fMRI signal dynamics in these regions revealed sustained activity during the anticipation of Uncertain Threat (**Fig. 3**). Analyses focused on the anticipation of temporally Certain Threat revealed a remarkably similar pattern, with voxels sensitive to both kinds of threat evident in key cortical and subcortical regions (**Figs. 2** and **S4**). Collectively, these observations suggest this network is sensitive to both certain and uncertain threat. Direct comparison of the two threat conditions demonstrated that the threat anticipation network can be fractionated: cortical regions showed relatively greater activity during the anticipation of Uncertain Threat, whereas the BST and dorsal amygdala showed relatively greater activity during the anticipation of Certain Threat (**Fig. 4**). While there is widespread agreement that the BST and dorsal amygdala play a critical role in orchestrating adaptive responses to danger, their precise contributions to human anxiety have remained contentious (3, 36, 66). Our results suggest that these regions respond similarly to different kinds of threat (**Figs. 2** and **4**). In fact, the present findings rigorously demonstrate that the BST and dorsal amygdala exhibit statistically indistinguishable hemodynamic responses to threat anticipation across a variety of 'head-to-head' comparisons (**Fig. 5**), reinforcing the possibility that they make similar contributions to human anxiety (3, 44).

Since the time of Freud (67), the distinction between certain (*'fear'*) and uncertain (*'anxiety'*) danger has been a key feature of prominent models of emotion and psychiatric disease (1-5, 43, 68). Our findings show that the brain regions recruited during the anticipation of Certain and Uncertain Threat are anatomically co-localized at the voxelwise level (**Fig. 2**). This common threat anticipation network encompasses subcortical regions—including the PAG, BST, and dorsal amygdala—that are critical for assembling appropriate defensive responses to uncertain threat in animals (3, 12, 69). But it also includes fronto-cortical regions—including the MCC, AI/FrO, and dlPFC/FP—that have received less empirical attention and are difficult or impossible to study in rodents (18-24). These frontal regions have traditionally been associated with the controlled processing and regulation of emotion, cognition, and action (70-75) and more recently implicated in the conscious experience of emotion (i.e. feelings) (76). As described in more detail in **Fig. S5**, the present results are well aligned with recent coordinate-based meta-analyses of neuroimaging studies of both 'fear' (54) and 'anxiety' (32). Across studies collectively encompassing hundreds of subjects, this body of research shows that the anticipation of *certain threat* (Pavlovian threat cues; the prototypical 'fear' stimulus in laboratory studies) and *uncertain threat* (instructed 'threat-of-shock') recruit an overlapping network of core regions, including the BST, consistent with the present within-subjects results.



Supplementary Figure S5. Certain and uncertain threat elicit broadly similar patterns of neural activity. Figure summarizes the results of two coordinate-based meta-analyses of functional neuroimaging studies. *Top-left* inset depicts the results for 27 'fear conditioning' studies (*N*=677), highlighting regions showing consistently greater activity during the anticipation of certain threat (CS+ > CS-) (71). *Bottom-right* inset depicts the results for 18 'threat-of-shock' studies (*N*=693), highlighting regions showing consistently greater activity during the anticipation of uncertain threat (Threat > Safe) (5). Visual inspection of the results (*red clusters*) suggests that the anticipation of certain and uncertain threat elicits qualitatively similar patterns, including heightened activity in the region of the BST. This impression is reinforced by the substantial correlation between the two whole-brain patterns, r = .69. Note: The pattern correlation was estimated in Neurovault (72) using a brain-masked, 4-mm transformation of the publicly available, vectorized meta-analytic maps. For illustrative purposes, every 10th voxel is depicted in the scatter plot. Abbreviations—BST, bed nucleus of the stria terminalis; L, left hemisphere; R, right hemisphere.

Our results provide fresh insight into the functional architecture of the threat anticipation network, demonstrating that fronto-cortical regions prefer Uncertain over Certain Threat, whereas the BST and dorsal amygdala show the reverse preference—*a difference in degree, not in kind*. In contrast to prior research, trivial differences cannot account for this nuance; the two threat conditions were pseudo-randomly intermixed and nearly identical in terms of their perceptual, nociceptive, motor, and statistical features (**Fig. 1**). So, what might explain the observed regional preferences? Aside from temporal certainty, the most conspicuous difference between the threat conditions is the degree of cognitive scaffolding. During Certain Threat trials, the descending stream of integers provides a precise and predictable index of momentary changes in threat imminence, encouraging a reactive, stimulus-bound cognitive mode. During Uncertain Threat trials this support is absent, necessitating a greater reliance on the kinds of sustained, endogenous representations that are the hallmark of fronto-cortical regions (77, 78). A second notable difference between the two threat conditions is the intensity of anxiety. Consistent with prior work (37, 79-81), the anticipation of Uncertain Threat was associated with greater distress and arousal (**Fig. 1**). The observed increase in fronto-cortical activity could reflect either heightened anxiety or compensatory processes aimed at downregulating distress and arousal. Testing these non-exclusive hypotheses will require a multi-pronged approach that encompasses carefully optimized tasks and mechanistic interventions (e.g. transcranial magnetic stimulation, acute anxiolytic challenges, explicit regulation manipulations). Multivariate classifier approaches are likely to be useful for linking specific facets of anxiety, such as feelings, to variation in the function of the threat anticipation network, and determining whether this reflects expressive or regulatory processes (82-84).

The present results add to a growing body of evidence indicating that the BST and dorsal amygdala, while certainly not interchangeable (cf. **Fig. 4, right column**), are more alike than different (3, 31). Anatomically, the BST and dorsal amygdala are characterized by similar patterns of connectivity, cellular

composition, neurochemistry, and gene expression (58). Both regions are poised to trigger defensive responses to threat via dense projections to brainstem and subcortical effector regions (58, 85). Consistent with this perspective, neuroimaging studies in monkeys and humans have documented similar physiological responses in the two regions to a broad spectrum of threats, including sustained exposure to novelty ('diffuse' threat; 86, 87) and intruder threat (88, 89); brief exposure (800 ms) to aversive images (90); an unpredictably approaching tarantula (91); and 'jump-scares' in horror movies (34)[2]. Work in rodents reinforces the hypothesis that the BST and dorsal amygdala (Ce) are crucial substrates for human anxiety, showing that specific microcircuits within and between these two regions bi-directionally control defensive responses to a range of threatening cues and contexts (3, 12, 44-48). Notably, work using a variant of the MTC paradigm in mice demonstrates that cannabinoid projections from the Ce to the BST are necessary for mounting defensive responses to temporally uncertain shock (51), consistent with the present conclusions. While our understanding remains far from complete, these observations collectively underscore the need to revise models of anxiety—including the NIMH RDoC framework—that imply a strict regional segregation of certain and uncertain threat processing (4, 43). At minimum, the present results imply that the magnitude of regional differences in threat reactivity is small, conditional on known perceptual confounds or unknown moderators, or simply non-existent.

In conclusion, the neural networks recruited by uncertain and certain threat are not categorically different, at least when viewed through the macroscopic lens of fMRI. We see evidence of anatomical co-localization, not segregation. This shared threat anticipation system can be functionally fractionated, with fronto-cortical regions showing relatively stronger engagement during the anticipation of uncertain threat, and the BST and dorsal amygdala showing the reverse pattern. Across a range of comparisons, the BST and dorsal amygdala evinced statistically equivalent responses during the anticipation of certain and

[2] https://identifiers.org/neurovault.collection:6237 and personal communication with Dr. Matthew Hudson (2/14/2020).

uncertain threat, reinforcing the possibility that they make similar contributions to human anxiety. These observations provide a novel framework for conceptualizing fear and anxiety, and for guiding the development of mechanistic work in humans and animals aimed at developing more effective intervention strategies for extreme anxiety (12, 92, 93). A relatively large sample, well-controlled task, and advanced techniques for fMRI data acquisition and processing enhance confidence in the robustness and translational relevance of these results.

METHODS

As detailed in the **Supplement**, neuroimaging, behavioral, and psychophysiological data were collected, processed, and analyzed using well-established approaches.

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AUTHOR CONTRIBUTIONS

A.J.S., K.A.D., and J.F.S. designed the study. J.F.S. developed and optimized the imaging paradigm. K.A.D. managed data collection and study administration. K.A.D., J.F.S., A.S.A, J.K., and R.M.T. collected data. K.A.D., J.H.,and A.J.S. processed and analyzed phenotypic data. J.F.S. developed data processing and analytic software. J.H., J.F.S., H.C.K., and R.M.T. processed imaging data. J.F.S., J.H., A.J.S., and A.S.F. developed the analytic strategy. J.F.S., J.H., and A.J.S. analyzed data. J.H., A.J.S., J.F.S., and A.S.F. interpreted data. J.H., J.F.S., A.S.F., and A.J.S. wrote the paper. J.H., M.K., and A.J.S. created figures. J.H., H.C.K., and A.J.S. created tables. A.J.S. funded and supervised all aspects of the study. All authors contributed to reviewing and revising the paper and approved the final version.

DATA SHARING

Raw data have been or will be made available via the National Institute of Mental Health's RDoC Database. Key statistical maps and regions-of-interest have been or will be uploaded to NeuroVault.org.

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