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1 Anxiety and the neurobiology of temporally uncertain threat anticipation

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13

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22

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43 **AUTHOR CONTRIBUTIONS**

44 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.
45 managed data collection and study administration. K.A.D., J.F.S., A.S.A, J.K., and R.M.T. collected data.
46 K.A.D., J.H., and A.J.S. processed and analyzed phenotypic data. J.F.S. developed data processing and
47 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.
48 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
49 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.
50 created tables. A.J.S. funded and supervised all aspects of the study. All authors contributed to reviewing
51 and revising the paper and approved the final version.

52 **RESOURCE SHARING**

53 Raw data are available at the National Institute of Mental Health's Data Archive. Key statistical maps are
54 or will be publicly available at NeuroVault.org.

55 **ABSTRACT**

56 When extreme, anxiety—a state of distress and arousal prototypically evoked by uncertain danger—can
57 be debilitating. Uncertain anticipation is a shared feature of situations that elicit signs and symptoms of
58 anxiety across psychiatric disorders, species, and assays. Despite the profound significance of anxiety for
59 human health and wellbeing, the neurobiology of uncertain-threat anticipation remains unsettled.
60 Leveraging a paradigm adapted from animal research and optimized for functional MRI signal
61 decomposition, we examined the neural circuits engaged during the anticipation of temporally uncertain
62 and certain threat in 99 men and women. Results revealed that the neural systems recruited by uncertain
63 and certain threat anticipation are anatomically co-localized in fronto-cortical regions, extended
64 amygdala, and periaqueductal gray. Comparison of the threat conditions demonstrated that this circuitry
65 can be fractionated, with fronto-cortical regions showing relatively stronger engagement during the
66 anticipation of uncertain threat, and the extended amygdala showing the reverse pattern. Although there
67 is widespread agreement that the bed nucleus of the stria terminalis and dorsal amygdala—the two
68 major subdivisions of the extended amygdala—play a critical role in orchestrating adaptive responses to
69 potential danger, their precise contributions to human anxiety have remained contentious. Follow-up
70 analyses demonstrated that these regions show statistically indistinguishable responses to temporally
71 uncertain and certain threat anticipation. These observations provide a framework for conceptualizing
72 anxiety and fear, for understanding the functional neuroanatomy of threat anticipation in humans, and
73 for accelerating the development of more effective intervention strategies for pathological anxiety.

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77 **SIGNIFICANCE STATEMENT**

78 Anxiety—an emotion prototypically associated with the anticipation of uncertain harm—has profound
79 significance for public health, yet the underlying neurobiology remains unclear. Leveraging a novel
80 neuroimaging paradigm in a relatively large sample, we identify a core circuit responsive to both
81 uncertain and certain threat anticipation, and show that this circuitry can be fractionated into
82 subdivisions with a bias for one kind of threat or the other. The extended-amygdala occupies center-stage
83 in neuropsychiatric models of anxiety, but its functional architecture has remained contentious. Here we
84 demonstrate that its major subdivisions show statistically indistinguishable responses to temporally
85 uncertain and certain threat. Collectively, these observations indicate the need to revise how we think
86 about the neurobiology of anxiety and fear.

87

88

89

90 **INTRODUCTION**

91 Anxiety is widely conceptualized as a state of distress and arousal elicited by the anticipation of uncertain
92 danger (Davis et al., 2010; Grupe and Nitschke, 2013). Anxiety lies on a continuum and, when extreme,
93 can be debilitating (Salomon et al., 2015; Conway et al., 2019). Anxiety disorders are the most common
94 family of psychiatric illnesses and existing treatments are inconsistently effective or associated with
95 significant adverse effects, underscoring the urgency of developing a clearer understanding of the
96 underlying neurobiology (Griebel and Holmes, 2013; Global Burden of Disease Collaborators, 2016;
97 Craske et al., 2017).

98

99 Perturbation and recording studies in mice have begun to reveal the specific molecules and cellular
100 ensembles that underlie defensive responses to uncertain threat (Fadok et al., 2018; Fox and Shackman,
101 2019), but the relevance of these tantalizing discoveries to the complexities of human anxiety is unclear.
102 Humans and mice diverged ~75 MYA, leading to marked behavioral, genetic, and neurobiological
103 differences between the two species (Van Essen et al., 2019). The role of fronto-cortical regions that are
104 especially well-developed in humans—including the midcingulate cortex (MCC), anterior insula (AI), and
105 dorsolateral prefrontal cortex (dlPFC)—also remains opaque, reflecting equivocal or absent anatomical
106 homologies and the use of disparate paradigms across species (Vogt and Paxinos, 2014; Shackman et al.,
107 2016; Carlén, 2017; Roberts, 2020).

108

109 Beneath the neocortex, the role of the central extended amygdala—including the dorsal amygdala in the
110 region of the central nucleus (Ce) and the bed nucleus of the stria terminalis (BST)—remains particularly
111 contentious (Fox and Shackman, 2019). Inspired by an earlier-generation of lesion studies in rodents
112 (Davis, 2006), it is widely believed that these regions are functionally dissociable, with the amygdala
113 mediating phasic responses to clear-and-immediate danger ('acute threat') and the BST mediating

114 sustained responses to uncertain-or-remote danger ('potential threat') (e.g., Sylvers et al., 2011;
115 Somerville et al., 2013; Avery et al., 2016; LeDoux and Pine, 2016; Klumpers et al., 2017; Watson et al.,
116 2017). This 'strict-segregation' hypothesis has even been enshrined in the National Institute of Mental
117 Health's (NIMH) Research Domain Criteria (RDoC) framework (National Institute of Mental Health, 2011,
118 2020a, b). Yet, a growing body of optogenetic, chemogenetic, and electrophysiological work in rodents
119 demonstrates that defensive responses elicited by the anticipation of uncertain threat (e.g. elevated-plus
120 maze) are assembled by microcircuits encompassing both regions (Gungor and Paré, 2016; Lange et al.,
121 2017; Ahrens et al., 2018; Pomrenze et al., 2019a; Pomrenze et al., 2019b; Ressler et al., 2020; Griessner
122 et al., *in press*), motivating the competing hypothesis that the dorsal amygdala and BST are both
123 important substrates for human anxiety (Shackman and Fox, 2016; Fox and Shackman, 2019).

124

125 To address these fundamental questions, we combined fMRI with a novel threat-anticipation task in 99
126 adults. Advanced data acquisition and processing techniques enhanced resolution of subcortical regions.
127 Building on earlier work (e.g., Somerville et al., 2013; Grupe et al., 2016), the Maryland Threat
128 Countdown (MTC) paradigm is an fMRI-optimized variant of assays that have been validated using fear-
129 potentiated startle and acute pharmacological manipulations in rodents (Miles et al., 2011; Daldrup et al.,
130 2015), and humans (Hefner et al., 2013), maximizing translational relevance. It takes the form of a 2
131 (*Valence*: Threat/Safety) × 2 (*Temporal Certainty*: Uncertain/Certain) randomized event-related design
132 (**Fig. 1**). On Certain Threat trials, subjects saw a descending stream of integers for 18.75 s, sufficiently
133 long to enable the dissection of onset-evoked from sustained hemodynamic responses. To ensure robust
134 emotion, this anticipatory epoch ('countdown') always culminated with the delivery of a multi-modal
135 reinforcer (aversive shock, photograph, and audio-clip). Uncertain Threat trials were similar, but the
136 integer stream was randomized and presented for an uncertain and variable duration ($M=18.75$ s,
137 Range=8.75-30.00). Here, subjects knew the threat was coming, but they did not know *when* it would

138 occur. Safety trials were similar, but terminated in benign reinforcers. Comparison of the well-matched
139 anticipatory epochs enabled us to rigorously isolate circuits recruited during uncertain-threat
140 anticipation.

141

142 MATERIALS AND METHODS

143 Subjects

144 As part of an on-going prospective-longitudinal study focused on the emergence of anxiety disorders and
145 depression, we used well-established measures of dispositional negativity (often termed neuroticism or
146 negative emotionality; Shackman et al., 2018; Hur et al., 2019; Hur et al., *in press*) to screen 6,594 young
147 adults (57.1% female; 59.0% White, 19.0% Asian, 9.9% African American, 6.3% Hispanic, 5.8%
148 Multiracial/Other; $M=19.2$ years, $SD=1.1$ years). Screening data were stratified into quartiles (top quartile,
149 middle quartiles, bottom quartile) separately for men and women. Individuals who met preliminary
150 inclusion criteria were independently recruited from each of the resulting six strata. Given the focus of
151 the larger study, approximately half the subjects were recruited from the top quartile, with the remainder
152 split between the middle and bottom quartiles (i.e., 50% high, 25% medium, and 25% low), enabling us
153 to sample a wide range of risk for the development of internalizing disorders. A total of 121 subjects were
154 recruited. Of these, 2 withdrew during the imaging assessment due to excess distress. Of the 119 subjects
155 who completed the imaging assessment, a total of 20 were excluded from analyses due to incidental
156 neurological findings ($n=4$), scanner problems ($n=2$), insufficient fMRI data (<2 usable scans, $n=1$),
157 excessive global motion artifact (see below; $n=3$), or excessive task-correlated motion (see below, $n=10$).
158 This yielded a final sample of 99 subjects (52 females; 65.7% White, 17.2% Asian, 8.1% African American,
159 3.0% Hispanic, 6.1% Multiracial/Other; $M=18.8$ years, $SD=0.4$ years), providing substantially greater
160 power to detect medium-sized ($0.5 < \text{Cohen's } d < 0.8$) statistical effects (Geuter et al., 2018) compared to

161 typical fMRI studies of uncertain threat anticipation (median N=29; range=15-108; Chavanne and
162 Robinson, in press). All subjects had normal or corrected-to-normal color vision; and reported the
163 absence of lifetime neurological symptoms, pervasive developmental disorder, very premature birth,
164 medical conditions that would contraindicate MRI, and prior experience with noxious electrical
165 stimulation. All subjects were free from a lifetime history of psychotic and bipolar disorders; a current
166 diagnosis of a mood, anxiety, or trauma disorder (past 2 months); severe substance abuse; active
167 suicidality; and on-going psychiatric treatment as determined by an experienced masters-level
168 diagnostician using the Structured Clinical Interview for DSM-5 (First et al., 2015). Subjects provided
169 informed written consent and all procedures were approved by the Institutional Review Board at the
170 University of Maryland, College Park.

171

172 **Maryland Threat Countdown (MTC) fMRI Paradigm**

173 **Paradigm Structure and Design Considerations.** Building on earlier imaging work (Somerville et al.,
174 2013; Grupe et al., 2016; Pedersen et al., 2019), the Maryland Threat Countdown (MTC) paradigm is an
175 fMRI-optimized version of temporally uncertain-threat assays that have been validated using fear-
176 potentiated startle and acute anxiolytic administration (e.g. benzodiazepine) in mice (Daldrup et al.,
177 2015; Lange et al., 2017), rats (Miles et al., 2011), and humans (Hefner et al., 2013), enhancing its
178 translational relevance. The MTC paradigm takes the form of a 2 (*Valence*: Threat/Safety) × 2 (*Temporal*
179 *Certainty*: Uncertain/Certain) randomized event-related design (3 scans; 6 trials/condition/scan).
180 Simulations were used to optimize the detection and deconvolution of task-related hemodynamic signals
181 (variance inflation factors <1.54). Stimulus presentation and ratings acquisition were controlled using
182 Presentation software (version 19.0, Neurobehavioral Systems, Berkeley, CA).

183

184 On Certain Threat trials, subjects saw a descending stream of integers ('count-down;' e.g. 30, 29, 28...3, 2,
185 1) for 18.75 s. To ensure robust emotion, this anticipatory epoch always culminated with the delivery of a
186 noxious electric shock, unpleasant photographic image (e.g. mutilated body), and thematically related
187 audio clip (e.g. scream, gunshot). Uncertain Threat trials were similar, but the integer stream was
188 randomized and presented for an uncertain and variable duration (8.75-30.00 s; $M=18.75$ s). Here,
189 subjects knew that something aversive was going to occur, but they had no way of knowing precisely
190 when it would occur. Consistent with recent recommendations (Shackman and Fox, 2016), the average
191 duration of the anticipatory epoch was identical across conditions, ensuring an equal number of
192 measurements (TRs/condition). Mean duration was chosen to enhance detection of task-related
193 differences in the blood oxygen level-dependent (BOLD) signal (Henson, 2007), and to enable dissection
194 of onset from genuinely sustained responses. Safety trials were similar, but terminated with the delivery
195 of benign reinforcers (see below). Valence was continuously signaled during the anticipatory epoch by
196 the background color of the display. Temporal certainty was signaled by the nature of the integer stream.
197 Certain trials always began with the presentation of the number 30 (**Fig. 1**). On uncertain trials integers
198 were randomly drawn from a near-uniform distribution ranging from 1 to 45 to reinforce the impression
199 that Uncertain trials could be much longer than Certain ones and to minimize incidental temporal
200 learning ('time-keeping'). To mitigate potential confusion and eliminate mnemonic demands, a lower-
201 case 'c' or 'u' was presented at the lower edge of the display throughout the anticipatory epoch. White-
202 noise visual masks (3.2 s) were presented between trials to minimize persistence of the visual reinforcers
203 in iconic memory. Subjects provided ratings of anticipatory fear/anxiety for each trial type during each
204 scan using an MRI-compatible response pad (MRA, Washington, PA; **Fig. 1**). Subjects were instructed to
205 rate the intensity of the fear/anxiety experienced during the prior anticipatory ('countdown') epoch

206 using a 1 (*minimal*) to 4 (*maximal*) scale. Subjects were prompted to rate each trial type once per scan. A
207 total of 6 additional echo-planar imaging (EPI) volumes were acquired at the beginning and end of each
208 scan (see below).

209

210 **Procedures.** Prior to scanning, subjects practiced an abbreviated version of the paradigm—without
211 electrical stimulation—until they indicated and staff confirmed that they understood the task. Benign and
212 aversive electrical stimulation levels were individually titrated. *Benign Stimulation.* Subjects were asked
213 whether they could “reliably detect” a 20 V stimulus and whether it was “at all unpleasant.” If the subject
214 could not detect the stimulus, the voltage was increased by 4 V and the process repeated. If the subject
215 indicated that the stimulus was unpleasant, the voltage was reduced by 4V and the process repeated. The
216 final level chosen served as the benign electrical stimulation during the imaging assessment ($M=20.67$,
217 $SD=6.23$). *Aversive Stimulation.* Subjects received a 100 V stimulus and were asked whether it was “as
218 unpleasant as you are willing to tolerate.” If the subject indicated that they were willing to tolerate more
219 intense stimulation, the voltage was increased by 10 V and the process repeated. If the subject indicated
220 that the stimulus was too intense, the voltage was reduced by 5 V and the process repeated. The final
221 level chosen served as the aversive electrical stimulation during the imaging assessment ($M=115.21$,
222 $SD=25.05$). Following each scan of the MTC paradigm, we re-assessed whether stimulation was
223 sufficiently intense and re-calibrated as necessary. In total, 32.3% of subjects adjusted the level of benign
224 or aversive stimulation at least once during the imaging assessment.

225

226 **Electrical Stimuli.** Electrical stimuli (100 ms; 2 ms pulses every 10 ms) were generated using an MRI-
227 compatible constant-voltage stimulator system (STMEPM-MRI; Biopac Systems, Inc., Goleta, CA). Stimuli

228 were delivered using MRI-compatible, disposable carbon electrodes (Biopac) attached to the fourth and
229 fifth phalanges of the non-dominant hand.

230

231 **Visual Stimuli.** Visual stimuli (1.8 s) were digitally back-projected (Powerlite Pro G5550, Epson America,
232 Inc., Long Beach, CA) onto a semi-opaque screen mounted at the head-end of the scanner bore and
233 viewed using a mirror mounted on the head-coil. A total of 72 photographs were selected from the
234 International Affective Picture System (IAPS identification numbers)—*Benign*: 1670, 2026, 2038, 2102,
235 2190, 2381, 2393, 2397, 2411, 2850, 2870, 2890, 5390, 5471, 5510, 5740, 7000, 7003, 7004, 7014, 7020,
236 7026, 7032, 7035, 7050, 7059, 7080, 7090, 7100, 7140, 7187, 7217, 7233, 7235, 7300, 7950. *Aversive*:
237 1300, 3000, 3001, 3010, 3015, 3030, 3051, 3053, 3061, 3062, 3063, 3069, 3100, 3102, 3150, 3168, 3170,
238 3213, 3400, 3500, 6022, 6250, 6312, 6540, 8230, 9042, 9140, 9253, 9300, 9405, 9410, 9414, 9490, 9570,
239 9584, 9590. (Lang et al., 2008). Based on normative ratings, the aversive images were significantly more
240 negative and arousing than the benign images, $t(70)>24.3$, $p<.001$. On a 1 (*negative/low-arousal*) to 9
241 (*positive/high-arousal*) scale, the mean valence and arousal scores were 2.2 ($SD=0.6$) and 6.3 ($SD=0.6$) for
242 the aversive images, and 5.2 ($SD=0.4$) and 2.8 ($SD=0.3$) for the benign images.

243

244 **Auditory Stimuli.** Auditory stimuli (0.80 s) were delivered using an amplifier (PA-1 Whirlwind) with in-
245 line noise-reducing filters and ear buds (S14; Sensimetrics, Gloucester, MA) fitted with noise-reducing ear
246 plugs (Hearing Components, Inc., St. Paul, MN). A total of 72 auditory stimuli (half aversive, half benign)
247 were adapted from open-access online sources.

248

249 **Peripheral Physiology Data Acquisition**

250 Peripheral physiology was continuously acquired during each fMRI scan using a Biopac system (MP-150).
251 Skin conductance (250 Hz; 0.05 Hz high-pass) was measured using MRI-compatible disposable electrodes
252 (EL507) attached to the second and third phalanges of the non-dominant hand. For imaging analyses,
253 measures of respiration and breathing were also acquired using a respiration belt and photo-
254 plethysmograph (first phalange of the non-dominant hand).

255

256 **MRI Data Acquisition**

257 MRI data were acquired using a Siemens Magnetom TIM Trio 3 Tesla scanner (32-channel head-coil).
258 Foam inserts were used to immobilize the participant's head within the head-coil and mitigate potential
259 motion artifact. Subjects were continuously monitored from the control room using an MRI-compatible
260 eye-tracker (Eyelink 1000; SR Research, Ottawa, Ontario, Canada). Head motion was monitored using the
261 AFNI real-time plugin (Cox, 1996). Sagittal T1-weighted anatomical images were acquired using a
262 magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence (TR=2,400 ms; TE=2.01 ms;
263 inversion time=1060 ms; flip angle=8°; sagittal slice thickness=0.8 mm; in-plane=0.8 × 0.8 mm;
264 matrix=300 × 320; field-of-view=240 × 256). A T2-weighted image was collected co-planar to the T1-
265 weighted image (TR=3,200 ms; TE=564 ms; flip angle=120°). To enhance resolution, a multi-band
266 sequence was used to collect oblique-axial echo planar imaging (EPI) volumes (multiband acceleration=6;
267 TR=1,250 ms; TE=39.4 ms; flip angle=36.4°; slice thickness=2.2 mm, number of slices=60; in-plane
268 resolution=2.1875 × 2.1875 mm; matrix=96 × 96). Images were collected in the oblique axial plane
269 (approximately -20° relative to the AC-PC plane) to minimize potential susceptibility artifacts. Three
270 478-volume EPI scans were acquired. The scanner automatically discarded 7 volumes prior to the first

271 recorded volume. To enable fieldmap correction, two oblique-axial spin echo (SE) images were collected
272 in each of two opposing phase-encoding directions (rostral-to-caudal and caudal-to-rostral) at the same
273 location and resolution as the functional volumes (i.e., co-planar; TR=7,220 ms; TE=73 ms). Following the
274 last scan, subjects were removed from the scanner, debriefed, compensated, and discharged.

275

276 **Skin Conductance Data Pipeline**

277 Skin conductance data were processed using PsPM (version 4.0.2) and in-house Matlab code (Bach and
278 Friston, 2013; Bach et al., 2018). Data from each scan were band-pass filtered (0.01-0.25 Hz), resampled
279 to match the TR used for fMRI data acquisition (1.25 s), and z-transformed. Using standard Matlab
280 functions, SCR data were modeled in a manner that approximated that used for the fMRI data. A GLM was
281 used to estimate skin conductance levels during the anticipatory epoch of each condition of the MTC
282 paradigm (see above) for each subject (Bach et al., 2009; Bach et al., 2013; Bach, 2014). Predictors from
283 the first-level fMRI model (see below) were convolved with a canonical skin conductance response
284 function (Bach et al., 2010; Gerster et al., 2018), bandpass filtered to match the data, and z-transformed.

285

286 **MRI Data Pipeline**

287 Methods were optimized to minimize spatial normalization error and other potential sources of noise.
288 Structural and functional MRI data were visually inspected before and after processing for quality
289 assurance.

290

291 **Anatomical Data Processing.** Methods were similar to those described in other recent reports by our
292 group (Hur et al., 2018; Smith et al., 2018; Tillman et al., 2018). T1-weighted images were inhomogeneity
293 corrected using N4 (Tustison et al., 2010) and filtered using the denoise function in ANTS (Avants et al.,
294 2011). The brain was then extracted using a variant of the BEaST algorithm (Eskildsen et al., 2012) with
295 brain-extracted and normalized reference brains from the IXI database ([https://brain-](https://brain-development.org/ixi-dataset)
296 [development.org/ixi-dataset](https://brain-development.org/ixi-dataset)). Brain-extracted T1 images were normalized to a version of the brain-
297 extracted 1-mm T1-weighted MNI152 (version 6) template (Grabner et al., 2006) modified to remove
298 extracerebral tissue. This was motivated by evidence that brain-extracted T1 images and a brain-
299 extracted template enhance the quality of spatial normalization (Fein et al., 2006; Acosta-Cabronero et al.,
300 2008; Fischmeister et al., 2013). Normalization was performed using the diffeomorphic approach
301 implemented in SyN (version 1.9.x.2017-09.11; Klein et al., 2009; Avants et al., 2011). T2-weighted
302 images were rigidly co-registered with the corresponding T1 prior to normalization and the brain
303 extraction mask from the T1 was applied. Tissue priors (Lorio et al., 2016) were unwarped to the native
304 space of each T1 using the inverse of the diffeomorphic transformation. Brain-extracted T1 and T2
305 images were simultaneously segmented using native-space priors generated using FAST (FSL version
306 5.0.9) (Zhang et al., 2001) for use in T1-EPI co-registration (see below).

307

308 **Fieldmap Data Processing.** SE images were used to create a fieldmap in topup (Andersson et al., 2003;
309 Smith et al., 2004; Graham et al., 2017). Fieldmaps were converted to radians, median filtered, and
310 smoothed (2-mm). The average of the distortion-corrected SE images was inhomogeneity-corrected
311 using N4, and brain-masked using 3dSkullStrip in AFNI (version 17.2.10; Cox, 1996). The resulting mask
312 was minimally eroded to further exclude extracerebral voxels.

313

314 **Functional Data Processing.** EPI files were de-spiked using 3dDespike and slice-time corrected (to the
315 center of the TR) using 3dTshift, inhomogeneity corrected using N4, and motion corrected to the first
316 volume using a 12-parameter affine transformation implemented in ANTs. Recent work indicates that de-
317 spiking is more effective than ‘scrubbing’ for attenuating motion-related artifacts (Jo et al., 2013; Siegel et
318 al., 2014; Power et al., 2015). Transformations were saved in ITK-compatible format for subsequent use.
319 The first volume was extracted for EPI-T1 co-registration. The reference EPI volume was simultaneously
320 co-registered with the corresponding T1-weighted image in native space and corrected for geometric
321 distortions using boundary-based registration (Greve and Fischl, 2009). This step incorporated the
322 previously created fieldmap, undistorted SE, T1, white matter (WM) image, and masks. The spatial
323 transformations necessary to transform each EPI volume from native space to the reference EPI, from the
324 reference EPI to the T1, and from the T1 to the template were concatenated and applied to the processed
325 (de-spiked and slice-time corrected) EPI data in a single step to minimize incidental spatial blurring.
326 Normalized EPI data were resampled to 2-mm isotropic voxels using fifth-order b-splines and smoothed
327 (6-mm FWHM) using 3DblurInMask.

328

329 **Data Exclusions.** To assess residual motion artifact, we computed the number of times the brain showed
330 a volume-to-volume displacement >0.5 mm using the motion-corrected data. Scans with excess artifact
331 ($\geq 7.5\%$) were discarded. Three subjects with insufficient usable data (<2 scans) were excluded from
332 analyses, while 6 subjects with 2 usable scans were retained. To assess task-correlated motion, we
333 computed correlations between the design matrix and the motion estimates (see above). Scans showing
334 extreme correlations ($>2 SD$) were discarded. On this basis, ten subjects with insufficient usable data (<2
335 scans) were excluded from analyses, while 19 subjects with 2 usable scans were retained.

336

337 **Canonical First-Level fMRI Modeling.** Modeling was performed using SPM12 (version 6678;
338 <https://www.fil.ion.ucl.ac.uk/spm>). Band-pass was set to the hemodynamic response function (HRF) and
339 128 s for low and high pass, respectively. The MTC paradigm was modeled using variable-duration
340 rectangular ('box-car') regressors time-locked to the anticipatory epochs of the Uncertain Threat, Certain
341 Threat, and Uncertain Safety trials. Certain Safety trials were treated as an unmodeled ('implicit') high-
342 level baseline. EPI volumes collected before the first trial, during intertrial intervals, and following the
343 final trial were also unmodeled, and contributed to the baseline estimate. Regressors were convolved
344 with a canonical HRF and its temporal derivative. The periods corresponding to the delivery of the four
345 reinforcers and rating trials were modeled using a similar approach (Fig. 1). Volume-to-volume
346 displacement and motion parameters (including 1- and 2-volume lagged versions) were also included,
347 similar to other recent work (Reddan et al., 2018). To further attenuate potential noise, cerebrospinal
348 fluid (CSF) time-series, instantaneous pulse and respiration rates, and their estimated effect on the BOLD
349 time-series were also included as nuisance variates. ICA-AROMA (Pruim et al., 2015) was used to model
350 several other potential sources of noise (brain-edge, CSF-edge, WM). These and the single ICA component
351 showing the strongest correlation with motion estimates were included as additional nuisance variates.
352 EPI volumes with excessive volume-to-volume displacement (>0.25 mm), as well as those during and
353 immediately following the delivery of aversive reinforcers, were censored.

354

355 **Decomposing Canonical Effects Using Finite Impulse Response (FIR) Modeling.** The canonical
356 modeling approach estimates the amplitude of anticipatory activity under the assumption that it
357 approximates a 'boxcar'-like square-wave function. This makes it tempting to conclude that regions

358 showing significant activation represent sustained responses. Yet there is ample evidence that a variety
359 of other signals are plausible (e.g. Gonzalez-Castillo et al., 2015; Gungor and Paré, 2016; Sreenivasan and
360 D'Esposito, 2019) and, importantly, can yield similarly strong statistical effects (Fig. 2). Addressing this
361 ambiguity necessitates a finer decomposition of the signal underlying significant 'omnibus' effects
362 revealed by canonical modeling—a surprisingly rare approach in the neuroimaging literature. To do so,
363 we identified the most extreme peak (e.g. BST) in each of the major regions identified in our canonical
364 analyses. These peak locations were then interrogated using a finite impulse response (FIR) analysis,
365 which provides an estimate of the magnitude *and* shape of anticipatory activity (Glover, 1999; Ollinger et
366 al., 2001; for a similar approach by our group, see Guller et al., 2012). To perform the FIR modeling,
367 variance related to reinforcer delivery, ratings, and the nuisance variables was removed from the
368 preprocessed data using the canonical approach described above. Residualized data were bandpass
369 filtered (.007813-0.2667 Hz) and normalized to the SD of the Certain Safety trials (i.e. the implicit
370 baseline in the canonical HRF GLM). We then estimated the mean response at each TR of the anticipatory
371 epoch for each condition of the MTC paradigm for each subject.

372

373 **Experimental Design and Statistical Analyses**

374 **Overview.** Study design is described in 'Maryland Threat Countdown (MTC) fMRI Paradigm.' The number
375 of usable datasets, data exclusions, and power considerations are detailed in 'Subjects.'

376

377 **In-Scanner Fear/Anxiety Ratings and Skin Conductance.** Data were analyzed using standard repeated-
378 measures GLM approaches with Huynh-Feldt correction for potential non-sphericity implemented in
379 SPSS (version 24; IBM, Inc., Armonk, NY). Significant interactions were decomposed using simple effects.

380 Figures were created using R Studio (<http://www.rstudio.com>) and yarrr (version 0.1.5) for R (version
381 3.6.1.; <https://www.R-project.org>).

382

383 **Canonical Second-Level GLM.** Standard whole-brain voxelwise GLMs (random effects) were used to
384 compare anticipatory hemodynamic activity elicited by each threat-anticipation condition and its
385 corresponding control condition (e.g. Uncertain Threat vs. Uncertain Safety). Significance was assessed
386 using FDR $q < .05$, whole-brain corrected. As in prior work by our group (Shackman et al., 2013; Shackman
387 et al., 2017), a minimum conjunction (logical ‘AND’) was used to identify voxels sensitive to both
388 temporally certain *and* temporally uncertain threat anticipation (Nichols et al., 2005). We also directly
389 examined potential differences in anticipatory activity between the two threat conditions (Certain Threat
390 vs. Uncertain Threat). We did not examine hemodynamic responses to reinforcer delivery given the
391 possibility of artifact. Some figures were created using MRIcron
392 (<http://people.cas.sc.edu/rorden/mricron>). Clusters and local maxima were labeled using a combination
393 of the Allen Institute, Harvard–Oxford, and Mai atlases (Frazier et al., 2005; Desikan et al., 2006b; Makris
394 et al., 2006; Hawrylycz et al., 2012; Mai et al., 2015) and a recently established consensus nomenclature
395 (ten Donkelaar et al., 2018).

396

397 **Descriptive Decomposition of Canonical Effects Using FIR Modeling**

398 To decompose the signal underlying significant canonical effects, we identified the most extreme peak in
399 each of the major regions (e.g. amygdala) identified in our canonical analyses (indicated by a black-and-
400 white asterisk in the accompanying figures). These peaks were then descriptively interrogated using FIR
401 models. As shown in Fig. 1, the duration of anticipatory epoch differed between certain (18.75 s) and

402 uncertain trials (8.75-32.5 s; $M=18.75$ s), necessitating slightly different procedures for specific contrasts.
403 For the comparison of Certain Threat to Certain Safety, responses were modeled for 15 TRs (1.25 s TR;
404 total=18.125 s). Given the temporal resolution and autocorrelation of the BOLD signal, data were
405 averaged for 3 windows (TR-1 to TR-5, TR-6 to TR-10, TR-11 to TR-15). For the comparison of Uncertain
406 Threat to Uncertain Safety, responses were modeled for 24 TRs (total=30.00 s) and averaged for 4
407 windows, the first three corresponding to those used for certain trials and a fourth spanning TR-16 to TR-
408 24. This choice was partially motivated by the modest number of trials with the longest anticipatory
409 epoch (**Fig. 1**). For the comparison of the two threat conditions, responses were modeled for 15 TRs and
410 averaged across 3 windows, as above. ‘Sustained’ activity was operationally defined as greater mean
411 activity across two consecutive windows. Using this criterion, descriptive tests indicated nominally
412 significant ($p<.05$) evidence of sustained responses for most of the key regions for most of the contrasts
413 (e.g. Uncertain Threat vs. Uncertain Safety). Exceptions were the PAG for the Certain Threat vs. Certain
414 Safety contrast, and the PAG and BST for the Certain Threat vs. Uncertain Threat contrast. Because this
415 approach yields optimistically biased effect-size estimates (Kriegeskorte et al., 2010; Davenport and
416 Nichols, 2020), we refrain from reporting exact p -values, and instead provide standard errors of the
417 mean as a descriptive guide to the size of observed effects. Naturally, any inferences drawn from
418 inspection of the standard errors only apply to the peak voxels depicted in the accompanying figures
419 (black-and-white asterisks) and not necessarily to the entire parent region (e.g. amygdala).

420

421 **Testing Whether the BST and Amygdala Show Different Responses to Threat.** To test hypothesized
422 regional differences in threat sensitivity (see the Introduction), we used a combination of anatomical and
423 functional criteria to independently identify BST and amygdala voxels that were most sensitive to each
424 kind of threat. As shown in **Fig. 3**, each region was anatomically defined using an *a priori* probabilistic

425 region of interest (ROIs; Frazier et al., 2005; Desikan et al., 2006a; Theiss et al., 2017). Next, we extracted
426 and averaged standardized regression coefficients for voxels showing significant (FDR $q < .05$, whole-brain
427 corrected) activation for each of the relevant contrasts, separately for each region: Uncertain Threat >
428 Uncertain Safety, Certain Threat > Certain Safety, and Certain Threat > Uncertain Threat. Potential
429 regional differences (i.e. Region \times Condition interactions) were then assessed using standard repeated-
430 measures GLM approaches implemented in SPSS. Nonparametric tests (Wilcoxon signed rank) yielded
431 identical conclusions (not reported). Inferences necessarily apply only to the subset of BST or amygdala
432 voxels that proved sensitive to one or more of the threat contrasts, *not* the entire region.

433

434 **Testing Whether the BST and Amygdala Show Equivalent Responses to Threat.** Consistent with prior
435 work by our group (McMenamin et al., 2009; McMenamin et al., 2010), we used the well-established two
436 one-sided tests (TOST) procedure for formally testing whether the BST and amygdala show statistically
437 equivalent activity during threat anticipation. While it is not possible to definitively show that the true
438 difference in regional activity is zero, TOST provides a well-established and widely used framework for
439 testing whether mean differences are small enough to be considered equivalent (Lakens, 2017; Lakens et
440 al., 2018). Regression coefficients were extracted and averaged using the approach detailed in the prior
441 section. For present purposes, we considered regional differences smaller than a ‘medium’ standardized
442 effect for dependent means (Cohen’s $d_z = .35$) to be equivalent (Lakens, 2017). TOST procedures were
443 performed using the TOSTER package (version 0.3.4) for R.

444

445 RESULTS

446 **Temporally Uncertain Threat anticipation elicits robust symptoms and signs of anxiety**

447 As shown in **Fig. 1**, threat anticipation markedly increased subjective symptoms (in-scanner ratings) and
448 objective signs (skin conductance) of anxiety, and this was particularly evident when the timing of
449 aversive stimulation was *uncertain*. **Anticipatory feelings**. Subjects reported experiencing more intense
450 fear/anxiety when anticipating aversive outcomes ($F(1,98)=543.27, p< .001$), and when anticipating
451 outcomes with uncertain timing ($F(1,98)=85.46, p<.001$). The impact of threat on fear/anxiety ratings
452 was potentiated by temporal uncertainty (Valence × Uncertainty: $F(1,98)=13.08, p<.001$; Uncertain
453 Threat > Certain Threat: $t(98)=7.58, p<.001$; Uncertain Safety > Certain Safety: $t(98)=4.90, p<.001$;
454 Uncertain Threat > Uncertain Safety: $t(98)=21.98, p<.001$; Certain Threat > Certain Safety: $t(98)=20.36,$
455 $p<.001$), consistent with prior work (Grillon et al., 2006; Nelson and Shankman, 2011; Somerville et al.,
456 2013; Bennett et al., 2018). **Anticipatory arousal**. Subjects showed elevated skin conductance levels
457 when anticipating aversive outcomes ($F(1,98)=345.31, p<.001$), and when anticipating outcomes with
458 uncertain timing ($F(1,98)=85.86, p<.001$). The impact of threat on skin conductance was potentiated by
459 temporal uncertainty (Valence × Uncertainty: $F(1,98)=93.63, p<.001$; Uncertain Threat > Certain Threat:
460 $t(98) = 11.53, p<.001$; Uncertain Safety > Certain Safety: $t(98) = -3.99, p< .001$; Uncertain Threat >
461 Uncertain Safety: $t(98)=25.59, p<.001$; Certain Threat > Certain Safety: $t(98)=9.84, p<.001$). Taken
462 together, these results confirm the validity of the MTC paradigm for understanding the neural circuits
463 underpinning human anxiety.

464

465 **Temporally Uncertain Threat anticipation recruits a distributed network of subcortical and cortical**
466 **regions**

467 Next, a voxelwise GLM was used to identify brain regions recruited during the anticipation of temporally
468 Uncertain Threat (Uncertain Threat > Uncertain Safety; FDR $q<.05$, whole-brain corrected). As shown in
469 **Fig. 4**, this highlighted a widely distributed network of regions previously implicated in the expression
470 and regulation of human fear and anxiety (Fullana et al., 2016; Qi et al., 2018; Fox and Shackman, 2019;

471 Chavanne and Robinson, in press), including the MCC; AI extending into the frontal operculum (FrO);
472 dlPFC extending to the frontal pole (FP); brainstem encompassing the periaqueductal grey (PAG); basal
473 forebrain, in the region of the BST; and dorsal amygdala, in the region of the central and medial nuclei.
474 Heightened activity during the anticipation of Uncertain Threat was also evident in the orbitofrontal
475 cortex, basal ganglia, hippocampus, and ventrolateral amygdala in the region of the lateral nucleus
476 (**Extended Data Fig. 4-1**). Consistent with prior work (Choi et al., 2012; Grupe et al., 2016), Uncertain
477 Threat anticipation was associated with *reduced* activity in a set of midline regions that resembled the
478 default mode network (e.g. anterior rostral sulcus/ventromedial prefrontal cortex, postcentral gyrus, and
479 precuneus), as well as the posterior insula and parahippocampal gyrus (**Extended Data Fig. 4-2**).
480 Reduced activity was also observed in the most rostral tip of the amygdala, underscoring the functional
481 heterogeneity of this complex structure.

482

483 ***Temporally Uncertain Threat anticipation elicits sustained hemodynamic activity***

484 Anxiety is widely conceptualized as a sustained state (Davis et al., 2010; Tye et al., 2011; LeDoux and Pine,
485 2016; Mobbs, 2018), and it is tempting to interpret clusters of enhanced activity (e.g. **Fig. 4**) through this
486 lens. But do we actually see evidence of sustained responses during the anticipation of temporally
487 Uncertain Threat? Although a wide variety of other signals are physiologically plausible (**Fig. 2**), the vast
488 majority of fMRI studies never address this question; they simply assume the shape of the hemodynamic
489 response and focus on estimates of response magnitude ('activation'). To address this ambiguity, we used
490 a finite impulse response (FIR) approach to estimate responses elicited by the anticipation of Uncertain
491 Threat and Uncertain Safety on a finer time-scale. Descriptively, this revealed sustained activity (see
492 Materials and Methods) across key cortical (MCC, AI/FrO, dlPFC/FP) and subcortical (PAG, BST, dorsal
493 amygdala) regions (Uncertain Threat > Uncertain Safety; 6.25-30 s; **Fig. 5**).

494

495 **Temporally Certain Threat anticipation recruits an anatomically and functionally similar network**

496 Having identified a distributed neural circuit sensitive to Uncertain Threat, we used a parallel approach
497 to identify regions recruited during the anticipation of temporally *Certain Threat* (*Certain Threat* >
498 *Certain Safety*; FDR $q < .05$, whole-brain corrected). As shown in **Fig. 4**, results were similar to those found
499 for Uncertain Threat (**Extended Data Figs. 4-3 and 4-4**). In fact, a minimum conjunction analysis
500 (Logical ‘AND;’ Nichols et al., 2005) revealed voxelwise co-localization in every key cortical and
501 subcortical region, including the BST and dorsal amygdala in the region of the central and medial nuclei
502 (**Fig. 4** and **Extended Data Fig. 4-5**). FIR results also suggested functional convergence across conditions,
503 with all but one of these key regions (PAG) showing sustained levels of heightened hemodynamic activity
504 during the anticipation of Certain Threat (see Materials and Methods; **Fig. 6**). Taken together, these
505 results suggest that this network of subcortical and cortical regions is sensitive to multiple kinds of threat
506 anticipation, both certain and uncertain.

507

508 **The threat anticipation network can be fractionated into subdivisions**

509 To determine whether regions recruited during threat anticipation are sensitive to temporal uncertainty,
510 we directly compared the Uncertain and Certain Threat conditions (FDR $q < .05$, whole-brain corrected).
511 This indicated that the threat anticipation network can be fractionated into subdivisions. As shown in **Fig.**
512 **7**, key cortical regions (MCC, AI/FrO, and dlPFC/FP) showed a further increase in activity during the
513 anticipation of *Uncertain Threat* (**Extended Data Fig. 7-1**). In contrast, the BST and dorsal amygdala
514 (adjacent to the central nucleus, in the region of the cortical nucleus and Amygdala-Hippocampal
515 Transition Area) showed the reverse pattern, with relatively greater activity during the anticipation of
516 *Certain Threat* (**Extended Data Fig. 7-2**). The PAG did not discriminate the two threat conditions. FIR
517 results suggest a similar conclusion (**Fig. 7**).

518

519 **The anticipation of temporally Uncertain and Certain Threat elicits statistically indistinguishable**
520 **responses in the extended amygdala**

521 Our results indicate that the BST and dorsal amygdala—the two major subdivisions of the EA—respond
522 similarly to threat anticipation. Both regions show signs of elevated activity during threat anticipation,
523 and this is evident whether or not the timing of aversive stimulation is uncertain (**Fig. 4**). Furthermore,
524 both regions showed parallel increases in activity during the anticipation of Certain Threat (**Figs. 6-7**).
525 Yet it remains possible that the BST and the amygdala exhibit subtler differences in threat sensitivity. To
526 rigorously test this, we directly compared regional responses for each of the threat contrasts (e.g.
527 Uncertain Threat vs. Uncertain Safety), equivalent to testing the Region × Condition interactions. As
528 shown in **Fig. 8**, mean differences were small to very-small ($d_z < .17$) and all non-significant (**Extended**
529 **Data Fig. 8-1**). Likewise, the proportion of subjects showing numerically greater activity in one region or
530 the other never exceeded 55% (**Fig. 8**). Naturally, these results do not license strong claims of regional
531 equivalence. While it is impossible to demonstrate that the true difference in regional activity is zero, the
532 two one-sided tests (TOST) procedure provides a well-established and widely used framework for testing
533 whether mean differences—here, in regional activity—are small enough to be considered statistically
534 equivalent (Lakens, 2017; Lakens et al., 2018). For present purposes, we considered differences smaller
535 than a ‘medium’ standardized effect (Cohen’s $d_z = .35$) to be statistically equivalent. Using the voxels that
536 were most sensitive to each threat contrast (see Materials and Methods), our results revealed significant
537 equivalence for all three contrasts ($p_s = .001-.03$; **Fig. 8** and **Extended Data Fig. 8-1**). Although these
538 statistical findings do not demonstrate that the amygdala and the BST are functionally interchangeable
539 (“the same”), they do enable us to decisively reject claims of strict functional segregation (i.e. that the BST

540 is sensitive to uncertain danger, whereas the amygdala is not) for the subset of these regions engaged by
541 the MTC paradigm.

542

543 DISCUSSION

544 Uncertain-threat anticipation is the prototypical trigger of anxiety, a core theme that cuts across
545 psychiatric disorders, species, and assays, including novelty, darkness, and other ‘diffuse’ threats. Despite
546 the immense significance of anxiety for public health, the neural systems recruited by uncertain threat
547 have remained unclear. Leveraging a translationally relevant paradigm optimized for fMRI signal
548 decomposition (**Fig. 1**), our results reveal that the anticipation of temporally uncertain aversive
549 stimulation recruits a distributed network of fronto-cortical (MCC, AI/FrO, and dlPFC/FP) and subcortical
550 (PAG, BST, and dorsal amygdala) regions (**Fig. 4**), mirroring robust changes in experience and
551 psychophysiology (**Fig. 1**). Closer inspection of signal dynamics in these regions provided descriptive
552 support for sustained activity during the anticipation of Uncertain Threat (**Fig. 5**). Analyses focused on
553 the anticipation of temporally Certain Threat revealed a similar pattern, with voxels sensitive to both
554 kinds of threat evident in key cortical and subcortical regions (**Fig. 4**), suggesting that this circuitry is
555 sensitive to both certain and uncertain threat. Direct comparison of the two threat conditions
556 demonstrated that this network can be fractionated: cortical regions showed relatively greater activity
557 during the anticipation of Uncertain Threat, whereas the extended amygdala showed relatively greater
558 activity during the anticipation of Certain Threat (**Fig. 7**). While there is consensus that the BST and
559 dorsal amygdala play a critical role in orchestrating adaptive responses to danger, their precise
560 contributions to human anxiety have remained contentious. Our results suggest that these regions
561 respond similarly to different kinds of threat anticipation. In fact, we show that the BST and dorsal
562 amygdala exhibit statistically indistinguishable responses to threat anticipation across a variety of

563 comparisons (**Fig. 8**), reinforcing the possibility that they make broadly similar contributions to human
564 anxiety (Gungor and Paré, 2016; Fox and Shackman, 2019).

565

566 Since the time of Freud (Freud, 1920), the distinction between certain ('fear') and uncertain ('anxiety')
567 danger has been a key feature of neuropsychiatric models of emotion (Davis et al., 2010; LeDoux and Pine,
568 2016; Mobbs, 2018). Our findings show that the regions recruited during the anticipation of Certain and
569 Uncertain Threat are co-localized in several key regions (**Fig. 4**). This common threat anticipation
570 network encompasses subcortical regions that are critical for assembling defensive responses to
571 uncertain threat in animals (Fox and Shackman, 2019). But it also includes fronto-cortical regions—like
572 the MCC, AI/FrO, and dlPFC/FP—that have received less empirical attention and are challenging to study
573 in rodents (e.g. Carlén, 2017). These regions have traditionally been associated with the controlled
574 processing and regulation of emotion and cognition (Shackman et al., 2011; Morawetz et al., 2017;
575 Langner et al., 2018; Kroes et al., 2019; Picó-Pérez et al., 2019; Morawetz et al., *in press*) and more
576 recently implicated in the conscious experience of emotion (LeDoux, 2020). As shown in **Fig. 9**, the
577 present results are well aligned with recent meta-analyses of neuroimaging studies of 'fear' (Fullana et al.,
578 2016) and 'anxiety' (Chavanne and Robinson, *in press*). Across studies encompassing tens of studies and
579 hundreds of subjects, this work demonstrates that the anticipation of *certain*-threat (Pavlovian threat
580 cues; the prototypical 'fear' stimulus in laboratory studies) and *uncertain*-threat (instructed 'threat-of-
581 shock') recruit an overlapping network of core regions, including the BST (but not the Ce; see below).
582 This similarity cannot be dismissed as an artifact of neuroimagers' penchant for partial-reinforcement
583 Pavlovian paradigms, which render 'certain' threat uncertain (Fullana et al., 2016, median threat
584 probability=63%; Picó-Pérez et al., 2019, median threat probability=62%). In fact, the same general
585 pattern—including elevated activity in the region of the BST—is evident in large-scale studies of *certain*-
586 (Sjouwerman et al., 2020, Study 2, n=113, threat probability=100%,

587 <https://neurovault.org/collections/6031>) and *uncertain-threat* anticipation (Klumpers et al., 2017,
588 Sample 1: n=108, threat probability=33%), consistent with our results.

589

590 Our observations provide insight into the functional architecture of the threat anticipation network,
591 demonstrating that fronto-cortical regions prefer Uncertain over Certain Threat, whereas the BST and
592 dorsal amygdala show the reverse preference—*a difference in degree, not in kind*. Trivial differences
593 cannot account for this nuance; the two threat conditions were pseudo-randomly intermixed and nearly
594 identical in terms of their perceptual, nociceptive, motor, and statistical features (**Fig. 1**). What might
595 explain the observed regional preferences? Aside from temporal certainty, the most conspicuous
596 difference between the conditions is the degree of cognitive scaffolding. On Certain Threat trials, the
597 descending stream of integers provided a precise and predictable index of momentary changes in threat
598 imminence, encouraging a reactive, stimulus-bound cognitive mode. On Uncertain Threat trials this
599 support was absent, necessitating greater reliance on the kinds of sustained, endogenous representations
600 that are the hallmark of fronto-cortical regions (Badre and Nee, 2018). A second notable difference
601 between the two threat conditions is the intensity of anxiety. Uncertain-Threat anticipation was
602 associated with greater distress and arousal (**Fig. 1**). The observed increase in fronto-cortical activity
603 could reflect either heightened anxiety or compensatory processes aimed at downregulating distress and
604 arousal. Testing these non-exclusive hypotheses will require a multi-pronged approach that encompasses
605 carefully optimized tasks, mechanistic interventions, and a broader assessment of the nomological
606 network. Multivoxel classifier approaches are likely to be useful for linking specific facets of anxiety (e.g.
607 feelings) to particular elements of the threat anticipation network, and determining whether this reflects
608 expressive or regulatory processes (Chang et al., 2015).

609

610 The present results add to a growing body of evidence indicating that the BST and dorsal amygdala, while
611 certainly not interchangeable, are more alike than different (Fox and Shackman, 2019). The BST and
612 dorsal amygdala are characterized by broadly similar patterns of anatomical connectivity, cellular
613 composition, neurochemistry, and gene expression (Fox et al., 2015), although some differences in
614 functional connectivity have been identified (Gorka et al., 2018). Both regions are poised to trigger
615 defensive responses via dense projections to downstream effectors (Fox et al., 2015). Neuroimaging
616 studies have documented similar responses in the two regions to a range of anxiety-eliciting stimuli (Fox
617 and Shackman, 2019; Hudson et al., 2020, <https://neurovault.org/collections/6237>), and mechanistic
618 work in rodents reinforces the hypothesis that the BST and dorsal amygdala (Ce) are crucial substrates
619 for human anxiety (Fox and Shackman, 2019). In fact, work using a variant of the present paradigm in
620 mice shows that Ce-BST projections are necessary for mounting defensive responses during the
621 anticipation of temporally uncertain shock (Lange et al., 2017), consistent with our general conclusions.
622 While our understanding remains far from complete, this body of observations underscores the need to
623 revise models of anxiety, like RDoC, that imply a strict segregation of certain and uncertain threat
624 processing in the extended amygdala. The present results imply that the magnitude of regional
625 differences in hemodynamic sensitivity to threat-uncertainty is modest ($<dz=.35$); conditional on
626 perceptual confounds, collinearities, or other moderators; or simply non-existent. An important challenge
627 for the future will be to determine whether the *type* of threat uncertainty (e.g. temporal vs. likelihood) is
628 a crucial determinant of regional differences in function.

629

630 Our results indicate that the amygdala's response to threat anticipation is sparse, at least when compared
631 to widely used emotional face and scene paradigms. This was not unexpected. The amygdala is a
632 heterogeneous collection of at least 13 nuclei and cortical areas—not ‘a thing’ (Swanson and Petrovich,
633 1998; Yilmazer-Hanke, 2012)—and converging lines of mechanistic and imaging evidence point to the

634 special importance of the dorsal amygdala, in the region of the Ce (Davis et al., 2010; Fox and Shackman,
635 2019; Hur et al., 2019). In humans, Ce represents ~3% of total amygdala volume (Wegiel et al., 2014;
636 Avino et al., 2018). The dorsal amygdala clusters that we observed extend beyond the Ce to encompass
637 neighboring dorso-caudal aspects of the medial, lateral, and cortical nuclei, and amygdala-hippocampal
638 transition area (Extended Data). While meta-analyses of small-sample neuroimaging studies have failed
639 to detect significant amygdala responses to threat anticipation (Fullana et al., 2016, median n=16;
640 Chavanne and Robinson, in press, median n=29), the location and extent of the dorsal amygdala clusters
641 reported here align with more recent large-sample studies of *certain-* (Sjouwerman et al., 2020) and
642 *uncertain*-threat anticipation (Reddan et al., 2018, n=68, threat probability=33%). In sum, our results are
643 broadly aligned with amygdala anatomy, prior theory, and emerging neuroimaging evidence.

644

645 To conclude, the neural circuits recruited by temporally uncertain and certain threat are not categorically
646 different, at least when viewed through the macroscopic lens of fMRI. We see evidence of anatomical co-
647 localization—not segregation—in a number of key regions, in broad accord with animal models and
648 recent imaging meta-analyses. This shared threat-anticipation system can be fractionated, with fronto-
649 cortical regions showing relatively stronger engagement during the anticipation of temporally uncertain
650 threat, and the BST and dorsal amygdala showing the reverse pattern. In direct comparisons, the BST and
651 dorsal amygdala exhibited statistically indistinguishable responses, reinforcing the possibility that they
652 make similar contributions to human anxiety. These observations provide a framework for
653 conceptualizing fear and anxiety and for guiding mechanistic work aimed at developing more effective
654 intervention strategies for pathological anxiety. A large sample, well-controlled task, and advanced
655 techniques for data acquisition and processing enhance confidence in the robustness and translational
656 relevance of these results.

657

663 **FIGURE LEGENDS**

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

686

687

688 **Fig. 2. Interpretive ambiguities of canonical HRF modeling.** The canonical approach to fMRI analysis
689 models the amplitude of anticipatory activity (*solid black line*) under the assumption that it approximates
690 a 'boxcar'-like square-wave shape (*dotted line*; convolution of a canonical HRF with task duration). In
691 some cases, such as the upper-left panel, the hemodynamic signal and the model will match. But in others,
692 it will not. Importantly, a variety of physiologically plausible hemodynamic responses can produce
693 similarly strong and statistically significant results ($T = 52.556$ in this example), highlighting the
694 importance of modeling the BOLD signal on a finer temporal scale.

695

696 **Fig. 3. Amygdala and BST ROIs.** **BST.** The probabilistic BST ROI (*green*) is described in (Theiss et al.,
697 2017) and was thresholded at 0%. The seed mostly encompasses the supra-commissural BST, given the
698 difficulty of reliably discriminating the borders of regions below the anterior commissure on the basis of
699 T1-weighted images (Kruger et al., 2015). **Amygdala.** The Harvard-Oxford probabilistic amygdala (*cyan*)
700 is described in (Frazier et al., 2005; Desikan et al., 2006a) and conservatively thresholded at 50%.
701 Analyses employed ROIs decimated to the 2-mm resolution of the EPI data. For illustrative purposes, 1-
702 mm ROIs are shown. Single-subject data were visually inspected to ensure that the ROIs were correctly
703 aligned to the spatially normalized T1-weighted images. Abbreviation—BST, bed nucleus of the stria
704 terminalis.

705

706

707 **Fig. 4. The anticipation of temporally Uncertain and Certain Threat recruits broadly similar**
708 **neural systems.** Key regions (*cyan arrowheads*) showing significantly elevated activity during the
709 anticipation of Uncertain Threat (*left column*) and Certain Threat (*center column*) compared to their
710 respective control conditions. Voxels showing significantly increased activity in both contrasts are
711 depicted in the *right column*. BST and dorsal amygdala images are masked to highlight significant voxels
712 in extended amygdala (*green*). Coronal insets depict the thresholded statistical parametric maps without
713 the additional mask. Taken together, these observations indicate that these regions are sensitive to both
714 temporally certain and uncertain threat. For additional details, see **Extended Data Figs. 4-1 to 4-5.**
715 Abbreviations—Ant, anterior; BST, bed nucleus of the stria terminalis; dlPFC, dorsolateral prefrontal
716 cortex; FrO, frontal operculum; L, left; PAG, periaqueductal grey; WB, whole-brain corrected.
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728 **Fig. 5. Regions sensitive to temporally Uncertain Threat show sustained hemodynamic activity.**
729 Mean responses to the anticipatory epoch were estimated on a TR-by-TR (1.25 s) basis for Uncertain
730 Threat (*red*) and Uncertain Safety (*blue*) trials, using data from the local maxima of key clusters (*black-*
731 *and-white asterisks in the left panels*) identified using a canonical analytic approach. Given the temporal
732 resolution and autocorrelation of the hemodynamic signal, data were averaged for 4 windows (TR-1 to
733 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).
734 Windows are indicated by broken vertical lines. Shaded envelopes depict the standard error of the mean.
735 Abbreviations—Ant., anterior; BST, bed nucleus of the stria terminalis; dlPFC, dorsolateral prefrontal
736 cortex; FrO, frontal operculum; L, left; PAG, periaqueductal grey; TR, repetition time (the time needed to
737 acquire a single volume of fMRI data).

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742 **Fig. 6. Regions sensitive to temporally Certain Threat show sustained hemodynamic activity.** Mean
743 responses to the anticipatory epoch were estimated on a TR-by-TR (1.25 s) basis for Certain Threat (red)
744 and Certain Safety (blue) trials, using data from the local maxima of key clusters (*black-and-white*
745 *asterisks in the left panels*) identified using a canonical HRF GLM approach. Given the temporal resolution
746 and autocorrelation of the hemodynamic signal, data were averaged for 3 windows (TR-1 to TR-5, TR-6
747 to TR-10, and TR-11 to TR-15), spanning a total of 15 measurements (18.75 s). Windows are indicated by
748 broken vertical lines. Shaded envelopes depict the standard error of the mean. Abbreviations—Ant.,
749 anterior; BST, bed nucleus of the stria terminalis; dlPFC, dorsolateral prefrontal cortex; FrO, frontal
750 operculum; L, left; PAG, periaqueductal grey.

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754 **Fig. 7. The threat anticipation network can be fractionated into subdivisions.** The midcingulate
755 cortex, anterior insula/frontal operculum, and dlPFC showed greater activity during the anticipation of
756 Uncertain Threat (*left column*), whereas the BST and dorsal amygdala showed greater activity during the
757 anticipation of Certain Threat (*center column*). Thresholds and other conventions are identical to **Fig. 4**.
758 For additional details, see **Extended Data Figs. 7-1** and **7-2**. The *right column* depicts TR-by-TR (1.25 s)
759 hemodynamic responses during the anticipation of Uncertain Threat (*solid red line*) and Certain Threat
760 (*broken red line*). Data were extracted from the local maxima of key clusters (*black-and-white asterisks in*
761 *the left and center columns*) identified using a canonical HRF GLM approach. Given the temporal
762 resolution and autocorrelation of the hemodynamic signal, data were averaged for 3 windows (TR-1 to
763 TR-5, TR-6 to TR-10, and TR-11 to TR-15), spanning a total of 15 measurements (18.75 s). Windows are
764 indicated by broken vertical lines. Shaded envelopes depict the standard error of the mean.
765 Abbreviations—Ant, anterior; BST, bed nucleus of the stria terminalis; dlPFC, dorsolateral prefrontal
766 cortex; FrO, frontal operculum; L, left; PAG, periaqueductal grey.

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770 **Fig. 8. The BST and dorsal amygdala regions recruited by the Maryland Threat Countdown**
771 **paradigm show statistically indistinguishable responses during threat anticipation.** While it is
772 impossible to demonstrate that the true difference in regional hemodynamic activity is zero, the two one-
773 sided tests (TOST) procedure provides a well-established and widely used statistical framework for
774 testing whether mean differences in regional activity are small enough to be considered equivalent
775 (Lakens, 2017; Lakens et al., 2018). Using the subset of voxels that were most sensitive to each threat
776 contrast (see Materials and Methods), results revealed significant equivalence for all contrasts (**Extended**
777 **Data Fig. 8-1**). Figure depicts the data (*black points; individual participants*), density distribution (*bean*
778 *plots*), Bayesian 95% highest density interval (HDI; *colored bands*), and mean (*black bars*) for each
779 condition. HDIs permit population-generalizable visual inferences about mean differences and were
780 estimated using 1,000 samples from a posterior Gaussian distribution. Inset ring plots indicate the
781 percentage of subjects showing greater activity in the BST compared to the dorsal amygdala for each
782 contrast.

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787 **Fig. 9. Certain and uncertain threat anticipation elicit broadly similar patterns of neural activity.**
788 Figure summarizes the results of two coordinate-based meta-analyses (CBMA) of functional
789 neuroimaging studies. *Top-left* inset depicts the results for 27 ‘fear conditioning’ studies ($N=677$),
790 highlighting regions showing consistently greater activity during the anticipation of certain threat (CS+ >
791 CS-; <https://neurovault.org/collections/2472>; Fullana et al., 2016). *Bottom-right* inset depicts the results
792 for 18 ‘threat-of-shock’ studies ($N=693$), highlighting regions showing consistently greater activity during
793 the anticipation of uncertain threat (Threat > Safe; <https://neurovault.org/collections/6012>; Chavanne
794 and Robinson, in press). Visual inspection of the results (*red clusters*) suggests that the anticipation of
795 certain and uncertain threat elicits qualitatively similar patterns, including heightened activity in the
796 region of the BST. This impression is reinforced by the substantial correlation between the two whole-
797 brain patterns, $r = .69$. Consistent amygdala activity was not detected in either meta-analysis. Note: The
798 pattern correlation was estimated in NeuroVault using a brain-masked, 4-mm transformation of the
799 publicly available, vectorized meta-analytic maps (Gorgolewski et al., 2015). For illustrative purposes,
800 every 10th voxel is depicted in the scatter plot. Abbreviations—BST, bed nucleus of the stria terminalis;
801 CBMA, coordinate-based meta-analyses; L, left hemisphere; R, right hemisphere.

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