Adolescent social anxiety is associated with diminished discrimination of anticipated threat and safety

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AUTHOR CONTRIBUTIONS

R.M.T., A.J.S., and J.F.S. designed the overall study with guidance from A.D.L.R. and K.A.D. R.M.T., M.D.S., and J.F.S. developed and optimized the imaging paradigm. R.M.T. managed data collection, clinical assessment, and study administration with guidance from K.A.D. R.M.T., H.C.K., A.S.A., S.I., and M.D.S. collected data. J.F.S. developed data processing and analytic software for imaging analyses. J.F.S. and R.M.T. processed imaging data. R.M.T, J.F.S., J.H., and A.J.S. analyzed data. R.M.T. and A.J.S. developed the analytic strategy. R.M.T., J.H., A.D.L.R., and A.J.S. interpreted data. R.M.T., J.H., M.D.S., and A.J.S. wrote the paper. P.D., H.C.K., J.H., and R.M.T. created figures and tables. J.H. coordinated editorial communications. 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.

RESOURCE SHARING

Processed data are publicly available (https://osf.io/aj64g/). Key neuroimaging maps are publicly available (https://neurovault.org/collections/15620). Processed neuroimaging data have also been shared with the ENIGMA Consortium Anxiety Workgroup (https://enigma.ini.usc.edu/ongoing/enigma-anxiety).

ABSTRACT (248/250)

Social anxiety—which typically emerges in adolescence—lies on a continuum and, when extreme, can be devastating, with clear functional impairment evident even at subdiagnostic levels. Socially anxious individuals are prone to heightened fear, anxiety, and avoidance of social interactions and contexts associated with the potential for social scrutiny. To date, most neuroimaging research has focused on acute social threat. Much less scientific attention has been devoted to understanding the neural systems recruited during the anticipation of potential encounters with social threat. Here we combined functional MRI with a novel social threat-anticipation paradigm in a racially diverse sample of 66 adolescents selectively recruited to encompass a broad spectrum of social-anxiety symptoms and enriched for clinically significant levels of distress and impairment. Results demonstrated that socially anxious adolescents report potentiated distress when anticipating encounters with threatening faces and voices, and indiscriminately elevated distress during the anticipation of benign social cues. Adolescents with more severe symptoms also showed reduced discrimination of uncertain social threat and safety in the bed nucleus of the stria terminalis (BST), a key division of the central extended amygdala (EAc). Although the EAc was robustly engaged by the acute presentation of threatening faces and voices, variation in acute EAc reactivity was unrelated to symptom severity. These observations provide a neurobiologically grounded framework for conceptualizing adolescent social anxiety and set the stage for the kinds of prospective-longitudinal and mechanistic research that will be necessary to determine causation and, ultimately, to develop improved interventions for extreme early-life anxiety.

INTRODUCTION

Socially anxious individuals are prone to heightened fear, anxiety, and avoidance of social interactions and other contexts associated with potential social scrutiny (Alden & Taylor, 2004; Heimberg et al., 2014). Social anxiety lies on a continuum and, when extreme, can be devastating, with clear functional impairment evident even at subdiagnostic levels of severity (Fehm, Beesdo, Jacobi, & Fiedler, 2008; Hyett & McEvoy, 2018; Katzelnick et al., 2001; Kessler, 2003; Lipsitz & Schneier, 2000; Merikangas, Avenevoli, Acharyya, Zhang, & Angst, 2002; Schneier, Blanco, Antia, & Liebowitz, 2002). Social anxiety disorder (SAD) is among the most common psychiatric illnesses (lifetime prevalence: ~13%), typically emerges in adolescence, and confers heightened risk for other developmental, academic, and psychiatric problems, including co-morbid internalizing disorders and substance misuse (Angst et al., 2016; Beesdo-Baum & Knappe, 2012; Gregory et al., 2007; Hyett & McEvoy, 2018; Kessler, Petukhova, Sampson, Zaslavsky, & Wittchen, 2012; Koyuncu, İnce, Ertekin, & Tükel, 2019; Mathew, Pettit, Lewinsohn, Seeley, & Roberts, 2011; Schneier, Johnson, Hornig, Liebowitz, & Weissman, 1992; Stein et al., 2017). Existing treatments are inconsistently effective or associated with significant adverse effects, underscoring the urgency of developing a more complete understanding of the neural systems governing social anxiety in the first decades of life (Batelaan et al., 2017; Evans, Clark, & Leigh, 2021; James, Reardon, Soler, James, & Creswell, 2020; Rapee et al., 2023; Scholten et al., 2016; Scholten et al., 2013; Singewald, Sartori, Reif, & Holmes, 2023; Spinhoven et al., 2016; Strawn, Lu, Peris, Levine, & Walkup, 2021).

Socially anxious adults and youth are prone to elevated distress and arousal in two distinct contexts: **(a)** when social threat is certain-and-imminent ('acute'), as when performing in front of a group; and **(b)** when social threat is possible, but uncertain in timing or likelihood, as when first entering a classroom, conversation, or other socially enriched environments (APA, 2022; Cain, *in press;* Davis, Walker, Miles, & Grillon, 2010; NIMH, 2011). Converging lines of preclinical research in rodents, monkeys, and humans

suggest that heighted reactivity to both kinds of threat reflect functional alterations in the central extended amygdala (EAc), an anatomical macrocircuit encompassing the dorsal amygdala in the region of the central nucleus (Ce) and the neighboring bed nucleus of the stria terminalis (BST) (Fox & Shackman, 2019; Hur et al., 2020; Shackman & Fox, 2021).

Despite this progress, the relevance of these discoveries to pediatric social anxiety remains unclear. To date, the vast majority of human neuroimaging studies have focused on laboratory analogues of acute social threat, including virtual social interactions and 'threat-related' social cues, such as photographs of fearful and angry facial expressions (Jarcho et al., 2013). Meta-analyses show that the EAc is generally hyper-reactive to social cues among adults with SAD and adults with a childhood history of extreme shyness and behavioral inhibition, key temperamental risk factors for SAD (Brühl, Delsignore, Komossa, & Weidt, 2014; Chavanne & Robinson, 2021; Clauss & Blackford, 2012; Fox & Kalin, 2014; Gentili et al., 2016; Tan et al., *in press*). Among adults, EAc reactivity to acute social threat is dampened by clinically effective treatments for SAD, consistent with a causal role (Klumpp & Fitzgerald, 2018; Shackman et al., 2016). While the pediatric social anxiety literature is comparatively sparse, the available evidence suggests a broadly consistent pattern (Jarcho et al., 2013). Nevertheless, some comparatively large-scale studies have failed to detect exaggerated EAc reactivity to acute social threat. For example, Ziv and colleagues reported negligible effects of Generalized SAD on amygdala reactivity to either dynamically looming hostile faces or short film clips of actors delivering social criticism (n=67 unmedicated patients, n=28 matched controls; Ziv, Goldin, Jazaieri, Hahn, & Gross, 2013).

Much less scientific attention has been devoted to understanding the neural systems underlying heightened emotional reactivity during the anticipation of social threat. Early work using comparatively

low-resolution neuroimaging techniques—including ^{15}O-positron emission tomography (PET) and 1.5T fMRI—and small case-control designs (n<10/group) revealed evidence of heightened amygdala activity among adults with SAD during the certain anticipation of speaking challenges (Lorberbaum et al., 2004; Tillfors, Furmark, Marteinsdottir, & Fredrikson, 2002)—an effect confirmed in subsequent high-resolution studies (3T fMRI) and larger samples (Boehme et al., 2014; Davies et al., 2017). Group differences in BST activation were not reported. More recent work has extended this approach to encompass the temporally uncertain anticipation of social threat (Figel et al., 2019). Here, a cue presented at the outset of each trial signaled whether the participant's face would ('threat') or would not ('safety') be video-recorded. Cues were followed by a temporally variable and uncertain anticipation phase (3-16 s) that terminated with the presentation of a second cue indicating whether or not the camera was recording. Results revealed sustained levels of heightened BST activation in adults with SAD *and* matched controls during the uncertain anticipation of social threat encounters. Although patients showed numerically smaller responses, significant diagnostic-group differences were not evident in either the BST or the dorsal amygdala (Ce), and potential regional differences (BST vs. Ce) were not interrogated. While important, the relevance of this body of observations to maladaptive social anxiety earlier in life, during adolescence, remains little explored and largely unknown (but see Michalska et al., 2023 for related efforts in a mixed-anxiety sample of youth).

Here we combined fMRI with the Maryland Social Threat Countdown (MSTC) paradigm in a racially diverse group of adolescents (13-17 years; 50% adolescents of color). The sample was selectively recruited to encompass a broad spectrum of social-anxiety symptoms—without gaps or discontinuities—and enriched for clinically significant levels of distress and impairment (50% positive screen; 36% SAD diagnosis). The MSTC task takes the form of a 2 (*Valence:* Social Threat/Safety) × 2 (*Temporal Certainty:* Uncertain/Certain) × 2 (*Phase:* Anticipation/Presentation) event-related design, allowing us to separately

assess EAc activation during the anticipation and acute presentation of certain and uncertain social threat cues (**Figure 1**). On Certain Social Threat trials, participants saw a descending stream of integers ('count-down'). To maximize anxious distress, the anticipation period always culminated with the presentation of a multi-modal social threat that included a photograph of an angry young-adult face and an audio clip of a hostile verbal remark (e.g., *"No one wants you here"*). Uncertain Social Threat trials were similar, but the integer stream was randomized and presented for a temporally uncertain and variable duration. Here, participants knew that a social threat was coming, but they had no way of knowing precisely when the encounter would occur. Safety trials were similar, but terminated with the presentation of normatively benign photographs (happy faces) and audio clips (e.g., *"I play soccer"*).

Anatomically defined regions-of-interest (ROIs) and spatially unsmoothed fMRI data allowed us to rigorously quantify Ce and BST reactivity to the anticipation and presentation of uncertain and certain social threat, and determine whether one or both regions covary with the severity of social anxiety symptoms (**Figure 1**). Unlike voxelwise analyses—which screen thousands of voxels for evidence of statistical significance and yield optimistic effect size estimates in suprathreshold regions—anatomically defined ROIs 'fix' the outcomes of interest *a priori*, providing statistically unbiased estimates of brain-phenotype associations (Poldrack et al., 2017). Exploratory whole-brain voxelwise analyses were also performed, enabling more direct comparison with prior work in anxious adults and youth.

METHOD

Recruitment and Study Overview

Adolescents (13-17 years) were recruited using a multipronged strategy, including online advertisements (e.g., Facebook, parent listservs), flyers posted at community mental health clinics and

other high-traffic community settings (e.g., coffee shops, libraries, community centers), and referrals from on-going research studies. To capture a broad spectrum of social anxiety, separate advertisements were used to target adolescents with elevated (e.g., 'shy' teens) and low-to-middling symptoms. Following preliminary screening, potentially eligible individuals completed a single face-to-face session that included a self-report assessment, a structured clinical interview, and an MRI assessment. Guardians and adolescents provided informed written consent and assent, respectively. All procedures were approved by the University of Maryland Institutional Review Board (protocol #661215).

Participants and Eligibility Criteria

Participants were selectively recruited to ensure a broad spectrum of social anxiety symptoms, with substantial enrichment for elevated levels of distress and impairment. Potentially interested adolescents completed an on-line screening assessment, including the abbreviated Social Phobia and Anxiety Scale for Children (SPAIC-11), which quantifies the frequency of social-anxiety symptoms across different social settings (Bunnell, Beidel, Liu, Joseph, & Higa-McMillan, 2015). Three additional *ad hoc* items (*How much does anxiety bother you or cause you distress? How much does anxiety "mess things up" for you? How much of an effect does anxiety have on your life?*) were added to the screening assessment shortly after enrollment began and were used to probe the degree of symptom distress and impairment on a 5-point Likert scale ranging from 1 *(Not at all)* to 5 (*Extremely*). Adolescents were invited to enroll if, at the time of the preliminary screening, they showed evidence of either **(a)** *significant social anxiety*, as indexed by an abbreviated SPAIC-11 score of >15 or moderate-to-severe (≥3) distress/impairment on 1 or more of the 3 follow-up items; or **(b)** *middling-to-low social anxiety*, as indexed by an abbreviated SPAIC-11 score of <7 and, if available, little-to-no (<3) distress/impairment on the 3 additional items.

All participants and their caregivers indicated that they were right-handed, native English speakers, with a full-term pregnancy (>34 weeks) and normal or corrected-to-normal color vision. Participants reported that they were free from psychiatric medications; standard MRI contraindications; and a lifetime history of severe head injury, neurological signs and disorders, psychotic disorders, and developmental delays or disorders (e.g., autism).

In total, 78 adolescents and their caregivers participated. Twelve participants were excluded from analyses due to premature termination of the MRI assessment (n=3), inadequate compliance with the in-scanner rating task (<50% ratings completed; n=3), or insufficient usable data (n=6; see below for details). The final sample included 66 adolescents (M=15.4 years, SD=1.3; 60.6% female; 50% White, 30.3% African American/Black, 7.6% Hispanic, 6.1% Asian, 6.1% multiracial/other). The sample showed a broad spectrum of social-anxiety symptoms (SPAIC: M=10.0, SD=6.0, *Range*=0.0-21.3). Half of the sample (n=33, 60.6% female) met the SPAIC-11 cut-off (>9.17) for probable SAD (Bunnell et al., 2015). Consistent with this dimensional perspective, the sample was significantly enriched for DSM-IV SAD diagnoses (n=24, 58.3% female). As expected, co-morbidity was rampant, and the majority of adolescents with SAD (58.3%) received at least one other categorical diagnosis (Lahey, Tiemeier, & Krueger, 2022; Tiego et al., 2023). GAD (50.0%) and MDD (33.3%) were the most common co-morbidities.

Clinical Assessments

Abbreviated Social Phobia and Anxiety Scale for Children (SPAIC-11). The 11-item SPAIC was used to quantify dimensional variation in adolescent-reported social-anxiety symptoms (Bunnell et al., 2015). Items probe the frequency of fear or anxiety symptoms evoked by specific social situations (e.g., *"I feel scared when I have to speak or read in front of a group of people"*) on a 3-point Likert scale ranging from 0

(*never*) to 2 (*always*). Total SPAIC-11 sum scores showed acceptable reliability (α=0.94) and were unrelated to variation in age, sex, and race (not reported).

Mini-International Neuropsychiatric Interview (MINI) for Children and Adolescents. For descriptive purposes, diagnostic status was assessed by a masters-level clinician using the Mini International Neuropsychiatric Interview for Children and Adolescents (MINI) (Sheehan et al., 2010). To encourage frank reporting, diagnostic interviews were completed without the caregiver present.

Maryland Social Threat Countdown (MSTC) Paradigm

Task Structure. Building on prior work by our group using physically aversive reinforcers in adults (Grogans et al., *under review;* Hur et al., 2022; Hur et al., 2020; Kim et al., 2023), the MSTC paradigm takes the form of a 2 (*Valence:* Social Threat/Safety) × 2 (*Temporal Certainty:* Uncertain/Certain) × 2 (*Phase:* Anticipation/Presentation) repeated-measures, randomized event-related design (**Figure 1**). On Certain Social Threat trials, participants saw a descending stream of integers ('count-down') for 18 s. To maximize anxious distress, the anticipation period always culminated with the presentation of a multi-modal social threat (1.8 s), encompassing a photograph of an angry young-adult face and an audio clip of a hostile verbal remark (e.g., *"No one wants you here"*). Uncertain Social Threat trials were similar, but the integer stream was randomized and presented for a temporally uncertain and variable duration (*Range*=8-30 s; *M*=18 s). Participants knew that a social threat was coming, but had no way of knowing precisely when it would occur. On Uncertain trials, integers were randomly drawn from a near-uniform distribution ranging from 1 to 45 to reinforce the impression that Uncertain trials could be much longer than Certain ones and to minimize incidental temporal learning ('time-keeping'). White-noise visual masks (3.2 s) were presented between trials to minimize persistence of the visual reinforcers in iconic memory. Safety trials were similar but terminated with the delivery of normatively benign photographs (happy faces) and audio clips (*"I play soccer"*). Consistent with recent recommendations (Shackman & Fox,

2016), the mean duration of the anticipation phase was identical across conditions, ensuring an equal number of measurements (TRs/condition). Mean duration was chosen to enhance detection of task-related differences in the blood oxygen level-dependent (BOLD) signal (Henson, 2007). Valence was continuously signaled throughout the anticipation epoch by the background color of the display. Certainty was signaled by the nature of the integer stream. Participants were periodically prompted (following the offset of the visual mask) to rate the intensity of fear/anxiety experienced a few moments earlier, during the anticipation period of the prior trial, using a 1-4 (*least/most*) scale and an MRI-compatible response pad (MRA, Washington, PA). Participants rated each trial type once per scan (16.7% trials). Participants were completely informed about the task design and contingencies prior to scanning. The task was administered in 3 scans, with short breaks between scans. Simulations were used to optimize the detection and deconvolution of task-related hemodynamic signals (variance inflation factors < 1.68). Stimulus presentation and ratings acquisition were controlled using Presentation software (version 19.0, Neurobehavioral Systems, Berkeley, CA).

Procedures. Prior to fMRI scanning, participants practiced an abbreviated version of the MSTC paradigm until participants indicated and staff confirmed understanding. During scanning, foam inserts were used to immobilize the participant's head within the head-coil and mitigate potential motion artifact. Participants were continuously monitored using an MRI-compatible eye-tracker (Eyelink 1000; SR Research, Ottawa, Ontario, Canada) and the AFNI real-time motion plugin (Cox, 1996). Eye-tracking data were not recorded. Measures of respiration and breathing were continuously acquired during scanning using a Siemens respiration belt and photo-plethysmograph affixed to the first digit of the non-dominant hand. Following the last scan, participants were removed from the scanner, debriefed, compensated, and discharged.

Visual Stimuli. A total of 72 face photographs were drawn from the Chicago Face Database (versions 1-2) (Ma, Correll, & Wittenbrink, 2015). Stimuli included 36 young-adult models of varying race and sex, each depicting 1 angry and 1 happy expression. Visual stimuli were digitally back-projected onto a semi-opaque screen mounted at the head-end of the scanner bore and viewed using a mirror mounted on the head-coil (Powerlite Pro G5550, Epson America, Inc., Long Beach, CA).

Auditory Stimuli. A total of 72 custom auditory stimuli were created by recording 36 young-adult voice actors, recruited to match the apparent sex and race of the photograph models. Each voice actor provided 1 threatening and 1 benign audio statement, equated for number of syllables. Voice actors were carefully coached to deliver the threatening statements (e.g., *"I don't like you"*) in a hostile manner and to deliver the comparatively benign statements (e.g., *"Today is nice"*) in a neutral or mildly positive manner. Audio stimuli were volume standardized. To reinforce the naturalistic nature of the paradigm, each photograph was consistently paired with a specific sex- and race-matched voice actor. Auditory stimuli were delivered using an amplifier (PA-1 Whirlwind) with in-line noise-reducing filters and ear buds (S14; Sensimetrics, Gloucester, MA) fitted with noise-reducing ear plugs (Hearing Components, Inc., St. Paul, MN).

MRI Data Acquisition

MRI data were acquired using a Siemens Magnetom TIM Trio 3T scanner (32-channel head-coil). Sagittal T1-weighted anatomical images were acquired using a magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence (TR=1,900 ms; TE=2.32 ms; inversion=900 ms; flip= 9°; sagittal slice thickness=0.9 mm; in-plane=0.449 × 0.449mm; matrix=512 × 512; FOV=230 × 230). To enhance anatomical resolution, a multi-band sequence was used to collect oblique-axial echo planar imaging (EPI) volumes during the MSTC task (multiband acceleration=6; TR=1,000 ms; TE=39.4 ms; flip=36.4°; slice thickness=2.2 mm, number of slices=66; in-plane=2.1875 × 2.1875 mm; matrix=96 × 96). Images were

collected in the oblique-axial plane (approximately −20° relative to the AC-PC plane) to minimize potential susceptibility artifacts. A total of three 568-volume scans were acquired. The scanner automatically discarded 7 volumes prior to the first recorded volume. To enable field map distortion correction, a pair of oblique-axial co-planar spin echo images with opposing phase encoding direction was also acquired (TR=7,220 ms; TE=73 ms; slice thickness=2.2 mm; matrix=96 × 96).

MRI Data Pipeline

Methods were optimized to minimize spatial-normalization error and other potential sources of noise. Data were visually inspected before and after processing for quality assurance.

Anatomical Data Processing. Methods are similar to those described in other recent reports by our group (Grogans et al., *under review*; Hur et al., 2018; Hur et al., 2022; Hur et al., 2020; Kim et al., 2023). T1-weighted images were inhomogeneity corrected using *N4* (Tustison et al., 2010) and denoised using *ANTS* (Avants et al., 2011). The brain was then extracted using *BEaST* (Eskildsen et al., 2012) and brain-extracted and normalized reference brains from *IXI* (BIAC, 2022). Brain-extracted T1 images were normalized to a version of the brain-extracted 1-mm T1-weighted MNI152 (version 6) template (Grabner et al., 2006) modified to remove extracerebral tissue. Normalization was performed using the diffeomorphic approach implemented in *SyN* (version 2.3.4) (Avants et al., 2011). Brain-extracted T1 images were segmented—using native-space priors generated in *FAST* (version 6.0.4) (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012)—to enable T1-EPI co-registration (see below).

Fieldmap Data Processing. SE images and *topup* were used to create fieldmaps. Fieldmaps were converted to radians, median-filtered, and smoothed (2-mm). The average of the distortion-corrected SE

images was inhomogeneity corrected using *N4* and masked to remove extracerebral voxels using *3dSkullStrip* (version 19.1.00).

Functional Data Processing. EPI files were de-spiked using *3dDespike*, slice-time corrected to the TR center using *3dTshift*, and motion corrected to the first volume and inhomogeneity corrected using *ANTS* (12-parameter affine). Transformations were saved in ITK-compatible format for subsequent use (McCormick, Liu, Jomier, Marion, & Ibanez, 2014). The first volume was extracted for EPI-T1 coregistration. The reference EPI volume was simultaneously co-registered with the corresponding T1-weighted image in native space and corrected for geometric distortions using boundary-based registration (Jenkinson et al., 2012). This step incorporated the previously created fieldmap, undistorted SE, T1, white matter (WM) image, and masks. The spatial transformations necessary to transform each EPI volume from native space to the reference EPI, from the reference EPI to the T1, and from the T1 to the template were concatenated and applied to the processed EPI data in a single step to minimize incidental spatial blurring. Normalized EPI data were resampled (2 mm^3) using fifth-order b-splines. To maximize anatomical resolution, no additional spatial filters were applied, consistent with recent recommendations (Tillman et al., 2018) and other work by our group (Grogans et al., *under review*; Kim et al., 2023). Hypothesis testing focused on anatomically defined ROIs (see below). By convention, exploratory whole-brain voxelwise analyses employed data that were spatially smoothed (6-mm) using *3DblurInMask*.

fMRI Data Exclusions and Modeling

Data Exclusions. Participants who responded to <50% of rating prompts—indicating inadequate task engagement—were excluded from all analyses (n=3). Volume-to-volume displacement (>0.5 mm) was used to assess residual motion artifact. Scans with excessively frequent artifacts (≥2.5 *SD*) were

discarded. To assess task-correlated motion, we computed correlations between the design matrix and motion estimates (see above). Scans showing extreme correlations (≥ 2.5 *SD*) were discarded. Participants who failed to provide at least 2 scans of usable data were censored from analyses (n=6).

Canonical First-Level Modeling. Single-participant ('first-level') GLMs were implemented in *SPM12* (version 7771) using the default autoregressive model and the temporal band-pass filter set to the hemodynamic response function (HRF) and 128 s (Wellcome Centre for Human Neuroimaging, 2022). Anticipatory hemodynamic signals were modeled using variable-duration rectangular regressors time-locked to the anticipation ('countdown') epochs of the Uncertain Threat, Certain Threat, and Uncertain Safety trials; and convolved with a canonical HRF and its temporal derivative. To maximize design efficiency, the anticipation of Certain Safety—which is psychologically similar to a typical inter-trial interval—served as the implicit baseline. The epochs corresponding to the acute presentation of aversive and benign reinforcers (photographs and audio clips) were simultaneously modeled using the same approach, separately for each of the 4 trial types (**Figure 1**). Epochs corresponding to the presentation of the white-noise visual masks and rating prompts were modeled using the same approach and treated as nuisance regressors. Consistent with prior work using shock-reinforced variants of the countdown task in adults (Grogans et al., *under review;* Hur et al., 2022; Hur et al., 2020; Kim et al., 2023), additional nuisance variates included estimates of volume-to-volume displacement, motion (6 parameters, 0- and 1-volume lagged), cerebrospinal fluid (CSF) signal, instantaneous pulse and respiration rates, and ICA-derived nuisance signals (e.g. brain edge, CSF edge, global motion, white matter) (Pruim et al., 2015). Volumes with excessive volume-to-volume displacement (>0.33 mm) were censored.

Extended Amygdala Regions of Interest (ROIs). Consistent with prior work by our group, task-related Ce and BST activation was quantified using well-established, anatomically defined ROIs and spatially unsmoothed fMRI data (Hur et al., 2018; Kim et al., 2023) (**Figure 1**). The derivation of the Ce ROI is

detailed in Tillman et al. (Tillman et al., 2018). The probabilistic BST ROI was developed by Theiss and colleagues and thresholded at 25% (Theiss, Ridgewell, McHugo, Heckers, & Blackford, 2017). The BST ROI mostly encompasses the supra-commissural BST, given the difficulty of reliably discriminating the borders of regions below the anterior commissure in T1-weighted MRIs (Kruger, Shiozawa, Kreifelts, Scheffler, & Ethofer, 2015). Bilateral ROIs were decimated to the 2-mm resolution of the fMRI data. EAc ROI analyses used standardized regression coefficients extracted and averaged for each combination of task contrast (e.g., Uncertain Threat vs. Certain Safety anticipation), ROI, and participant. *A priori* anatomically defined ROIs provided statistically unbiased estimates of brain-phenotype associations (Poldrack et al., 2017).

Analytic Strategy

Overview. The overarching goal of this study was to determine the impact of dimensional variation in social-anxiety symptoms (mean-centered SPAIC-11) on reactivity to the MSTC paradigm (**Figure 1**). Hypothesis testing focused on two measurement modalities: **(a)** in-scanner ratings of subjective distress experienced during the certain or uncertain anticipation of social threat, and **(b)** and fMRI measures of EAc (Ce and BST ROIs) activation during the corresponding anticipation and presentation phases. Except where noted otherwise, analyses were performed using *SPSS* (Version 27.0.1). Predictor collinearity was acceptable for all analytic models (variance inflation factors < 3.26). Some figures were created using *R* (version 4.0.2), *RStudio*, and *ggplot2* (version 3.4.1) (R Core Team, 2022; RStudio Team, 2022; Wickham, 2016).

Behavioral Hypothesis Testing. A standard mixed-effects GLM was used to determine whether the anticipation of social threat evokes heightened distress, test whether variation in the severity of social-anxiety symptoms (mean-centered SPAIC-11) potentiate subjective distress, and explore the possibility

that these effects are conditional on the temporal certainty of social-threat encounters. In effect, the GLM allowed us to partition variance in anticipatory distress into that associated with the experimental task, with the severity of social-anxiety symptoms, and with their interaction. Significant interactions were decomposed using focal contrasts (paired Students *t*-tests) and regressions.

fMRI Hypothesis Testing. A series of mixed-effects GLMs was used to determine the association between dimensional variation in social-anxiety symptoms (mean-centered SPAIC-11) and EAc (BST/Ce ROIs) reactivity to the anticipation and presentation of social-threat cues, and to explore the possibility that these effects are conditional on the temporal certainty of threat encounters. For the *anticipation* phase, the GLM was implemented as a 3 (*Condition:* Uncertain Social Threat, Certain Social Threat, and Uncertain Safety) × 2 (*Region:* Ce, BST) design with social-anxiety symptoms (mean-centered SPAIC-11) incorporated as a fully-crossed dimensional predictor. This analytic choice was dictated by the fact that the anticipation phase of Certain Safety trials served as the implicit baseline condition for our first-level fMRI models (see above). Huynh-Feldt correction was used as necessary. For the *presentation* phase, the GLM was implemented as a 2 (*Valence:* Social Threat, Safety) × 2 (*Temporal Certainty:* Uncertain, Certain) × 2 (*Region:* Ce, BST) design with social-anxiety symptoms (mean-centered SPAIC-11) incorporated as a fully-crossed dimensional predictor. For both GLMs, significant interactions were decomposed using focal contrasts (paired Students *t*-tests) and regressions.

In head-to-head comparisons of criterion validity, simple paper-and-pencil measures often outperform more sophisticated brain imaging metrics (Shackman & Fox, 2018). To gauge the added explanatory value ('incremental validity') of statistically significant brain metrics, we re-computed the relevant multiple regressions while simultaneously incorporating paper-and-pencil distress ratings. Sensitivity analyses confirmed that key conclusions were minimally altered by the inclusion of nuisance variation in

mean-centered biological sex, pubertal status (Petersen, Crockett, Richards, & Boxer, 1988), or age (not reported).

Exploratory and Confirmatory Whole-Brain Voxelwise Analyses. On an exploratory basis, we performed a series of whole-brain voxelwise GLMs. The general analytic framework was similar to that employed for the 'offline' ROI analyses, and was implemented using a combination of *MRM* (version 1.1) and *SPM12* (McFarquhar et al., 2016). Significance was set at $p<0.05$, whole-brain-corrected, as determined using permutation (10,000 permutations) or Gaussian Random Field Theory ($p<0.001$, cluster-defining threshold) (Eklund, Nichols, & Knutsson, 2016; Smith & Nichols, 2009; Worsley et al., 1996). In both cases, significant interactions were decomposed using pairwise contrasts masked by the relevant omnibus *F*-test. A whole-brain voxelwise analysis was also used to confirm that the acute presentation of faces and voices—independent of their temporal certainty—recruited the amygdala and relevant areas of sensory cortex (e.g., fusiform face area, auditory cortex). In all cases, clusters and local maxima were labeled using the Harvard–Oxford atlas (Desikan et al., 2006; Frazier et al., 2005; Makris et al., 2006)

RESULTS

Social-threat anticipation evokes heightened distress

As shown in **Figure 2a**, in-scanner ratings of fearful and anxious distress were significantly elevated during the anticipation of social threat compared to safety, and during the anticipation of temporally uncertain compared to certain social cues (*Valence:* $F(1,64)=47.39$, $p<0.001$, $p\eta^2=0.43$; *Certainty:* $F(1,64)=18.18$, $p<0.001$, $p\eta^2=0.22$). These observations are broadly consistent with prior work using shock-reinforced threat-anticipation tasks in adults and reinforce the validity of the MSTC paradigm for probing social anxiety in adolescence (Grogans et al., *under review;* Hur et al., 2020; Kim et al., 2023).

Social anxiety amplifies task-related distress

As shown in **Figures 2b-c**, adolescents with more severe social-anxiety symptoms reported indiscriminately elevated anticipatory distress across threatening and benign trials ($F(1,64)=4.80$, $p=0.03$, $p\eta^2=.07$). Among the more anxious adolescents, distress was particularly intense during the anticipation of social threat (*Valence × Social Anxiety:* $F(1,64)=7.81$; $p=0.01$, $p\eta^2=0.11$). Other omnibus effects were not significant ($p>0.73$). In short, adolescents with elevated social anxiety show a mixture of indiscriminate ('overgeneralized') *and* threat-potentiated anticipatory distress in response to the MSTC paradigm.

The EAc is preferentially engaged by the anticipation of temporally uncertain social threat

We leveraged anatomically defined Ce and BST ROIs and spatially unsmoothed fMRI data to test whether more severe social-anxiety symptoms are associated with exaggerated EAc activation when anticipating encounters with social threat, and to explore the possibility that threat-potentiation depends on the temporal certainty of encounters. A mixed-effects GLM revealed significant effects of Region, reflecting greater anticipatory activation, on average, in the Ce compared to the BST ($F(1,64)=4.81$; $p=0.03$, $p\eta^2=0.07$). The omnibus effect of Condition was also significant ($F(2,128)=3.84$; $p=0.02$, $p\eta^2=0.06$). As shown in **Figure 3a,** focal contrasts demonstrated that the anticipation of Uncertain Social Threat elicited significantly greater EAc activation compared to Certain Social Threat and Uncertain Safety (*Certain Threat:* $t(64)=2.56$, $p=0.01$; *Uncertain Safety:* $t(64)=2.07$, $p=0.04$), which in turn did not significantly differ from one another ($t(64)=-0.39$, $p=0.70$). The Region × Condition interaction was not significant ($p=0.57$). Together, these results indicate that the EAc is preferentially recruited by the anticipation of temporally Uncertain Social Threat—consistent with prior work in adults with SAD (Figel et al., 2019)—

and the degree of neural engagement does not statistically differ across the Ce and BST, its two major subdivisions.

Social anxiety is associated with diminished BST discrimination of anticipated threat and safety

The omnibus GLM also revealed significant Condition × Social Anxiety ($F(2,128)=5.87$, $p=0.004$, $p\eta^2=0.08$) and Condition × Region × Social Anxiety interactions ($F(2,128)=4.75$; $p=0.01$, $p\eta^2=0.07$). Other omnibus effects were not significant ($p>0.46$). To decompose the significant interactions, we computed separate regression models for the Ce and BST ROIs, in each case including reactivity to each of the three anticipation conditions—Uncertain Social Threat, Certain Social Threat, and Uncertain Safety—as simultaneous predictors of social-anxiety symptoms. Results indicated that Ce reactivity is unrelated to dimensional variation in social anxiety ($|t|(62)<1.19$, $p>0.23$). On the other hand, *blunted* BST reactivity to Uncertain-Threat anticipation and *heightened* BST reactivity to Uncertain-Safety anticipation were both significantly and uniquely associated with more severe symptoms (*Uncertain Threat:* $t(62)=-2.52$, $p=0.01$; *Uncertain Safety:* $t(62)=2.34$, $p=0.02$), with a negligible effect evident for Certain-Threat anticipation ($t(62)=0.17$, $p=0.87$). As shown in **Figures 3b-c,** both associations remained significant while controlling for variation in Ce anticipatory activation, indicating a unique association between BST reactivity and the severity of social-anxiety symptoms (*Uncertain Threat:* $t(60)=-2.57$, $p=0.01$; *Uncertain Safety:* $t(60)=3.23$, $p=0.002$). Both associations also remained significant after censoring one outlying observation ($p<.05$).

The omnibus GLM results indicate that the most robust EAc activation occurs during the uncertain anticipation of social threat, with significantly lower levels evident during the uncertain anticipation of benign social cues (**Figure 3a**). Our results suggest that this normative mean difference across the two conditions is diminished in the BST of adolescents with more severe social-anxiety symptoms. Consistent

with this view, higher levels of social anxiety were also negatively associated with the simple arithmetic difference in BST activation across the two conditions (*Uncertain Social Threat – Uncertain Safety, anticipation:* $r(64)=-0.38$ [95% CI: 0.15, 0.57], $p=0.002$). Although exploratory voxelwise analyses revealed a number of regions that were recruited during the anticipation of social threat, they uncovered no whole-brain significant associations with the severity of social-anxiety symptoms (**Supplementary Tables S1-S7**).

To clarify the explanatory value of BST reactivity, we computed a new regression model, using a combination of the BST neuroimaging metrics *and* two self-report metrics—indiscriminate distress (Threat + Safety Ratings) and threat-potentiated distress (Threat – Safety Ratings)—to simultaneously predict the severity of social-anxiety symptoms (cf. **Figure 2**). Results indicated that BST activation during the anticipation of Uncertain Threat and Safety continued to explain significant variance in social-anxiety symptoms while controlling for both forms of subjective distress (*Uncertain Threat:* $t(61)=-2.85$, $p=.006$; *Uncertain Safety:* $t(61)=2.86$, $p=.006$; *Ratings:* $t(61)<1.48$, $p>.14$). The same conclusion was also evident using the arithmetic difference in anticipatory BST activation across the Uncertain-Threat and -Safety contexts ($|t|(62)=-2.76$, $p=0.08$) or when controlling for all four condition-specific distress ratings ($|t|(59)>2.23$, $p<0.03$). In short, BST anticipatory activation explains variance in adolescent social-anxiety symptoms above-and-beyond that accounted for by a more traditional paper-and-pencil measure of task-evoked fear and anxiety, underscoring the added explanatory value ('incremental validity') of the fMRI metrics. These observations also make it clear that the association between indiscriminate BST anticipatory activation and dimensional variation in social anxiety cannot be attributed to simple differences in subjective reactivity to the MSTC paradigm.

The EAc is robustly recruited by the acute presentation of social threat, independent of social-anxiety symptoms

As shown in **Figure 4**, a mixed-effects GLM indicated that the EAc (Ce and BST) was significantly more activated by the acute presentation of threatening, compared to benign, social cues (*Valence:* $F(1,64)=8.03$; $p=0.006$, $p\eta^2=0.11$). Results also demonstrated that the Ce was more responsive to the acute presentation of faces and voices—irrespective of their valence—when compared to the BST (*Region:* $F(1,64)=11.56$; $p=0.001$, $p\eta^2=0.15$). No other omnibus effects were significant ($p>0.18$). In sum, while the EAc, in aggregate, is robustly recruited by the acute presentation of threatening face-voice stimuli, this is largely independent of variation in the severity of social-anxiety symptoms.

As expected, a whole-brain voxelwise analysis confirmed that the acute presentation of faces and voices recruited the amygdala and relevant areas of sensory cortex, including the bilateral fusiform gyri, superior temporal sulci, and Heschl's gyri (**Supplementary Tables S8-S10**). Exploratory analyses did not detect any whole-brain significant voxelwise associations with the severity of social-anxiety symptoms (**Supplementary Tables S11-22**).

DISCUSSION

Clinically significant social-anxiety symptoms often emerge during adolescence. Among socially anxious adolescents, distress and impairment manifest in response to both acute social scrutiny (e.g., interacting with unfamiliar peers) and by situations where negative evaluations are possible, but uncertain in their timing or likelihood, as when first entering a public restroom or navigating a school hallway. Heightened anticipatory responses to social threat—whether certain or uncertain—promotes maladaptive avoidance strategies which, in turn, undermine opportunities for fear extinction, social skill and academic development, and friendship cultivation (Grupe & Nitschke, 2013; Heckman, 2006; Lee et al., 2014; Rubin, Barstead, Smith, & Bowker, 2018). Preclinical research in rodents, monkeys, and humans

motivates the hypothesis that exaggerated reactivity to certain and uncertain threat reflects functional alterations in the EAc—including the Ce and BST—but the relevance of this work to the complexities of the adolescent human brain and social anxiety have remained unclear (Fox & Kalin, 2014; Fox, Oler, Tromp, Fudge, & Kalin, 2015; Grogans et al., 2023; Shackman & Fox, 2021). To address this central question, we combined a novel fMRI paradigm (**Figure 1**) with a racially diverse adolescent sample enriched for clinically significant social anxiety symptoms. Half of the participants reported clinically significant social anxiety symptoms (SPAIC-11 > 9.17; 51.5%) and more than one-third met DSM-IV diagnostic criteria for SAD (36.4%). *A priori* anatomically defined ROIs provided statistically unbiased estimates of Ce and BST reactivity to the anticipation and acute presentation of multimodal social-threat cues. Results demonstrated that fearful and anxious feelings were significantly elevated during the anticipation of social threat, confirming the basic validity of the MSTC task as a probe of social anxiety (**Figure 2a**). Adolescents with elevated social-anxiety symptoms showed more intense subjective responses to the MSTC task, manifesting as potentiated distress when anticipating encounters with threatening faces and voices, and indiscriminately elevated distress during the anticipation of benign social stimuli (**Figures 2b-c**). Neuroimaging results revealed robust EAc activation (Ce/BST) during the temporally uncertain anticipation of encounters with social threat cues, relative to both certain threat and uncertain safety (**Figure 3a**). Adolescents with more severe social-anxiety symptoms showed *blunted* BST activation during the uncertain anticipation of social threat and *heightened* BST activation during the uncertain anticipation of benign social stimuli, suggesting diminished discrimination of the two contexts. Indeed, more severe symptoms were also associated with a reduction in the arithmetic difference in BST activation across the two conditions. These brain-symptom associations remained significant when controlling for either Ce reactivity or paper-and-pencil measures of task-revoked distress, underscoring the unique explanatory value of BST anticipatory activity (**Figures 3b-c**). The EAc (Ce/BST) was more strongly recruited by the acute presentation of threatening than benign faces and voices, but variation in EAc reactivity was unrelated to symptom severity (**Figure 4**).

Like other anxiety disorders, SAD is largely defined, diagnosed, and treated on the basis of subjective feelings of fear and anxiety (APA, 2022; Grogans et al., 2023; LeDoux & Pine, 2016; Taschereau-Dumouchel, Michel, Lau, Hofmann, & LeDoux, 2022). The present results demonstrate that adolescents with more severe social anxiety experience more intense fear and anxiety in response to the MSTC paradigm, manifesting as a mixture of heightened distress when anticipating encounters with hostile, threatening faces and voices (i.e., potentiated threat reactivity) *and* pervasively elevated distress across threatening and benign social contexts. Aspects of this mixed 'potentiated-and-pervasive' profile are evident among adults with SAD (Figel et al., 2019; Goldin, Manber, Hakimi, Canli, & Gross, 2009) and adults at-risk for developing anxiety disorders (Grogans et al., *under review;* Hur et al., 2022; Sep, Steenmeijer, & Kennis, 2019; Stegmann, Reicherts, Andreatta, Pauli, & Wieser, 2019). In mixed-anxiety samples of youth, aspects of this profile have been observed using other threat-anticipation paradigms and other measures (e.g., skin conductance) (Abend et al., 2020; Abend et al., 2022; Bernstein et al., *in press;* Britton et al., 2013; Chauret et al., 2019; Klein et al., *in press;* Reinhard et al., *in press*). From an etiological perspective, pervasive, contextually inappropriate threat responses are particularly noteworthy because they have been shown to promote instrumental avoidance, a key sign of pathological anxiety (Grillon, 2002; Hunt, Cooper, Hartnell, & Lissek, 2017; van Meurs, Wiggert, Wicker, & Lissek, 2014); to discriminate adult anxiety patients from controls (Duits et al., 2015; McGregor et al., 2021); and to prospectively predict the first onset of anxiety disorders in adolescents (Craske et al., 2012; Yarrington et al., 2022) and the intensification of internalizing symptoms in children and adults (Barker et al., 2015; Lenaert et al., 2014).

The present results show that, on average, the EAc (Ce/BST) is robustly engaged during the uncertain anticipation of hostile faces and voices, with significantly lower levels of activation evident during the uncertain anticipation of benign social cues. Our results further suggest that this normative difference

across the two conditions is diminished in the BST of adolescents with more severe social-anxiety symptoms. Might this reflect heightened BST reactivity to benign or mildly positive social cues (**Figure 1**)? After all, socially anxious adolescents and adults often experience heightened levels of dread and discomfort during the anticipation or experience of positive social interactions (e.g., receiving praise) (Fredrick & Luebbe, 2020; Weeks, Howell, Srivastav, & Goldin, 2019), perceive positive and neutral facial expressions as less approachable and more arousing (Kivity & Huppert, 2015), and in some cases have been shown to exhibit exaggerated amygdala activation to positive and neutral faces (Birbaumer et al., 1998; Cooney, Atlas, Joormann, Eugène, & Gotlib, 2006; Crane, Chang, Kinney, & Klumpp, 2021; Straube, Mentzel, & Miltner, 2005). Yet our results demonstrate that variation in social anxiety is unrelated to EAc reactivity to the acute presentation of social cues, consistent with a large-scale study of adults with GAD (n=67 unmedicated patients, n=28 matched controls; Ziv et al., 2013). When viewing faces and voices, the EAc is more responsive to threatening than benign cues, independent of symptom severity, contraindicating a straightforward 'hyper-reactivity to benign social cues' explanation. Variation in social-anxiety symptoms was also unrelated to EAc activation during the certain anticipation of social threat, contraindicating the possibility that socially anxious adolescents are simply more pessimistic in their expectations or generically more reactive to anticipated encounters with social threat.

During the anticipation phase of the MSTC task, the EAc is much more strongly recruited, on average, by Uncertain Social Threat than either Certain Social Threat or Uncertain Safety, suggesting that temporally uncertain threat is a particularly potent driver of EAc activation. Within the BST (but not the Ce), these mean differences are further modulated by dimensional variation in social anxiety symptoms. Socially anxious adolescents show blunted BST activation during the uncertain anticipation of threatening social cues, and amplified activation during the uncertain anticipation of benign cues. Building on Grupe and Nitschke's theoretical framework—which emphasizes the importance of both threat uncertainty and indiscriminate defensive responses to normatively safe cues and contexts—this pattern suggests that,

among adolescents with more severe social anxiety, the amplifying consequences of uncertainty are especially potent when anticipating low-intensity or ambiguous social encounters (i.e., the benign faces and voices of unfamiliar adults) and comparatively weaker when anticipating encounters with more intense, normatively threatening cues (Grupe & Nitschke, 2013). That is, among adolescents with more severe social anxiety, uncertainty appears to play a key role in moderating the impact of anticipated encounters with social threat. While speculative, this hypothesis accounts for the overall pattern of mean differences and the opposing consequences of social anxiety on BST anticipatory activity, and it is broadly consistent with evidence highlighting the BST's role in assembling responses to uncertain potential threats (Avery, Clauss, & Blackford, 2016; Fox & Shackman, 2019). It is also well aligned with socially anxious individuals' exaggerated emotional responses to benign-but-unscripted everyday social encounters, such as anticipating an interaction with an instructor.

Clearly, a number of other important challenges remain for the future. First, our study was focused on a racially diverse sample of adolescents. Moving forward, it will be useful to expand this to encompass more clinically and nationally representative samples. Second, our analyses relied on adolescent-reported social-anxiety-symptoms. While this approach is common in both research and clinical settings, recent work highlights the value of using more sophisticated multi-informant approaches to quantify the depth and breadth of social anxiety (De Los Reyes et al., 2023; Makol et al., 2020). Third, our cross-sectional design does not license mechanistic claims. Prospective-longitudinal and intervention research will be necessary to determine whether diminished discrimination of uncertain threat and safety forecasts the emergence of clinically significant distress and impairment, and whether this bias is normalized by clinically effective treatments. Fourth, although our results highlight the importance of the BST, social anxiety is a complex phenotype that likely reflects multiple distributed networks. It will be important to understand how functional interactions between the BST and other brain regions support variation in pediatric social anxiety.

In conclusion, social anxiety lies on a continuum and, when extreme, can be devastating, with clear impairment evident even at subdiagnostic levels of severity. Social-anxiety symptoms often emerge in adolescence, promote other developmental problems and psychiatric illnesses, and often prove challenging to treat. Among socially anxious adolescents and adults, distress and impairment are triggered by social scrutiny, but they can also be evoked by situations where encounters with social threat are possible, but uncertain in their timing, likelihood, or severity. To date, the vast majority of human neuroimaging studies have focused on laboratory analogues of acute social threat. Much less scientific attention has been devoted to understanding the neural systems underlying aberrant responses to anticipated encounters with social threat. The present results indicate that socially anxious adolescents experience elevated fear and anxiety when anticipating encounters with threatening faces and voices, and indiscriminately elevated distress during the anticipation of benign social stimuli. Adolescents with more severe symptoms also showed reduced discrimination of uncertain social threat and safety. These observations provide a neuroscientifically grounded framework for conceptualizing early life social anxiety and set the stage for the prospective-longitudinal and mechanistic research that will be necessary to determine causation and, ultimately, to develop improved intervention strategies. A diverse sample enriched for clinically significant levels of social anxiety, a well-controlled task, and a statically unbiased (anatomical ROI) analytic approach enhance confidence in the robustness and translational relevance of these results.

FIGURES AND CAPTIONS

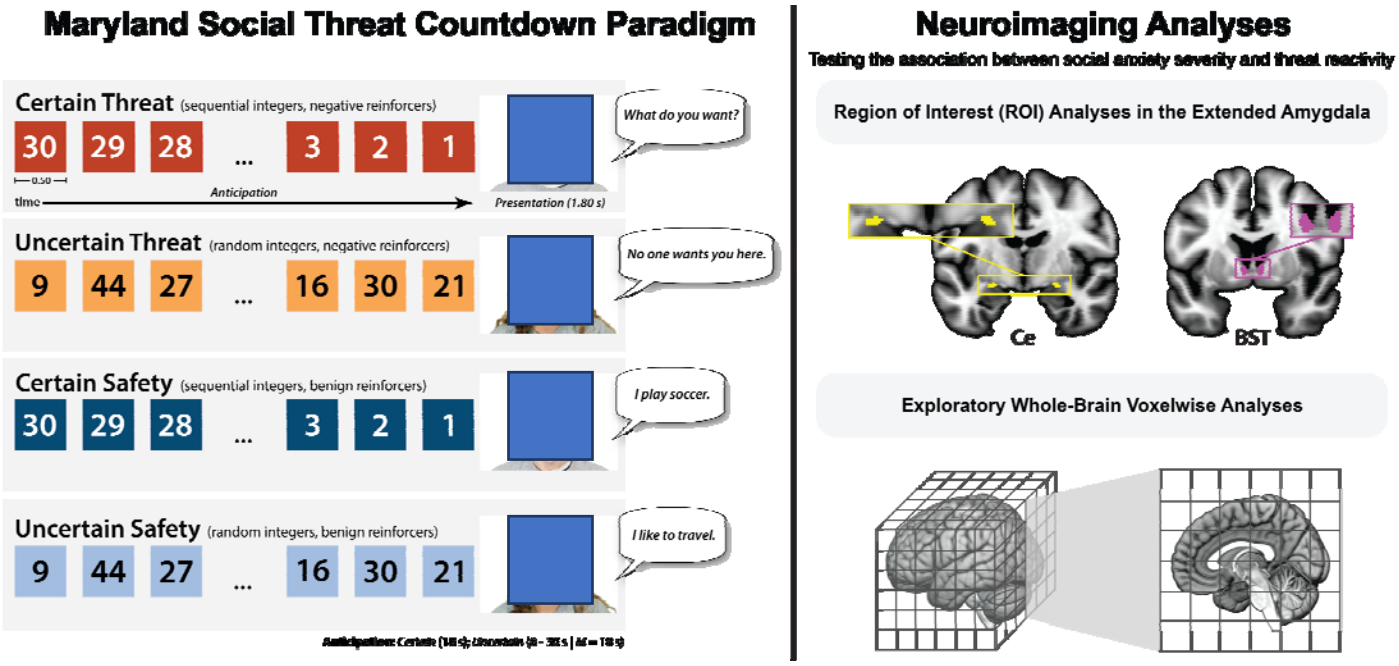

Figure 1. ***Study overview.*** **The Maryland Social Threat Countdown (MSTC) paradigm.** As shown schematically in the left panel, the MSTC paradigm takes the form of a 2 (*Valence:* Social Threat/Safety) × 2 (*Temporal Certainty:* Uncertain/Certain) × 2 (*Phase:* Anticipation/Presentation) repeated-measures randomized event-related design. On Certain Social Threat trials, participants saw a descending stream of integers ('count-down') for 18 s. To maximize distress, the anticipation epoch always culminated with the presentation of a multi-modal social threat (1.8 s), encompassing a photograph of an angry face and an audio clip of a hostile verbal remark (e.g., *"No one wants you here"*). Uncertain Social Threat trials were similar, but the integer stream was randomized and presented for an uncertain and variable duration (8-30 s; *M*=18 s). Participants knew that a social threat would occur, but had no way of knowing precisely when the encounter would occur. Safety trials were similar but terminated with the delivery of normatively benign photographs (happy faces) and audio clips (e.g., *"I play soccer"*). **Neuroimaging analyses.** As shown schematically in the top-right panel, two analytic approaches were used to determine the impact of social-anxiety symptoms on neural reactivity to social threat. Hypothesis testing focused on two well-established, anatomically defined EAc regions-of-interest (ROIs): the Ce (*yellow*) and BST (*magenta*). Because the ROIs (i.e., voxel-level measurements) were chosen *a priori*—on the basis of neuroanatomy, rather than suprathreshold threat reactivity—this approach provides unbiased effect-size estimates (Poldrack et al., 2017). Standardized regression coefficients were extracted and averaged across voxels for each combination of ROI, condition, and participant. Hypothesis testing used a standard general linear model (GLM). For illustrative purposes, 1-mm ROIs are shown. Analyses employed ROIs decimated to the 2-mm resolution of the fMRI data. As shown schematically in the bottom-right panel, exploratory whole-brain voxelwise analyses were also performed. Abbreviations—BST, bed nucleus of the stria terminalis; Ce, central nucleus of the amygdala; *M*, mean; s, seconds. ***Note: Faces are masked to comply with restrictions imposed by the preprint server.***

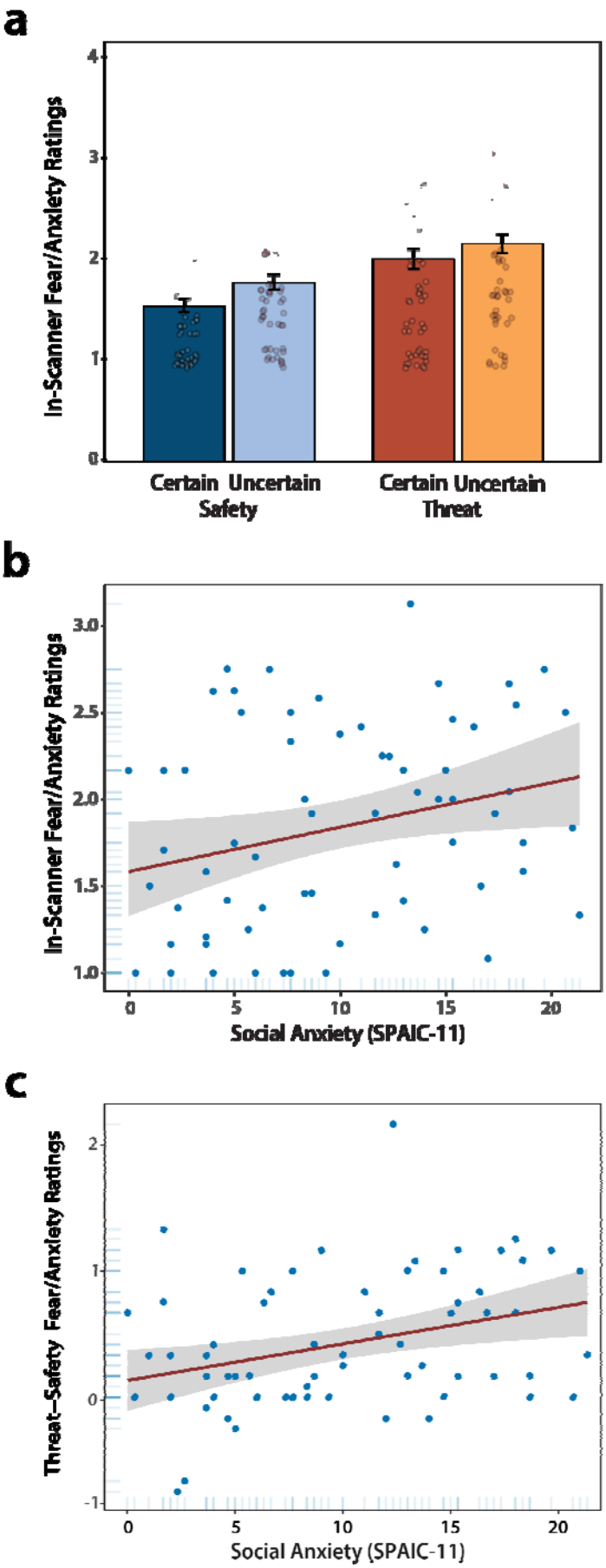

Figure 2. ***In-scanner ratings of subjective distress.*** **a.** Anticipatory fear and anxiety were significantly elevated during the anticipation of social threat compared to safety, and during the anticipation of temporally uncertain compared to certain reinforcers (p<0.001). Error bars depict the standard error of the mean. Dots indicate individual participants. **b.** Socially anxious adolescents experience indiscriminately elevated anticipatory distress across conditions, both threatening and benign (p=0.03). **c.** Socially anxious adolescents experience heightened distress during the anticipation of threatening, compared to benign, social stimuli (p=0.01). Red line depicts the regression slope. Gray envelope depicts the 95% confidence interval. Dots indicate individual participants.

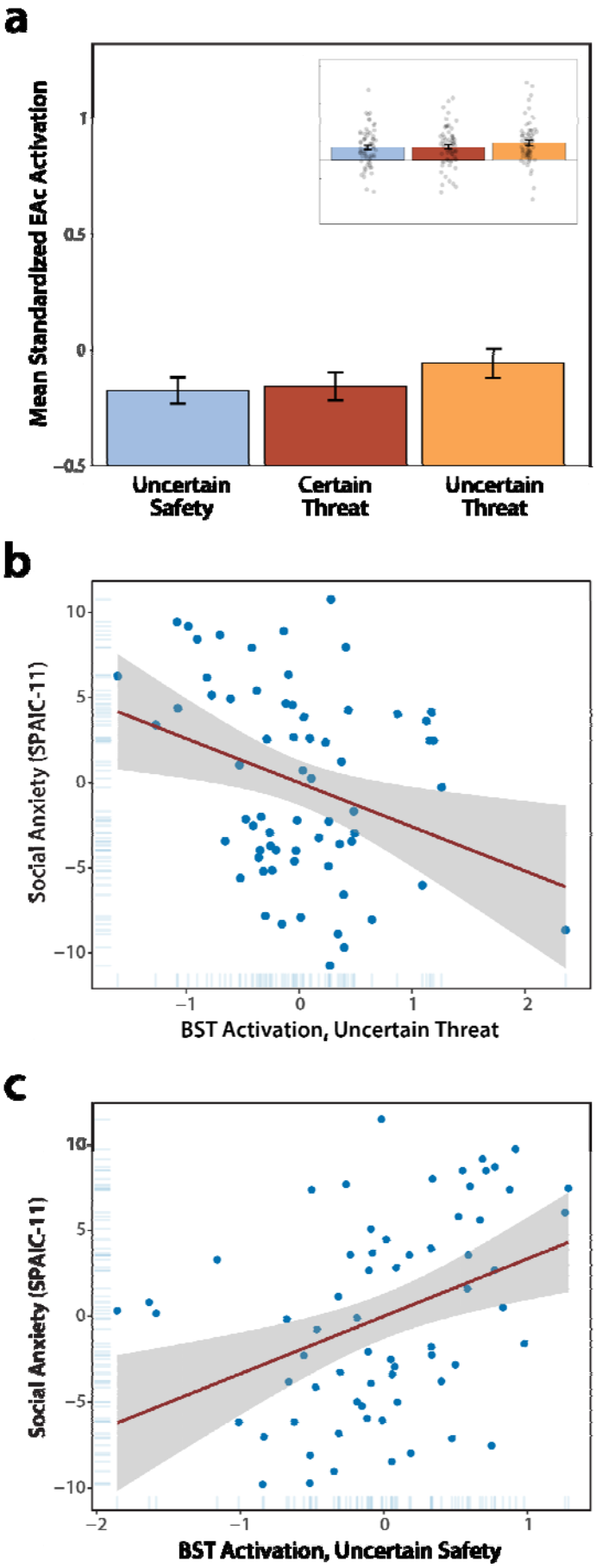

Figure 3. ***Anticipation phase of the Maryland Social Threat Countdown paradigm.*** **a.** The EAc is preferentially engaged by the temporally uncertain anticipation of social threat (p=0.03). Inset depicts individual participants. Error bars depict the standard error of the mean. **b.** Socially anxious adolescents show blunted BST reactivity during the temporally uncertain anticipation of social threat, while controlling for differences in Ce anticipatory activation (p=0.01). **c.** Socially anxious adolescents show amplified BST reactivity during the temporally uncertain anticipation of normatively benign social stimuli, while controlling for differences in Ce anticipatory activation (p=0.002). Both associations remained significant after censoring one outlying observation (p<.05). Conventions are identical to **Figure 2**. Abbreviations—BST, bed nucleus of the stria terminalis.

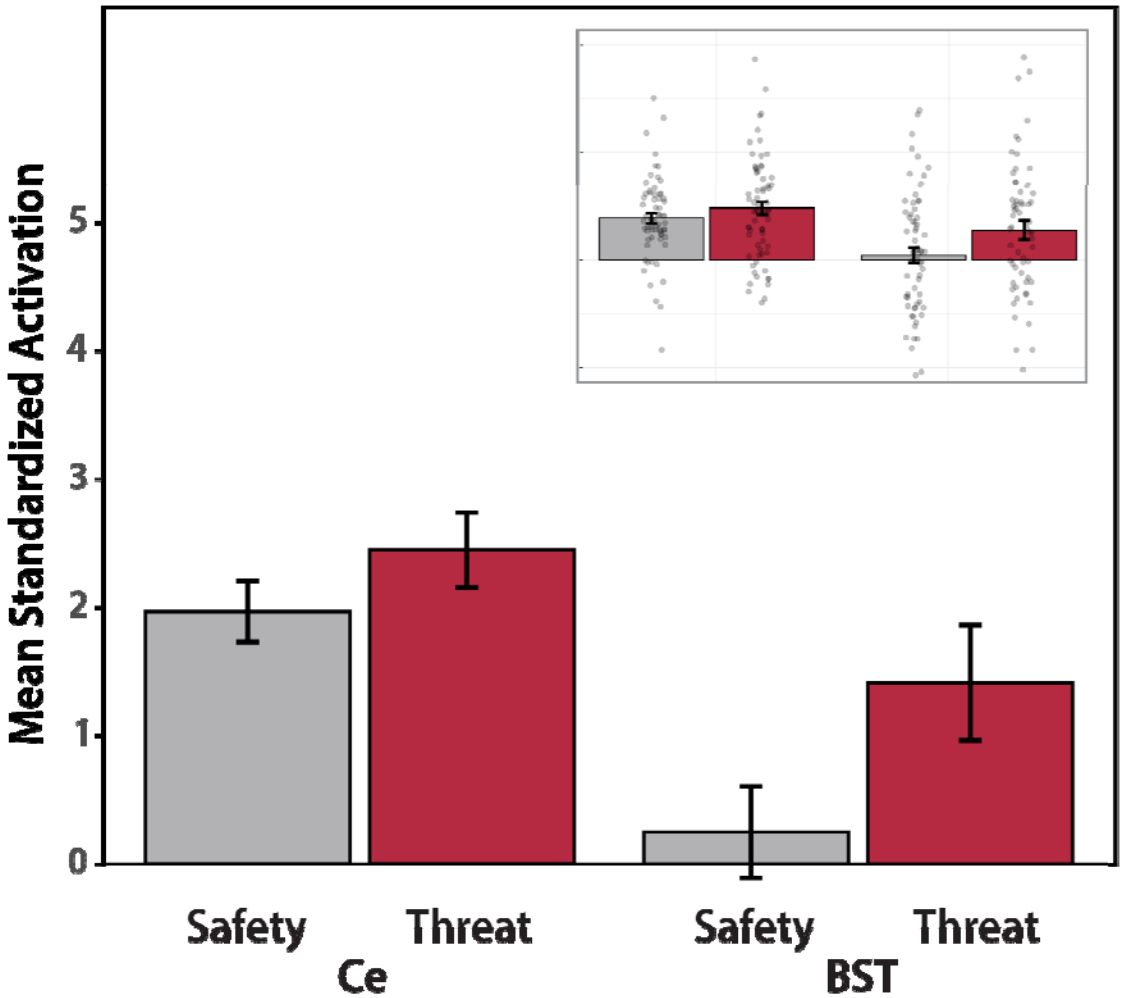

Figure 4. ***Presentation phase of the Maryland Social Threat Countdown paradigm.*** GLM results demonstrated that the EAc was more strongly recruited by the acute presentation of threatening (relative to benign) social stimuli (p=0.006) and that the Ce is more responsive (relative to the BST) to faces and voices, irrespective of their valence (p=0.001). Inset depicts individual participants. Other conventions are identical to **Figure 2**. Abbreviations—BST, bed nucleus of the stria terminalis.

REFERENCES

Abend, R., Gold, A. L., Britton, J. C., Michalska, K. J., Shechner, T., Sachs, J. F., Winkler, A. M., Leibenluft, E., Averbeck, B. B., & Pine, D. S. (2020). Anticipatory threat responding: Associations with anxiety, development, and brain structure. *Biol Psychiatry, 87*, 916-925.

Abend, R., Ruiz, S. G., Bajaj, M. A., Harrewijn, A., Linke, J. O., Atlas, L. Y., Winkler, A. M., & Pine, D. S. (2022). Threat imminence reveals links among unfolding of anticipatory physiological response, cortical-subcortical intrinsic functional connectivity, and anxiety. *Neurobiol Stress, 16*, 100428.

Alden, L. E., & Taylor, C. T. (2004). Interpersonal processes in social phobia. *Clin Psychol Rev, 24*(7), 857-882.

Angst, J., Paksarian, D., Cui, L., Merikangas, K. R., Hengartner, M. P., Ajdacic-Gross, V., & Rössler, W. (2016). The epidemiology of common mental disorders from age 20 to 50: results from the prospective Zurich cohort Study. *Epidemiology and psychiatric sciences, 25*, 24-32.

APA. (2022). *Diagnostic and statistical manual of mental disorders, text revision (DSM-5-TR)* (5 ed.). Washington, DC: American Psychiatric Publishing.

Avants, B. B., Tustison, N. J., Song, G., Cook, P. A., Klein, A., & Gee, J. C. (2011). A reproducible evaluation of ANTs similarity metric performance in brain image registration. *Neuroimage, 54*, 2033-2044.

Avery, S. N., Clauss, J. A., & Blackford, J. U. (2016). The human BNST: Functional role in anxiety and addiction. *Neuropsychopharmacology, 41*, 126-141.

Barker, T. V., Reeb-Sutherland, B., Degnan, K. A., Walker, O. L., Chronis-Tuscano, A., Henderson, H. A., Pine, D. S., & Fox, N. A. (2015). Contextual startle responses moderate the relation between behavioral inhibition and anxiety in middle childhood. *Psychophysiology, 52*, 1544-1549.

Batelaan, N. M., Bosman, R. C., Muntingh, A., Scholten, W. D., Huijbregts, K. M., & van Balkom, A. (2017). Risk of relapse after antidepressant discontinuation in anxiety disorders, obsessive-compulsive

disorder, and post-traumatic stress disorder: systematic review and meta-analysis of relapse prevention trials. *BMJ, 358*, j3927.

Beesdo-Baum, K., & Knappe, S. (2012). Developmental epidemiology of anxiety disorders. *Child Adolesc Psychiatr Clin N Am, 21*(3), 457-478.

Bernstein, R. A., Smith, A. R., Kitt, E. R., Cardinale, E. M., Harrewijn, A., Abend, R., Michalska, K. J., Pine, D. S., & Kircanski, K. (*in press*). Threat appraisal and pediatric anxiety: Proof of concept of a latent variable approach. *Clinical Psychological Science*.

BIAC. (2022). IXI Dataset. Retrieved from https://brain-development.org/ixi-dataset/

Birbaumer, N., Grodd, W., Diedrich, O., Klose, U., Erb, M., Lotze, M., Schneider, F., Weiss, U., & Flor, H. (1998). fMRI reveals amygdala activation to human faces in social phobics. *Neuroreport, 9*(6), 1223-1226.

Boehme, S., Ritter, V., Tefikow, S., Stangier, U., Strauss, B., Miltner, W. H., & Straube, T. (2014). Brain activation during anticipatory anxiety in social anxiety disorder. *Soc Cogn Affect Neurosci, 9*, 1413-1418.

Britton, J. C., Grillon, C., Lissek, S., Norcross, M. A., Szuhany, K. L., Chen, G., Ernst, M., Nelson, E. E., Leibenluft, E., Shechner, T., & Pine, D. S. (2013). Response to learned threat: An FMRI study in adolescent and adult anxiety. *Am J Psychiatry, 170*(10), 1195-1204.

Brühl, A. B., Delsignore, A., Komossa, K., & Weidt, S. (2014). Neuroimaging in social anxiety disorder—a meta-analytic review resulting in a new neurofunctional model. *Neurosci Biobehav Rev, 47*, 260-280.

Bunnell, B. E., Beidel, D. C., Liu, L., Joseph, D. L., & Higa-McMillan, C. (2015). The SPAIC-11 and SPAICP-11: Two brief child- and parent-rated measures of social anxiety. *J Anxiety Disord, 36*, 103-109.

Cain, C. K. (*in press*). Beyond fear, extinction, and freezing: Strategies for improving the translational value of animal conditioning research. *Curr Top Behav Neurosci*.

Chauret, M., Suffren, S., Pine, D. S., Nassim, M., Saint-Amour, D., & Maheu, F. S. (2019). Fear conditioning and extinction in anxious youth, offspring at-risk for anxiety and healthy comparisons: An fMRI study. *Biol Psychol, 148*, 107744.

Chavanne, A. V., & Robinson, O. J. (2021). The overlapping neurobiology of adaptive and pathological anxiety: a meta-analysis of functional neural activation. *American Journal of Psychiatry, 178*, 156-164.

Clauss, J. A., & Blackford, J. U. (2012). Behavioral inhibition and risk for developing social anxiety disorder: a meta-analytic study. *J Am Acad Child Adolesc Psychiatry, 51*, 1066-1075

Cooney, R. E., Atlas, L. Y., Joormann, J., Eugène, F., & Gotlib, I. H. (2006). Amygdala activation in the processing of neutral faces in social anxiety disorder: is neutral really neutral? *Psychiatry Res, 148*(1), 55-59.

Cox, R. W. (1996). AFNI: Software for analysis and visualization of functional magnetic resonance neuroimages. *Computers and Biomedical Research, 29*, 162-173.

Crane, N. A., Chang, F., Kinney, K. L., & Klumpp, H. (2021). Individual differences in striatal and amygdala response to emotional faces are related to symptom severity in social anxiety disorder. *Neuroimage Clin, 30*, 102615.

Craske, M. G., Wolitzky-Taylor, K. B., Mineka, S., Zinbarg, R., Waters, A. M., Vrshek-Schallhorn, S., Epstein, A., Naliboff, B., & Ornitz, E. (2012). Elevated responding to safe conditions as a specific risk factor for anxiety versus depressive disorders: evidence from a longitudinal investigation. *J Abnorm Psychol, 121*, 315-324.

Davies, C. D., Young, K., Torre, J. B., Burklund, L. J., Goldin, P. R., Brown, L. A., Niles, A. N., Lieberman, M. D., & Craske, M. G. (2017). Altered time course of amygdala activation during speech anticipation in social anxiety disorder. *J Affect Disord, 209*, 23-29.

Davis, M., Walker, D. L., Miles, L., & Grillon, C. (2010). Phasic vs sustained fear in rats and humans: Role of the extended amygdala in fear vs anxiety. *Neuropsychopharmacology, 35*, 105-135.

De Los Reyes, A., Wang, M., Lerner, M. D., Makol, B. A., Fitzpatrick, O. M., & Weisz, J. R. (2023). The Operations Triad Model and youth mental health assessments: Catalyzing a paradigm shift in measurement validation. *Journal of Clinical Child & Adolescent Psychology, 52*, 19-54.

Desikan, R. S., Ségonne, F., Fischl, B., Quinn, B. T., Dickerson, B. C., Blacker, D., Buckner, R. L., Dale, A. M., Maguire, R. P., Hyman, B. T., Albert, M. S., & Killiany, R. J. (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage, 31*, 968-980.

Duits, P., Cath, D. C., Lissek, S., Hox, J. J., Hamm, A. O., Engelhard, I. M., Hout, M. A., & Baas, J. M. (2015). Updated meta-analysis of classical fear conditioning in the anxiety disorders. *Depression and anxiety, 32*, 239-253.

Eklund, A., Nichols, T. E., & Knutsson, H. (2016). Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates. *Proc Natl Acad Sci U S A, 113*, 7900-7905.

Eskildsen, S. F., Coupé, P., Fonov, V., Manjón, J. V., Leung, K. K., Guizard, N., Wassef, S. N., Østergaard, L. R., Collins, D. L., & Alzheimer's Disease Neuroimaging Initiative. (2012). BEaST: brain extraction based on nonlocal segmentation technique. *Neuroimage, 59*, 2362-2373.

Evans, R., Clark, D. M., & Leigh, E. (2021). Are young people with primary social anxiety disorder less likely to recover following generic CBT compared to young people with other primary anxiety disorders? A systematic review and meta-analysis. *Behav Cogn Psychother, 49*(3), 352-369.

Fehm, L., Beesdo, K., Jacobi, F., & Fiedler, A. (2008). Social anxiety disorder above and below the diagnostic threshold: prevalence, comorbidity and impairment in the general population. *Soc Psychiatry Psychiatr Epidemiol, 43*, 257-265.

Figel, B., Brinkmann, L., Buff, C., Heitmann, C. Y., Hofmann, D., Bruchmann, M., Becker, M. P. I., Herrmann, M. J., & Straube, T. (2019). Phasic amygdala and BNST activation during the anticipation of temporally unpredictable social observation in social anxiety disorder patients. *Neuroimage Clin, 22*, 101735.

Fox, A. S., & Kalin, N. H. (2014). A translational neuroscience approach to understanding the development of social anxiety disorder and its pathophysiology. *American Journal of Psychiatry, 171*, 1162-1173.

Fox, A. S., Oler, J. A., Tromp, D. P., Fudge, J. L., & Kalin, N. H. (2015). Extending the amygdala in theories of threat processing. *Trends Neurosci, 38*, 319-329.

Fox, A. S., & Shackman, A. J. (2019). The central extended amygdala in fear and anxiety: Closing the gap between mechanistic and neuroimaging research. *Neuroscience letters, 693*, 58-67.

Frazier, J. A., Chiu, S., Breeze, J. L., Makris, N., Lange, N., Kennedy, D. N., Herbert, M. R., Bent, E. K., Koneru, V. K., Dieterich, M. E., Hodge, S. M., Rauch, S. L., Grant, P. E., Cohen, B. M., Seidman, L. J., Caviness, V. S., & Biederman, J. (2005). Structural brain magnetic resonance imaging of limbic and thalamic volumes in pediatric bipolar disorder. *American Journal of Psychiatry, 162*, 1256-1265.

Fredrick, J. W., & Luebbe, A. M. (2020). Fear of positive evaluation and social anxiety: A systematic review of trait-based findings. *J Affect Disord, 265*, 157-168.

Gentili, C., Cristea, I. A., Angstadt, M., Klumpp, H., Tozzi, L., Phan, K. L., & Pietrini, P. (2016). Beyond emotions: A meta-analysis of neural response within face processing system in social anxiety. *Exp Biol Med (Maywood), 241*(3), 225-237.

Goldin, P. R., Manber, T., Hakimi, S., Canli, T., & Gross, J. J. (2009). Neural bases of social anxiety disorder: emotional reactivity and cognitive regulation during social and physical threat. *Arch Gen Psychiatry, 66*(2), 170-180.

Grabner, G., Janke, A. L., Budge, M. M., Smith, D., Pruessner, J., & Collins, D. L. (2006). Symmetric atlasing and model based segmentation: an application to the hippocampus in older adults. *Med Image Comput Comput Assist Interv Int Conf Med Image Comput Comput Assist Interv, 9*, 58–66.

Gregory, A. M., Caspi, A., Moffitt, T. E., Koenen, K., Eley, T. C., & Poulton, R. (2007). Juvenile mental health histories of adults with anxiety disorders. *Am J Psychiatry, 164*, 301-308.

Grillon, C. (2002). Associative learning deficits increase symptoms of anxiety in humans. *Biol Psychiatry, 51*, 851-858.

Grogans, S. E., Bliss-Moreau, E., Buss, K. A., Clark, L. A., Fox, A. S., Keltner, D., Cowen, A. S., Kim, J. J., Kragel, P. A., MacLeod, C., Mobbs, D., Naragon-Gainey, K., Fullana, M. A., & Shackman, A. J. (2023). The nature and neurobiology of fear and anxiety: State of the science and opportunities for accelerating discovery. *Neuroscience & Biobehavioral Reviews, 151*, 105237.

Grogans, S. E., Hur, J., Barstead, M. G., Anderson, A. S., Islam, S., Kuhn, M., Tillman, R. M., Fox, A. S., Smith, J. F., DeYoung, K. A., & Shackman, A. J. (*under review*). Neuroticism/negative emotionality is associated with increased reactivity to uncertain threat in the bed nucleus of the stria terminalis, not the amygdala. *bioRxiv*.

Grupe, D. W., & Nitschke, J. B. (2013). Uncertainty and anticipation in anxiety: an integrated neurobiological and psychological perspective. *Nat Rev Neurosci, 14*, 488-501.

Heckman, J. J. (2006). Skill formation and the economics of investing in disadvantaged children. *Science, 312*, 1900-1902.

Heimberg, R. G., Hofmann, S. G., Liebowitz, M. R., Schneier, F. R., Smits, J. A., Stein, M. B., Hinton, D. E., & Craske, M. G. (2014). Social anxiety disorder in DSM-5. *Depression and anxiety, 31*(6), 472-479.

Henson, R. (2007). Efficient experimental design for fMRI. In K. Friston, J. Ashburner, S. Kiebel, T. Nichols, & W. Penny (Eds.), *Statistical parametric mapping: The analysis of functional brain images* (pp. 193-210). New York, NY: Academic Press.

Hunt, C., Cooper, S. E., Hartnell, M. P., & Lissek, S. (2017). Distraction/Suppression and Distress Endurance diminish the extent to which generalized conditioned fear is associated with maladaptive behavioral avoidance. *Behaviour Research & Therapy, 96*, 90-105.

Hur, J., Kaplan, C. M., Smith, J. F., Bradford, D. E., Fox, A. S., Curtin, J. J., & Shackman, A. J. (2018). Acute alcohol administration dampens central extended amygdala reactivity. *Scientific Reports, 8*, 16702.

Hur, J., Kuhn, M., Grogans, S. E., Anderson, A. S., Islam, S., Kim, H. C., Tillman, R. M., Fox, A. S., Smith, J. F., DeYoung, K. A., & Shackman, A. J. (2022). Anxiety-related frontocortical activity is associated with dampened stressor reactivity in the real world. *Psychological Science, 33*, 906-924.

Hur, J., Smith, J. F., DeYoung, K. A., Anderson, A. S., Kuang, J., Kim, H. C., Tillman, R. M., Kuhn, M., Fox, A. S., & Shackman, A. J. (2020). Anxiety and the neurobiology of temporally uncertain threat anticipation. *Journal of Neuroscience, 40*, 7949-7964.

Hyett, M. P., & McEvoy, P. M. (2018). Social anxiety disorder: looking back and moving forward. *Psychol Med, 48*, 1937-1944.

James, A. C., Reardon, T., Soler, A., James, G., & Creswell, C. (2020). Cognitive behavioural therapy for anxiety disorders in children and adolescents. *Cochrane Database Syst Rev, 11*, Cd013162.

Jarcho, J. M., Leibenluft, E., Walker, O. L., Fox, N. A., Pine, D. S., & Nelson, E. E. (2013). Neuroimaging studies of pediatric social anxiety: paradigms, pitfalls and a new direction for investigating the neural mechanisms. *Biol Mood Anxiety Disord, 3*, 14.

Jenkinson, M., Beckmann, C. F., Behrens, T. E., Woolrich, M. W., & Smith, S. M. (2012). FSL. *Neuroimage, 62*, 782-790.

Katzelnick, D. J., Kobak, K. A., DeLeire, T., Henk, H. J., Greist, J. H., Davidson, J. R., Schneier, F. R., Stein, M. B., & Helstad, C. P. (2001). Impact of generalized social anxiety disorder in managed care. *Am J Psychiatry, 158*, 1999-2007.

Kessler, R. C. (2003). The impairments caused by social phobia in the general population: implications for intervention. *Acta Psychiatr Scand, 108 (Suppl. 417)*, 19–27.

Kessler, R. C., Petukhova, M., Sampson, N. A., Zaslavsky, A. M., & Wittchen, H. U. (2012). Twelve-month and lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in the United States. *Int J Methods Psychiatr Res, 21*, 169-184.

Kim, H. C., Kaplan, C. M., Islam, S., Anderson, A. S., Piper, M. E., Bradford, D. E., Curtin, J. J., DeYoung, K. A., Smith, J. F., Fox, A. S., & Shackman, A. J. (2023). Acute nicotine abstinence amplifies subjective withdrawal symptoms and threat-evoked fear and anxiety, but not extended amygdala reactivity. *PLoS One, 18*, e0288544.

Kivity, Y., & Huppert, J. D. (2015). Emotional reactions to facial expressions in social anxiety: A meta-analysis of self-reports. *Emotion Review, 8*, 367-375.

Klein, Z., Shner-Livne, G., Danon-Kraun, S., Ginat-Frolich, R., Pine, D. S., & Shechner, T. (*in press*). Enhanced late positive potential to conditioned threat cue during delayed extinction in anxious youth. *Journal of Child Psychology and Psychiatry*.

Klumpp, H., & Fitzgerald, J. M. (2018). Neuroimaging predictors and mechanisms of treatment response in Social Anxiety Disorder: An overview of the amygdala. *Curr Psychiatry Rep, 20*, 89.

Koyuncu, A., İnce, E., Ertekin, E., & Tükel, R. (2019). Comorbidity in social anxiety disorder: diagnostic and therapeutic challenges. *Drugs Context, 8*, 212573.

Kruger, O., Shiozawa, T., Kreifelts, B., Scheffler, K., & Ethofer, T. (2015). Three distinct fiber pathways of the bed nucleus of the stria terminalis to the amygdala and prefrontal cortex. *Cortex, 66*, 60-68.

Lahey, B. B., Tiemeier, H., & Krueger, R. F. (2022). Seven reasons why binary diagnostic categories should be replaced with empirically sounder and less stigmatizing dimensions. *JCPP Advances, 2*, e12108.

LeDoux, J. E., & Pine, D. S. (2016). Using neuroscience to help understand fear and anxiety: A two-system framework. *Am J Psychiatry, 173*, 1083-1093.

Lee, F. S., Heimer, H., Giedd, J. N., Lein, E. S., Sestan, N., Weinberger, D. R., & Casey, B. J. (2014). Mental health. Adolescent mental health--opportunity and obligation. *Science, 346*, 547-549.

Lenaert, B., Boddez, Y., Griffith, J. W., Vervliet, B., Schruers, K., & Hermans, D. (2014). Aversive learning and generalization predict subclinical levels of anxiety: a six-month longitudinal study. *J Anxiety Disord, 28*, 747-753.

Lipsitz, J. D., & Schneier, F. R. (2000). Social phobia. Epidemiology and cost of illness. *PharmacoEconomics, 18*, 23-32.

Lorberbaum, J. P., Kose, S., Johnson, M. R., Arana, G. W., Sullivan, L. K., Hamner, M. B., Ballenger, J. C., Lydiard, R. B., Brodrick, P. S., Bohning, D. E., & George, M. S. (2004). Neural correlates of speech anticipatory anxiety in generalized social phobia. *Neuroreport, 15*, 2701-2705.

Ma, D. S., Correll, J., & Wittenbrink, B. (2015). The Chicago face database: A free stimulus set of faces and norming data. *Behav Res Methods, 47*(4), 1122-1135.

Makol, B. A., Youngstrom, E. A., Racz, S. J., Qasmieh, N., Glenn, L. E., & De Los Reyes, A. (2020). Integrating multiple informants' reports: How conceptual and measurement models may address long-standing problems in clinical decision-making. *Clinical Psychological Science, 8*, 953-970.

Makris, N., Goldstein, J. M., Kennedy, D., Hodge, S. M., Caviness, V. S., Faraone, S. V., Tsuang, M. T., & Seidman, L. J. (2006). Decreased volume of left and total anterior insular lobule in schizophrenia. *Schizophrenia Research, 83*, 155-171.

Mathew, A. R., Pettit, J. W., Lewinsohn, P. M., Seeley, J. R., & Roberts, R. E. (2011). Co-morbidity between major depressive disorder and anxiety disorders: shared etiology or direct causation? *Psychol Med, 41*, 2023-2034.

McCormick, M., Liu, X., Jomier, J., Marion, C., & Ibanez, L. (2014). ITK: enabling reproducible research and open science. *Frontiers in Neuroinformatics, 8*, 13.

McFarquhar, M., McKie, S., Emsley, R., Suckling, J., Elliott, R., & Williams, S. (2016). Multivariate and repeated measures (MRM): A new toolbox for dependent and multimodal group-level neuroimaging data. *Neuroimage, 132*, 373-389.

McGregor, T., Purves, K. L., Constantinou, E., Baas, J. M. P., Barry, T. J., Carr, E., Craske, M. G., Lester, K. J., Palaiologou, E., Breen, G., Young, K. S., & Eley, T. C. (2021). Large-scale remote fear conditioning: Demonstration of associations with anxiety using the FLARe smartphone app. *Depression and anxiety, 38*, 719-730.

Merikangas, K. R., Avenevoli, S., Acharyya, S., Zhang, H., & Angst, J. (2002). The spectrum of social phobia in the Zurich cohort study of young adults. *Biological Psychiatry, 51*, 81-91.

Michalska, K. J., Benson, B., Ivie, E. J., Sachs, J. F., Haller, S. P., Abend, R., McFarlin, D. R., Blackford, J. U., & Pine, D. S. (2023). Neural responding during uncertain threat anticipation in pediatric anxiety. *Int J Psychophysiol, 183*, 159-170.

NIMH. (2011). Negative valence systems: Workshop proceedings (March 13, 2011 – March 15, 2011; Rockville, Maryland). Retrieved from https://www.nimh.nih.gov/research/research-funded-by-nimh/rdoc/negative-valence-systems-workshop-proceedings.shtml

Petersen, A. C., Crockett, L., Richards, M., & Boxer, A. (1988). (1988). Pubertal Development Scale (PDS). *APA PsycTests*.

Poldrack, R. A., Baker, C. I., Durnez, J., Gorgolewski, K. J., Matthews, P. M., Munafo, M. R., Nichols, T. E., Poline, J. B., Vul, E., & Yarkoni, T. (2017). Scanning the horizon: towards transparent and reproducible neuroimaging research. *Nat Rev Neurosci, 18*, 115-126.

Pruim, R. H. R., Mennes, M., van Rooij, D., Llera, A., Buitelaar, J. K., & Beckmann, C. F. (2015). ICA-AROMA: a robust ICA-based strategy for removing motion artifacts from fMRI data. *Neuroimage, 112*, 267-277.

R Core Team. (2022). R: A language and environment for statistical computing. In. Vienna, Austria: R Foundation for Statistical Computing.

Rapee, R. M., McLellan, L. F., Carl, T., Trompeter, N., Hudson, J. L., Jones, M. P., & Wuthrich, V. M. (2023). Comparison of transdiagnostic treatment and specialized social anxiety treatment for children and adolescents with Social Anxiety Disorder: A randomized controlled trial. *J Am Acad Child Adolesc Psychiatry, 62*, 646-655.

Reinhard, J., Mittermeier, A., Brandstetter, L., Mowat, K., Slyschak, A., Reiter, A. M. F., Gamer, M., & Romanos, M. (*in press*). Fear conditioning and fear generalization in children and adolescents with anxiety disorders. *European Child & Adolescent Psychiatry*.

RStudio Team. (2022). RStudio: Integrated Development for R. In. Boston, MA: RStudio PBC.

Rubin, K. H., Barstead, M. G., Smith, K. A., & Bowker, J. C. (2018). Peer relations and the behaviorally inhibited child. In K. Pérez-Edgar & N. A. Fox (Eds.), *Behavioral Inhibition: Integrating theory, research, and clinical perspectives* (pp. 157-184). Cham, Switzerland: Springer-Nature.

Schneier, F. R., Blanco, C., Antia, S. X., & Liebowitz, M. R. (2002). The social anxiety spectrum. *Psychiatr Clin North Am, 25*, 757-774.

Schneier, F. R., Johnson, J., Hornig, C. D., Liebowitz, M. R., & Weissman, M. M. (1992). Social phobia: comorbidity and morbidity in an epidemiologic sample. *Archives of General Psychiatry, 49*(4), 282-288.

Scholten, W. D., Batelaan, N. M., Penninx, B. W., van Balkom, A. J., Smit, J. H., Schoevers, R. A., & van Oppen, P. (2016). Diagnostic instability of recurrence and the impact on recurrence rates in depressive and anxiety disorders. *J Affect Disord, 195*, 185-190.

Scholten, W. D., Batelaan, N. M., van Balkom, A. J., Wjh Penninx, B., Smit, J. H., & van Oppen, P. (2013). Recurrence of anxiety disorders and its predictors. *J Affect Disord, 147*, 180-185.

Sep, M. S. C., Steenmeijer, A., & Kennis, M. (2019). The relation between anxious personality traits and fear generalization in healthy subjects: A systematic review and meta-analysis. *Neurosci Biobehav Rev, 107*, 320-328.

Shackman, A. J., & Fox, A. S. (2016). Contributions of the central extended amygdala to fear and anxiety. *Journal of Neuroscience, 36*, 8050-8063.

Shackman, A. J., & Fox, A. S. (2018). Getting serious about variation: Lessons for clinical neuroscience. *Trends in cognitive sciences, 22*, 368-369.

Shackman, A. J., & Fox, A. S. (2021). Two decades of anxiety neuroimaging research: New insights and a look to the future *American Journal of Psychiatry, 178*, 106-109.

Shackman, A. J., Stockbridge, M. D., Tillman, R. M., Kaplan, C. M., Tromp, D. P. M., Fox, A. S., & Gamer, M. (2016). The neurobiology of anxiety and attentional biases to threat: Implications for understanding anxiety disorders in adults and youth. *Journal of Experimental Psychopathology, 7*, 311-342.

Sheehan, D. V., Sheehan, K. H., Shytle, R. D., Janavs, J., Bannon, Y., Rogers, J. E., Milo, K. M., Stock, S. L., & Wilkinson, B. (2010). Reliability and validity of the Mini International Neuropsychiatric Interview for Children and Adolescents (MINI-KID). *J Clin Psychiatry, 71*(3), 313-326.

Singewald, N., Sartori, S. B., Reif, A., & Holmes, A. (2023). Alleviating anxiety and taming trauma: Novel pharmacotherapeutics for anxiety disorders and posttraumatic stress disorder. *Neuropharmacology, 226*, 109418.

Smith, S. M., & Nichols, T. E. (2009). Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage, 44*(1), 83-98.

Spinhoven, P., Batelaan, N., Rhebergen, D., van Balkom, A., Schoevers, R., & Penninx, B. W. (2016). Prediction of 6-yr symptom course trajectories of anxiety disorders by diagnostic, clinical and psychological variables. *J Anxiety Disord, 44*, 92-101.

Stegmann, Y., Reicherts, P., Andreatta, M., Pauli, P., & Wieser, M. J. (2019). The effect of trait anxiety on attentional mechanisms in combined context and cue conditioning and extinction learning. *Sci Rep, 9*, 8855.

Stein, D. J., Lim, C. C. W., Roest, A. M., de Jonge, P., Aguilar-Gaxiola, S., Al-Hamzawi, A., Alonso, J., Benjet, C., Bromet, E. J., Bruffaerts, R., de Girolamo, G., Florescu, S., Gureje, O., Haro, J. M., Harris, M. G., He, Y., Hinkov, H., Horiguchi, I., Hu, C., Karam, A., Karam, E. G., Lee, S., Lepine, J.-P., Navarro-Mateu, F., Pennell, B.-E., Piazza, M., Posada-Villa, J., ten Have, M., Torres, Y., Viana, M. C., Wojtyniak, B., Xavier, M., Kessler, R. C., Scott, K. M., & WHO World Mental Health Survey Collaborators. (2017). The cross-national epidemiology of social anxiety disorder: Data from the World Mental Health Survey Initiative. *BMC Medicine, 15*, 143.

Straube, T., Mentzel, H. J., & Miltner, W. H. (2005). Common and distinct brain activation to threat and safety signals in social phobia. *Neuropsychobiology, 52*(3), 163-168.

Strawn, J. R., Lu, L., Peris, T. S., Levine, A., & Walkup, J. T. (2021). Pediatric anxiety disorders – what have we learnt in the last 10 years? *Journal of Child Psychology and Psychiatry, 62*, 114-139.

Tan, E., Zeytinoglu, S., Morales, S., Buzzell, G. A., Almas, A. N., Degnan, K. A., Chronis-Tuscano, A., Henderson, H., Pine, D. S., & Fox, N. A. (*in press*). Social versus non-social behavioral inhibition: Differential prediction from early childhood of long-term psychosocial outcomes. *Developmental Science*.

Taschereau-Dumouchel, V., Michel, M., Lau, H., Hofmann, S. G., & LeDoux, J. E. (2022). Putting the "mental" back in "mental disorders": a perspective from research on fear and anxiety. *Mol Psychiatry, 27*, 1322-1330.

Theiss, J. D., Ridgewell, C., McHugo, M., Heckers, S., & Blackford, J. U. (2017). Manual segmentation of the human bed nucleus of the stria terminalis using 3T MRI. *Neuroimage, 146*, 288-292.

Tiego, J., Martin, E., DeYoung, C. G., Hagan, K., Cooper, S. E., Pasion, R., Satchell, L., Shackman, A. J., Bellgrove, M. A., Fornito, A., & The HiTOP Neurobiological Foundations Work Group. (2023). Precision behavioral phenotyping as a strategy for uncovering the biological correlates of psychopathology. *Nature Mental Health, 1*, 304-315.

Tillfors, M., Furmark, T., Marteinsdottir, I., & Fredrikson, M. (2002). Cerebral blood flow during anticipation of public speaking in social phobia: a PET study. *Biol Psychiatry, 52*, 1113-1119.

Tillman, R. M., Stockbridge, M. D., Nacewicz, B. M., Torrisi, S., Fox, A. S., Smith, J. F., & Shackman, A. J. (2018). Intrinsic functional connectivity of the central extended amygdala. *Human Brain Mapping, 39*, 1291-1312.

Tustison, N. J., Avants, B. B., Cook, P. A., Zheng, Y. J., Egan, A., Yushkevich, P. A., & Gee, J. C. (2010). N4ITK: Improved N3 bias correction. *IEEE Transactions on Medical Imaging, 29*, 1310-1320.

van Meurs, B., Wiggert, N., Wicker, I., & Lissek, S. (2014). Maladaptive behavioral consequences of conditioned fear-generalization: a pronounced, yet sparsely studied, feature of anxiety pathology. *Behav Res Ther, 57*, 29-37.

Weeks, J. W., Howell, A. N., Srivastav, A., & Goldin, P. R. (2019). "Fear guides the eyes of the beholder": Assessing gaze avoidance in social anxiety disorder via covert eye tracking of dynamic social stimuli. *J Anxiety Disord, 65*, 56-63.

Wellcome Centre for Human Neuroimaging. (2022). SPM. Retrieved from https://fil.ion.ucl.ac.uk/spm/

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis* (2nd ed.). New York, NY: Springer-Verlag.

Worsley, K. J., Marrett, S., Neelin, P., Vandal, A. C., Friston, K. J., & Evans, A. C. (1996). A unified statistical approach for determining significant signals in images of cerebral activation. *Hum Brain Mapp, 4*(1), 58-73.

Yarrington, J. S., Vinograd, M., Williams, A. L., Wolitzky-Taylor, K. B., Zinbarg, R. E., Mineka, S., Waters, A. M., & Craske, M. G. (2022). Fear-potentiated startle predicts longitudinal change in transdiagnostic symptom dimensions of anxiety and depression. *Journal of Affective Disorders, 311*, 399-406.

Ziv, M., Goldin, P. R., Jazaieri, H., Hahn, K. S., & Gross, J. J. (2013). Is there less to social anxiety than meets the eye? Behavioral and neural responses to three socio-emotional tasks. *Biol Mood Anxiety Disord, 3*, 5.