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Anticipatory Threat Responding: Associations With Anxiety, Development, and Brain Structure

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ABSTRACT

BACKGROUND: While translational theories link neurodevelopmental changes in threat learning to pathological anxiety, findings from studies in patients inconsistently support these theories. This inconsistency may reflect difficulties in studying large patient samples with wide age ranges using consistent methods. A dearth of imaging data in patients further limits translational advances. We address these gaps through a psychophysiology and structural brain imaging study in a large sample of patients across the lifespan.

METHODS: A total of 351 participants (8–50 years of age; 209 female subjects; 195 healthy participants and 156 medication-free, treatment-seeking patients with anxiety) completed a differential threat conditioning and extinction paradigm that has been validated in pediatric and adult populations. Skin conductance response indexed psychophysiological response to conditioned (CS+, CS−) and unconditioned threat stimuli. Structural magnetic resonance imaging data were available for 250 participants. Analyses tested anxiety and age associations with psychophysiological response in addition to associations between psychophysiology and brain structure.

RESULTS: Regardless of age, patients and healthy comparison subjects demonstrated comparable differential threat conditioning and extinction. The magnitude of skin conductance response to both conditioned stimulus types differentiated patients from comparison subjects and covaried with dorsal prefrontal cortical thickness; structure–response associations were moderated by anxiety and age in several regions. Unconditioned responding was unrelated to anxiety and brain structure.

CONCLUSIONS: Rather than impaired threat learning, pathological anxiety involves heightened skin conductance response to potential but not immediately present threats; this anxiety-related potentiation of anticipatory responding also relates to variation in brain structure. These findings inform theoretical considerations by highlighting anticipatory response to potential threat in anxiety.

Keywords: Anticipation, Anxiety, Conditioning, Development, Extinction, Threat

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Translational theories link neurodevelopmental changes in threat learning to pathological anxiety (1–5). However, findings in patients inconsistently support these theories (3,6,7). Disconnect between theory and data may reflect difficulties in recruiting large patient samples, inconsistent methods across studies, and failure to examine wide age ranges within studies. Furthermore, few studies in patients relate physiology to brain measures, limiting translational advances across developmental stages. Here, we address these gaps by integrating psychophysiology with structural brain imaging to study threat learning in individuals with anxiety and healthy individuals spanning childhood, adolescence, and adulthood (n = 351; 8–50 years of age).

Threat learning encompasses conditioning and extinction. Conditioning is a highly conserved process through which a neutral stimulus becomes associated with a threat, such that subsequent encounters with the stimulus elicit anticipatory responding to the danger that might follow; extinction reflects the attenuation of conditioned threat responding when the stimulus no longer predicts the occurrence of threat (2,8–11). A core feature of pathological anxiety is an exaggerated fear of anticipated threats (12,13), and contemporary theories attribute anxiety to aberrant threat learning, which is conceptualized as rapid or exaggerated conditioning or impaired extinction (1,3,4,14). Other data suggest that developmental changes in threat learning contribute to the emergence of anxiety disorders in late childhood and early adolescence (2,5,15–18). Research guided by these theories aims to inform anxiety treatment (1,2,5,19–23).

While studies in nonhuman animals and healthy humans provide support for these theories (2,19,24,25), meta-analyses of studies comparing threat learning between healthy participants and participants with anxiety in both pediatric (6) and adult (3,7) samples yield mixed findings. Thus, all meta-analyses find evidence of perturbed threat learning but differ in the specific affected processes they

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identify. Such inconsistency generates a need for more data on associations among pathological anxiety, age, and threat learning (6,25,26). The current report addresses this need.

Paradigms model threat learning by pairing a neutral conditioned stimulus (CS) with an unconditioned threat stimulus (UCS) (11,27); with learning, the CS comes to elicit a conditioned response in anticipation of danger (9,13,14,28,29). Threat learning may therefore rely on both conditioned and unconditioned threat responding (30). While ample research focuses on anxiety-related differences in conditioned responding (3,6,7,13,31), fewer studies address aspects of unconditioned threat responding (13,31), particularly as such responding changes with development (25,32). Thus, comparing conditioned and unconditioned threat response among pediatric and adult anxiety patients and healthy volunteers addresses important gaps.

Finally, we extended insight from neuroanatomical data. Substantial research, particularly in healthy participants, links conditioned threat responding to structure and function in the prefrontal cortex (PFC), amygdala, and hippocampus (2,8,33–40). Moreover, other data suggest that age moderates the neural architecture of threat learning (2,25,41). However, no previous studies have related individual differences in brain structure to psychophysiological threat response measures that relate most strongly to anxiety disorders across age. We first identified such skin conductance measures and then identified their structural correlates. Finally, we evaluated the moderation of these associations between skin conductance response and brain structure by age and anxiety diagnosis.

To achieve these goals, we studied a large sample of children, adolescents, and adults with anxiety and similarly aged subjects without anxiety (n = 351). All participants completed a differential (i.e., involving both threat and safety learning) conditioning and extinction paradigm that had been previously validated in pediatric and adult populations (32,42); a subset (n = 250) completed structural imaging. Analyses proceeded in 3 stages, testing specific hypotheses arising from prior research. First, we tested anxiety and age effects on skin conductance response (SCR) indices of conditioning and extinction. Based on prior findings (3,6,7), we hypothesized that there are comparable differential conditioning and extinction effects in patients and comparison subjects but enhanced anxiety-related responding to both conditioned threat and safety cues during the task, in both youths and adults. Second, we examined response to unconditioned threat; given no prior reports of anxiety-related differences in UCS responding and the prominence of anticipatory fears in anxiety, we hypothesized that anxiety effects manifest more strongly in response to conditioned than to unconditioned threats. Finally, we examined correlations between brain structure (cortical thickness and gray matter volume [GMV]) and conditioned responding, and their moderation by anxiety and age. Given prior research on structure−SCR associations (19,36–38,40,43), we hypothesized that effects emerge in prefrontal regions as well as the amygdala and hippocampus. Primary hypothesis tests in all 3 areas considered SCR, given data on reliability (44), the ease with which SCR responses to the CS and UCS can be compared, and the availability of prior data on brain structure correlates of SCR. Secondary analyses examined anxiety and age effects on startle-probe–related electromyography (EMG) and self-reported fear.

METHODS AND MATERIALS

Participants

A total of 387 individuals underwent conditioning and extinction; analyses included n = 351 (Table 1 and Supplement), with 195 healthy participants (108 female subjects; 8–46 years of age) and 156 participants with anxiety (101 female subjects; 8–50 years of age) who did not differ in age, sex, or IQ, with all p values $>$.08. All participants were studied at the National Institute of Mental Health. Written informed consent was acquired from adult participants and from parents of youth participants, and written assent was acquired from youth participants for an institutional review board–approved protocol. Previously reported psychophysiology data for 162 participants (72 with anxiety, 90 healthy) (32,44) were combined with unpublished data for 189 participants to generate the sample (n = 351).

Anxiety Diagnosis. Psychiatric status was determined using structured interviews by trained clinicians. Pediatric patients met criteria for generalized anxiety, social anxiety, and/or separation anxiety disorder as the primary diagnosis and the presenting complaint for treatment. Adult patients were additionally eligible for panic disorder. Healthy participants were diagnosis free. See the Supplement.

Auxiliary analyses used standard anxiety symptom questionnaires. Youths and their parents completed the Screen for Child Anxiety Related Emotional Disorders (45), and adults completed the trait subscale of the State-Trait Anxiety Inventory (46). Data were combined by z scoring (see the Supplement).

Table 1. Sample Demographic and Clinical Characteristics[a]

Characteristic	Healthy Subjects, n = 195	Subjects With Anxiety, n = 156
Age, Years, Mean (SD)	21.46 (9.14)	19.78 (9.99)
IQ, WASI, Mean (SD)	114.30 (11.55)	114.31 (13.33)
Female, n (%)	108 (55.4)	101 (64.7)
Diagnosis, n (%)		
Generalized anxiety disorder	–	121 (77.6)
Social anxiety disorder	–	94 (60.3)
Separation anxiety disorder	–	29 (18.6)
Specific phobia	–	28 (17.9)
Panic disorder	–	11 (7.1)
Attention-deficit/hyperactivity disorder	–	5 (3.2)
Major depressive disorder	–	4 (2.5)
Selective mutism	–	2 (1.3)
Oppositional defiant disorder	–	1 (0.6)

WASI, Wechsler Abbreviated Scale of Intelligence.
[a]Total n = 351.

Threat Conditioning and Extinction Task

We used an uninstructed, differential threat learning task that had been previously shown to produce conditioning with acceptable dropout rates in youths and adults (18,26,32,42,44,47,48). In the task (Figure 1), photographs of 2 women displaying neutral expressions (49) served as CS+ and CS−. The UCS (presented at CS+ offset) was a 1-second presentation of the CS+ woman displaying fear co-occurring with a 95-dB female scream for all participants. The task involved 3 phases. During preconditioning, each CS appeared 4 times. During conditioning, each CS appeared 10 times; the CS+ was followed by the UCS with an 80% reinforcement schedule. During extinction, CSs each appeared 8 times. See the Supplement for additional details.

Psychophysiology. SCR was determined by the square-root-transformed difference in base-to-peak amplitude within 5 seconds after stimulus onset, in line with previous studies (32,35,44,47,50). Additionally, startle probes were delivered 5 to 6 seconds after stimulus onset, and response was measured using eye-blink startle EMG. Primary analyses used SCR; the Supplement provides EMG methods, results, and discussion on combining psychophysiology measures.

Subjective Fear Ratings. Before and following conditioning, and following extinction, participants rated their fear of the CSs using a 10-point Likert scale (1 = no fear, 10 = extreme fear) (32,47). These ratings complemented psychophysiological responses to the CSs.

Brain Imaging

Magnetic resonance images (1 mm^3) were collected for 250 of the participants (71%; 145 healthy [82 female subjects, mean age = 21.3 years]; 105 with anxiety [71 female subjects, mean age = 19.0 years]) in a separate visit. Data were processed using FreeSurfer (http://surfer.nmr.mgh.harvard.edu/). Analyses tested associations between structural imaging measures and psychophysiological indices and the moderation of these associations by anxiety and age using permutation tests (51). We considered whole-brain cortical thickness, using the threshold-free cluster enhancement statistic (52), and subcortical GMV. Magnetic resonance imaging data from 115 participants appear in previous reports that use different analyses (41,53). Analyses applied familywise error rate correction. See the Supplement for additional details.

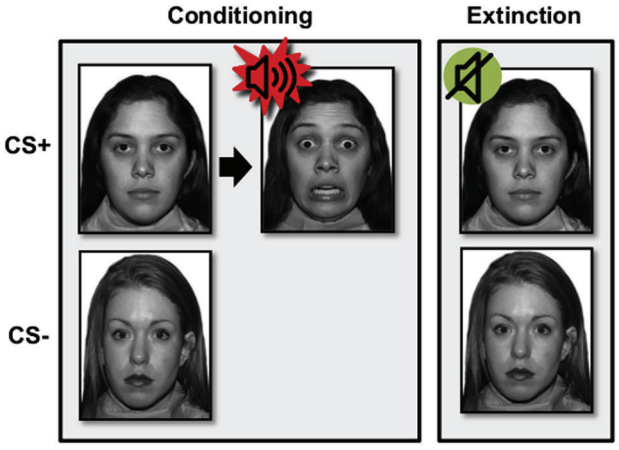

Figure 1. Schematic representation of the threat conditioning and extinction paradigm. During conditioning, one face (CS+) was repeatedly paired with a fearful face coterminating with a scream (unconditioned stimulus [UCS]); the other face (CS−) was never paired with the UCS. During extinction, both faces were presented in the absence of the UCS.

Data Analysis

First, we examined anxiety and age effects on differential threat conditioning and extinction through omnibus anxiety × age × phase × CS interactions on SCR to conditioned cues; trial-by-trial analyses complemented analyses on averaged SCR (11). In auxiliary analyses, EMG and self-reported data were analyzed in a similar manner. Second, effects on unconditioned responding were tested through the anxiety × age interaction on SCR to the UCS. Finally, we examined relationships between brain structure and SCR responses, with the primary analysis using the SCR measure that best differentiated healthy comparison subjects from patients, and moderation of structure−response relations by anxiety and age. To do so, we regressed SCR on cortical thickness and GMV measures. All analyses used general linear models, whereby anxiety status (with anxiety or healthy) was a between-subjects factor; age was a continuous covariate. Effect sizes are reported as η_p^2. All tests were 2-sided, and significance was set at $\alpha = .05$.

RESULTS

Response to Conditioned Cues

Skin Conductance Response. Averaged psychophysiological responding to conditioned cues by task phase is summarized in Figure 2A. Repeated-measures analysis of covariance testing the anxiety × age × phase × CS effect on averaged SCR yielded a significant phase × CS interaction, $F_{2,696} = 21.62$, $p < .001$, $\eta_p^2 = .06$, with follow-up paired-samples t tests indicating greater response to CS+ relative to CS− during conditioning, $t_{350} = 8.12$, $p < .001$, but not during preconditioning or extinction, p values $> .16$. This pattern indicates successful conditioning followed by extinction.

We also observed a main effect of anxiety on SCR, $F_{1,348} = 10.46$, $p = .001$, $\eta_p^2 = .03$, whereby patients exhibited greater mean response to the conditioned cues across the task relative to that of healthy control subjects. Further group comparisons indicated that patients generated stronger responses relative to that of control subjects to both CS− and CS+ in each task phase, and all p values were $< .032$.

Additionally, we noted a main effect of age, $F_{1,348} = 83.90$, $p < .001$, $\eta_p^2 = .19$, indicating decreasing response with increasing age. This effect was qualified by an age × phase × CS interaction, $F_{2,696} = 7.77$, $p < .001$, $\eta_p^2 = .02$. Follow-up analyses yielded a significant age × CS interaction during conditioning, $F_{1,348} = 27.91$, $p < .001$, $\eta_p^2 = .07$, indicating decreased differential conditioning with age but no age differences during preconditioning or extinction, p values $> .86$.

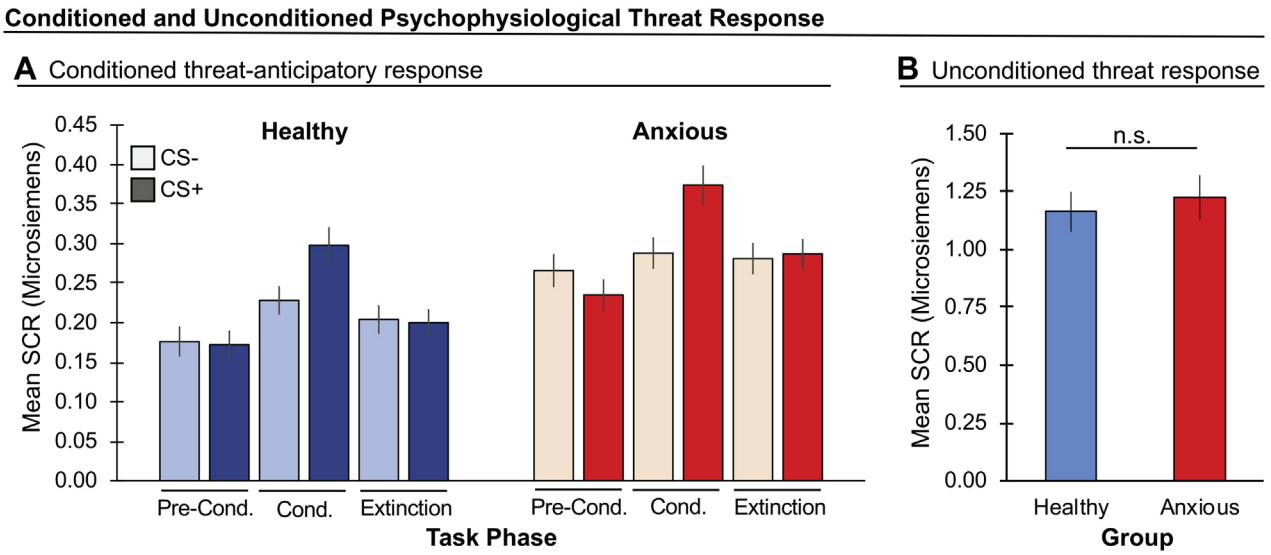

Figure 2. Conditioned and unconditioned psychophysiological threat response. **(A)** Conditioned skin conductance responses (SCRs) by stimulus type (CS−, CS+) averaged across each phase of the task (preconditioning, conditioning, extinction), by anxiety group (healthy or with anxiety). **(B)** Averaged SCR to the unconditioned stimulus by anxiety group (healthy or with anxiety). SCR data were square-root-transformed microsiemens. Error bars represent 1 standard error of the mean. Cond., Conditioning; n.s., not significant; Pre-Cond., preconditioning.

No significant anxiety interaction effects emerged, either across the task or separately during conditioning or extinction, indicating no difference in differential threat learning processes. Additional trial-by-trial SCR analyses are reported in the Supplement, indicating similar findings. A complementary dimensional analysis of anxiety-symptom severity indicated a significant positive association between symptoms and averaged SCR to the conditioned cues, $r = .14$, $p = .010$.

EMG analyses appear in the Supplement. These indicate two notable nonsignificant effects: phase × CS interaction ($p = .061$, CS+ > CS− only during conditioning and extinction) and anxiety main effect ($p = .090$, anxiety > healthy).

Self-reported Fear. Analyses of subjective fear responses are reported in the Supplement. Subjective fear paralleled SCR, demonstrating increased fear of CS+ relative to that of CS− following conditioning that was diminished following extinction. Moreover, fear of CS+ following conditioning correlated positively with the magnitude of SCR to CS+, supporting convergence of subjective and psychophysiological measures. Finally, as with SCR, we noted a main effect of anxiety on fear reports, indicating greater fear of conditioned cues throughout the task but no anxiety interaction effects.

Brain Structure Correlates. Since anxiety group differences emerged in SCR responding across CSs and task phases, averaged SCR across CSs and phases was used to index conditioned responding. Analyses tested the main effect of cortical thickness, as well as moderation by anxiety and age using the 3-way thickness × anxiety × age interaction, in predicting the magnitude of conditioned response. These analyses indicated a significant association between cortical thickness and conditioned response (controlling for age and anxiety) in a left-hemisphere cluster extending from the dorsomedial to dorsolateral PFC (Figure 3A, Table 2), whereby less thickness predicted greater conditioned response. Another cluster in the left retrosplenial cortex demonstrated a positive association between thickness and conditioned response. Furthermore, anxiety moderated the association between cortical thickness and conditioned responding in the bilateral ventral occipital cortex (Figure 3B) such that patients exhibited a more positive thickness–SCR association in this region. Finally, age moderated the thickness–response associations in several clusters (Figure 3C), including the bilateral posterior insula and temporal occipital cortex, right midcingulate cortex, and left middle-frontal gyrus. Analysis of GMV revealed age moderation in the bilateral hippocampus. The effect of age was consistent across all regions, such that among younger participants, thicker cortex or greater GMV was positively associated with conditioned responding, but with age this association became negative.

In summary, analyses of psychophysiological responses indicate comparable threat conditioning and extinction between patients and healthy control subjects. Compared with control subjects, patients demonstrated increased conditioned SCR responding to both CS− and CS+. The magnitude of this responding was inversely related to dorsal PFC thickness; anxiety and age moderation effects emerged in other cortical regions as well as bilateral hippocampus.

Response to UCS

Skin Conductance Response. Analysis of SCR to the UCS indicated comparable response to the unconditioned threat stimulus in the patient and healthy groups, $F_{1,348} = 0.24$, $p = .62$, $\eta_p^2 < .01$ (Figure 2B). We noted a significant main effect of age, $F_{1,348} = 34.29$, $p < .001$, $\eta_p^2 = .02$, indicating

Structural Correlates of Conditioned Response

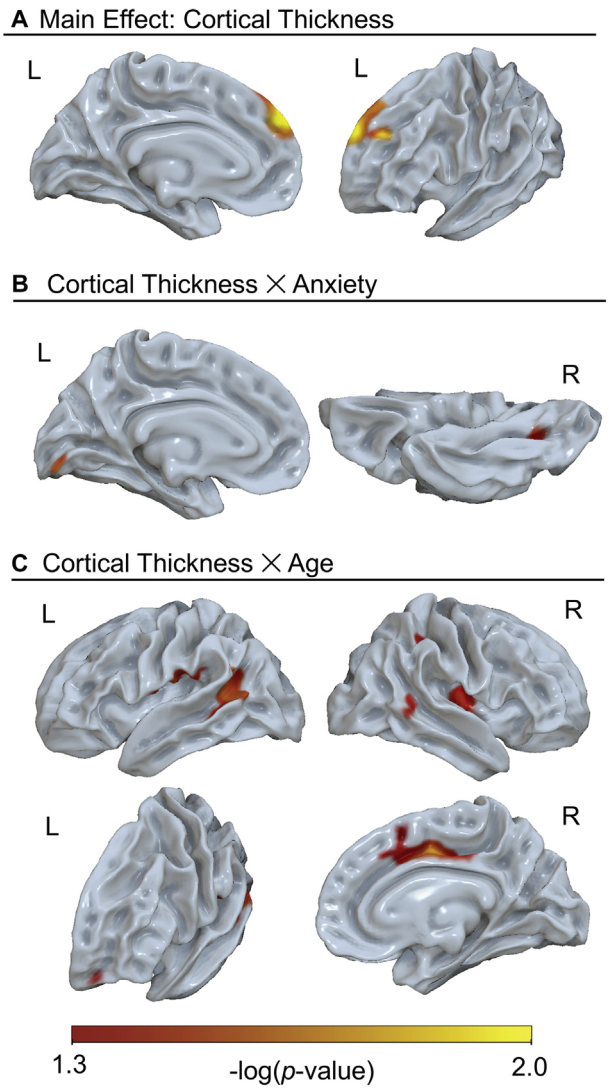

Figure 3. Brain structure correlates of psychophysiological response to conditioned cues. Result of analysis predicting individual averaged skin conductance responses to conditioned cues (CS−, CS+) across the task by cortical thickness, anxiety status (healthy or with anxiety), and age (in years). **(A)** Association between cortical thickness and conditioned response, controlling for anxiety status and age. **(B)** Moderation of association between cortical thickness and conditioned response by anxiety status. **(C)** Moderation of association between cortical thickness and conditioned response by age. Each surface's color reflects −log(*p* value) of the threshold-free cluster enhancement statistic; brighter colors represent stronger effects (threshold: $p_{FWE} < .05$). FWE, familywise error; L, left; R, right.

decreasing response with increasing age. Additional trial-by-trial analyses indicated diminishing unconditioned response across trials and with age (Supplement; Supplemental Figure S1B), but no anxiety effects. Of note, these analyses indicate that absence of anxiety effects on unconditioned responding is not due to ceiling effects.

An auxiliary analysis directly comparing averaged SCR to the conditioned cues with averaged SCR to the UCS within a single model yielded a significant stimulus × anxiety interaction, $F_{1,348} = 7.86$, $p = .005$, $\eta_p^2 = .02$, further indicating that the anxiety effect on response was specific to increased conditioned but not unconditioned responding. Dimensional analysis indicated that symptom severity and averaged SCR to the UCS were not correlated, $r_{319} = .03$, $p = .65$.

Self-reported Fear. Full statistics are provided in the Supplement. Subjective fear of the conditioned cues did not depend on the magnitude of unconditioned response to threat.

Brain Structure Correlates. Analyses of cortical thickness and GMV indicated no significant association, either direct or moderated by anxiety or age, between variation in brain structure measures and magnitude of unconditioned response.

In summary, the magnitude of unconditioned threat responses diminished with age. However, unlike anticipatory responses to the conditioned cues, response to the unconditioned stimulus did not differ as a function of anxiety, did not relate to conditioned subjective fear, and did not relate to variation in brain structure.

DISCUSSION

This study examined the associations that anxiety exhibits with conditioned and unconditioned threat responding across age. Three key findings emerged. First, as hypothesized, across age, patients with anxiety and healthy comparison subjects demonstrated comparable differential threat conditioning and extinction. Second, despite intact threat learning, the magnitude of conditioned SCR responding was greater to both CS− and CS+ in patients relative to that in healthy comparison subjects. The magnitude of such responding also covaried with subjective fear of the conditioned cues and brain structure in several hypothesized regions. Third, the magnitude of unconditioned psychophysiological responding did not relate to anxiety status, subjective fear of conditioned cues, or variation in brain structure. Together, these findings suggest that differential threat learning remains intact in pathological anxiety. Instead, anxiety involves heightened SCR to both CS+ and CS− but not UCS; the magnitude of such diagnosis-related SCRs also correlates with variation in brain structure.

This study is the largest single report comparing threat conditioning and extinction between patients with anxiety and healthy comparison subjects across development. The findings of comparable differential threat conditioning and extinction in patients and comparison subjects is consistent with prior meta-analyses (3,6,7). Thus, findings do not unequivocally support theories that relate anxiety to aberrant threat conditioning or extinction (3,4). Moreover, the use of a single, established paradigm informs theories on development and anxiety (2,6,25). Importantly, age did not moderate anxiety effects on these processes.

Instead, our findings highlight greater responding to both conditioned cues as differentiating participants with anxiety from healthy participants. This finding is consistent with previous findings generated in separate studies among youths

Table 2. Location, Peak Significance Level[a], and Size[b] of Clusters Showing Significant Associations Between Cortical Thickness or Gray Matter Volume and Magnitude of Conditioned Psychophysiological Response

Effect	Location	Peak *p* Value[c], FWE-Corrected	Cluster Size, No. of Vertices
Cortical Thickness	L dmPFC–dlPFC	.009	115
	L retrosplenial cortex	.021	27
Cortical Thickness × Anxiety	L visual association cortex	.029	16
	R visual association cortex	.039	15
Cortical Thickness × Age	R midcingulate cortex	.019	173
	L temporo-occipital cortex	.025	156
	L posterior insula	.025	146
	R posterior insula	.035	92
	R temporo-occipital cortex	.042	40
	R parieto-occipital cortex	.046	22
	L visual association cortex	.046	9
	L ventral medial frontal gyrus	.047	8
Gray Matter Volume × Age	R hippocampus	.017	–
	L hippocampus	.033	–

dlPFC, dorsolateral prefrontal cortex; dmPFC, dorsomedial prefrontal cortex; FWE, familywise error; L, left; R, right.
[a]$p_{FWE} < .05$.
[b]Number of vertices.
[c]For gray matter volume, *p* values refer to the entire structure. For cortical thickness, *p* values refer to the threshold-free cluster enhancement statistic.

and adults demonstrating anxiety-related enhancement of responding to CS−, CS+, or both (3,6,7). Greater response to CS− has been hypothesized to reflect anxiety-related aberrations in safety learning (54–56); greater response to CS+ has been hypothesized to reflect enhanced threat learning or reduced fear extinction (3,4,57). Both patterns emphasize some form of perturbed learning, and neither hypothesis is fully supported by our data. This finding calls for alternative explanations of the observed patterns.

A notable finding from the current study stems from comparing responses to the unconditioned stimulus. This finding indicates that enhanced SCRs in patients do not occur in response to the UCS, suggesting that there is perturbed anticipatory responding as opposed to acute-threat responding. This distinction could arise from differences in the function of conditioned and unconditioned responses to threat. Unconditioned threat stimuli signal unambiguous, immediate danger; as such, they elicit reflexive defensive responses that require minimal computation to execute (9,29). In contrast, conditioned stimuli predict only the potential for, as opposed to immediate occurrence of, danger; such prediction may be influenced by multiple processes that jointly estimate the probability, magnitude, or proximity of danger and accordingly influence adaptive defensive responding (55,58,59). Excessive conditioned responding may reflect perturbations in any of these processes, each involving biased threat estimates in response to any stimulus that predicts danger.

The uninstructed and probabilistic nature of the conditioning schedule used here may have led participants with anxiety relative to healthy participants to view both CS+ and CS− as conveying relatively high levels of danger, leading to greater anticipatory responses to both cues in patients (55,58). Such an effect was also observed prior to the presentation of the first UCS, whereby patients demonstrated greater responding to the initial face presentations during preconditioning (Supplement). As participants were aware of the aversive nature of the paradigm, patients may have shown increased anticipatory responding to the first stimuli presented in the task; this group difference diminished during preconditioning as the stimuli were continually nonreinforced.

Several brain structure correlates of anticipatory threat response were identified. Analyses specifically examined correlations with SCR measures that differentiated patients from healthy comparison subjects, to inform understanding of clinical psychophysiological correlates. Less left dorsomedial PFC and left dorsolateral PFC thickness was associated with greater anticipatory psychophysiological responding. Considerable functional imaging literature implicates these regions, particularly left-sided ones, in threat learning (33,34). Furthermore, other work suggests that altered function or structure in these regions contributes to maladaptive anticipation (55,60–62) and emotion regulation (63,64) processes. Our results bridge these findings, offering the possibility that dorsomedial PFC and dorsolateral PFC support effective regulation of anticipatory responses to potential danger. Indeed, preliminary findings from lesioned patients also support this possibility (65).

A positive association emerged between cortical thickness in left retrosplenial cortex and conditioned anticipatory response. The retrosplenial cortex is one of several regions implicated in threat conditioning (33), and it is suggested that it mediates the encoding of episodic or contextual memory of CS–UCS associations (66,67). Our results extend these findings by showing that structural variation in this region relates directly to the expression of conditioned psychophysiological responses indicative of diagnostic differences in physiology.

Additionally, the association between thickness in bilateral clusters in ventral visual association areas and anticipatory response varied with anxiety status. Prior research links structure and function in the occipital cortex to anxiety

disorder clinical features and treatment response (41,68–71). Reciprocal connections between the amygdala and visual cortex may account for such findings, as these connections are thought to facilitate the processing of biologically relevant stimuli in the context of threat conditioning (72–76). Our findings add to this literature, potentially linking patients' increased psychophysiological responding to visual threat stimuli to perturbations in cortical regions mediating visual processing. Additional research using conditioned stimuli of other modalities is needed to explore the specificity of this effect.

Age-dependent associations emerged between several structures and individual differences in conditioned responding. The consistent pattern of age moderation suggests that a group of regions may constitute a network supporting threat anticipation processes in ways that change with development. Broadly, other work finds these regions to show relatively protracted maturation with age (77,78). Some data suggest that the midcingulate cortex, particularly its anterior extent, acts as a key hub in networks mediating threat conditioning, modulation of negative affect, and anticipation (33,55,79). Consistent with these prior findings, our data may suggest that the midcingulate cortex supports anticipatory responding to threat. Similarly, prior functional and structural imaging work relates posterior insula to threat conditioning (33,37); this region has also been linked to the integration of interoceptive information (80). Given such prior work, our data also implicate the posterior insula in conditioned anticipatory preparation for harm. Finally, associations between GMV and anticipatory responding were also observed in bilateral hippocampus, a structure implicated in threat learning processes, potentially via context representation (33,39,81). As thinning in these regions is associated with greater anticipatory response, it is possible that some of their functions are regulatory, involving integration of somatic, affective, and contextual information. Nevertheless, it is important to emphasize that brain structure might not directly map onto function (82); thus, inferences on functional roles for these regions are limited. Longitudinal studies focusing on both the structure and function of these regions are necessary to more completely understand age moderation of associations among brain structure, function, and conditioned response to threat.

Of note, our findings suggest that neither aberrant differential conditioning nor extinction exhibits strong, direct associations with pathological anxiety across development. Nevertheless, it remains possible that more-nuanced anxiety differences in differential learning of anticipatory threat responses exist. One possibility is that analytical challenges in capturing dynamic learning processes mask such subtle differences. Methods that directly model associative learning processes (14,83,84) may be more powerful in identifying such differences. Alternatively, it has been suggested that other effects that derive from threat learning, such as tests of extinction recall or generalization of learned threat, may better capture anxiety deficits (19,26,56,85,86). Such effects may also reflect the elicitation of anticipatory responses (e.g., to generalized stimuli) and could prove valuable avenues for research linking anxiety, anticipation, and response to learned threat.

Exaggerated fear of potential danger is a core feature in the presentation of anxiety symptoms. Here, we identify a potential psychophysiological correlate of this maladaptive anticipatory fear response. Importantly, the magnitude of the anticipatory response differentiated between patients and healthy comparison subjects but also correlated with reported fear of the conditioned cues, thereby linking psychophysiological and subjective fear responses to potential threat. As such, this paradigm provides an experimental setting primed for uncovering the nature of associations among anticipatory psychophysiological responding, subjective fear, and anxiety symptoms. Follow-up studies could use repeated assessments of conditioned fear alongside anxiety ratings embedded in the threat learning paradigm to examine how anticipatory psychophysiological responses and subjective fear might interact to contribute to the experience of anxiety symptoms (10,26,87–89).

Along these lines, identifying a psychophysiological correlate of a pathological process in anxiety could potentially inform treatment development (5,90). For example, increased anticipatory psychophysiological response could serve as a specific target for interventions, such as particular forms of cognitive behavioral therapy and biofeedback techniques, that aim to directly reduce physiological arousal. Future research could explore whether neuroscience-guided interventions, such as brain stimulation methods and neurofeedback (91,92), could potentially downregulate neural processes mediating anticipatory responses or upregulate regulatory processes. Given the absence of anxiety differences in response to the unconditioned acute-threat stimulus, psychotherapy and cognitive behavioral therapy might focus on addressing anticipation-focused cognition and somatic responses. Additional research could further explore whether the magnitude of anticipatory psychophysiological response could serve as a biomarker for anxiety treatment outcome.

Several limitations should be acknowledged. First, this was a cross-sectional study, limiting the extent of inference about causality; a longitudinal design would allow stronger inferences about developmental and causal processes (93). Second, this study was not designed to directly link individual differences in threat learning and treatment outcome, thus limiting the scope of therapeutically relevant inference. Third, establishing baseline (11) for SCR to UCS is inherently challenging because of potential anticipation effects once associations have been learned. Here, CS+ and UCS events were separated by an adequate duration, as recommended (11); nevertheless, future research should consider this issue. Fourth, since brain structure variably maps onto function (82), inference on the functional role of identified brain regions is limited. Fifth, we measured both SCR and EMG; the use of multiple psychophysiological indicator variables could interfere with their indexing of the target processes (94). Sixth, we used a paradigm that is well suited for developmental research but uses a preset volume level for all participants; this limits comparison with prior studies in adults in which UCS aversiveness was set individually.

Several strengths mitigate these limitations and address general shortcomings in threat learning research (11,27). First, the large sample size increases precision in the estimates of associations (95). Second, participants were carefully assessed and free of medications known to impact threat learning and psychophysiology (11). Third, a wide age range

generates inferences on age differences with reasonable statistical power. Finally, task and setting were identical for all participants, reducing measurement confounds and noise.

In summary, the current study examined associations among conditioned and unconditioned responses to threat, anxiety, and age. Our findings highlight anticipatory threat responding as differentiating between patients and healthy control subjects and identify brain structure correlates of this response. These findings may bear implications for our conceptualization of anxiety and its treatment and study.

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ARTICLE INFORMATION

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