Biological Psychiatry: CNNI

Archival Report

Threat Neurocircuitry Predicts the Development of Anxiety and Depression Symptoms in a Longitudinal Study

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ABSTRACT

BACKGROUND: Owing to high heterogeneity and comorbidity, the shared and unique neural mechanisms underlying the development of anxiety and major depressive disorders remain unclear. Using a dimensional model describing shared versus unique symptoms associated with anxiety and depression, this study investigated how longitudinal changes in symptom dimensions relate to threat neurocircuitry.

METHODS: Participants were 18- to 19-year-olds (N = 279, 186 females) who completed self-report measures of anxiety and depression at baseline and at 10, 20, and 30 months. Linear slopes of symptom dimensions of general distress, fear, and anhedonia-apprehension were estimated through a trilevel factorial model. In addition, functional magnetic resonance imaging scans were obtained while participants performed Pavlovian fear conditioning tasks at baseline and 30 months, including three phases of fear acquisition, extinction, and extinction recall. Neural responses in regions of interest related to threat neural circuitry (e.g., amygdala, ventromedial prefrontal cortex, and subgenual anterior cingulate cortex) were extracted.

RESULTS: Linear mixed models used to estimate relationships between changes of symptom dimensions and neural responses revealed two major findings: 1) greater neural responses to threatening stimuli during fear acquisition at baseline were associated with a greater increase in fear symptoms during the 30-month prospective period; and 2) elevated neural responses to the extinguished stimulus during extinction recall at 30 months were negatively associated with changes in general distress, suggesting that greater increases in general distress are associated with larger deficits in extinction memory.

CONCLUSIONS: These findings improve our understanding of pathophysiological pathways underlying the development of anxiety and depression, while separating symptom dimensions that are shared versus unique between the two disorders.

https://doi.org/10.1016/j.bpsc.2021.12.013

Anxiety and major depressive disorders are among the most common and debilitating psychiatric illnesses. However, owing to high heterogeneity and comorbidity, the shared and unique neurobiological mechanisms underlying anxiety and major depressive disorders remain unclear. For anxiety disorders, studies in both humans and animals suggest that maladaptive fear learning is a central mechanism in illness development and treatment (1–6). At the neural level, anxiety is associated with dysregulation in regions of threat neurocircuitry, including the amygdala, insula, hippocampus, ventromedial prefrontal cortex (vmPFC), and anterior cingulate cortex (ACC) (7–10), which are critical for learning threat contingencies (11–17). For example, heightened activation in the amygdala, dorsal ACC, and insula in response to threat cues is associated with anxiety (18), increased vmPFC activity in response to extinguished cues is thought to partially underlie extinction learning (11,19–21), and impaired contextual encoding of memories in the hippocampus is associated with overgeneralized fear learning (20). Depression has also been found to be associated with disrupted functioning of threat neurocircuitry, particularly in the amygdala, ACC, and vmPFC (10,22–25).

The anatomical overlap in disrupted neural functioning may partially explain the high comorbidity between anxiety disorders and depression. Nevertheless, it is also possible that the functional neural dysregulation uniquely associated with each disorder has been clouded by overlapping symptoms common to both diagnostic categories. Disentangling the unique and shared neural dysregulation underlying anxiety and depression is of importance for understanding pathophysiological pathways to anxiety and depression and developing more objective clinical characterization and effective treatments that incorporate individual differences.

Dimensional models of anxiety and depression, which reduce large multidimensional sets of symptom data to smaller sets of latent variables using methods such as principal component or factor analysis (26,27), offer a potential solution

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for delineating shared and unique symptom clusters across psychiatric illnesses without being constrained by traditional diagnostic groupings (28). Such dimensional models may elucidate shared and unique neurobiological substrates of anxiety and depression (28). However, there have been challenges in relating the latent constructs from dimensional models of self-reported symptom measures to outcomes at other levels of measurement such as the brain, physiology, or behavior (29,30). As a result, evidence tying separable symptom dimensions of anxiety and depression to specific neural circuits is lacking.

Recent progress was made by examining the relationship between a previously established dimensional model of anxiety and depression (26,31–33) and neural activity during a widely used fear conditioning paradigm (20) in young adults (n = 229) (32). The trilevel dimensional model, which has been validated in both healthy (26,32,33) and clinical (31) samples, identifies three clusters of symptoms: a broad factor, general distress, representing negative affect that is common to anxiety and depression; and two intermediate factors, fears and anhedonia-apprehension, that are associated with subsets of anxious and depressive symptoms, respectively. Results showed that the anhedonia-apprehension factor was associated with activation of the bilateral amygdala, anterior insula, and dorsal ACC during late fear extinction, thus identifying potentially important neural markers that may be unique to depression (32).

Herein, we analyzed neural and symptom data collected from the same participants (32) at three additional time points extending over 30 months. Using data from (32) as a baseline, we investigated potential longitudinal associations between neural responses during fear conditioning and the same latent factors of general distress, fears, and anhedonia-apprehension. Whereas previous evidence has revealed longitudinal associations between the trilevel factors and outcomes such as neuroticism (34,35) and negative life events (36), there has been a call in the field to further map these longitudinal effects to changes in the brain (37).

With the current longitudinal approach, we addressed covariation between changes in the brain and symptom profiles and investigated several open questions regarding the association between the anhedonia-apprehension symptom dimension and neural responses during fear extinction observed at baseline (32). Does the association persist over time? What is the related directionality; does neurobiological disruption precede symptom onset? Similarly, does neural threat circuitry represent a malleable target for preventing the development of psychopathology? The identification of such long-term associations would represent a step forward for psychiatric classification approaches based on observable symptoms and neural signatures.

METHODS AND MATERIALS

Sampling and Participants

At baseline, 279 participants, aged 18 to 19 years (186 females, mean age = 19.65 years, SD = 0.53) (Table 1) were recruited at the University of California Los Angeles and Northwestern University. Participants were selected from a larger screening sample of 2461 individuals to represent a broad range of scores on self-reported trait neuroticism and reward sensitivity to maximize variance in threat- and reward-related sensitivity. All participants provided written informed consent. Of the 279 participants included at baseline, 273 completed Structured Clinical Interview for DSM-5 interviews. A total of 64 participants (23.4%) met criteria for a current anxiety disorder but no depressive disorder, 19 (6.96%) met criteria for current anxiety and depressive disorders, and 4 (1.47%) met criteria for a depressive disorder but no anxiety disorder. In the final analyses, at baseline, n = 273 had usable data for fear acquisition, n = 271 for fear extinction, and n = 265 for extinction recall. Only a subset of participants was contacted to conduct the neuroimaging measurement at 30 months due to budget limits, yielding n = 150 usable data for fear acquisition, n = 149 for fear extinction, and n = 146 for extinction recall. Results from the baseline time point of this dataset have been published in only one previous study (32).

Procedures

As shown in Figure 1, multidimensional self-reported symptoms of the trilevel model were assessed at baseline (T1) and 10, 20, and 30 months (T2, T3, and T4, respectively). Overall, 157 participants finished T4 measurements. Neural responses

Table 1. Sample Characteristics of Subjects Enrolled at Each Site at Baseline

Characteristic	All, N = 279	UCLA, n = 132	NU, n = 147
Age, Years, Mean (SD)	19.65 (0.53)	19.57 (0.53)	19.72 (0.52)
Women, n (%)	186 (66.7%)	87 (66%)	99 (67.3%)
Race/Ethnicity, n (%)			
Hispanic/Latino	73 (26.2%)	39 (29.5%)	34 (23.1%)
Non-Hispanic Asian	75 (26.9%)	47 (35.6%)	28 (19%)
Non-Hispanic White	93 (33.3%)	37 (28%)	56 (38.1%)
Other	38 (13.6%)	9 (6.8%)	29 (19.7%)
SCID-5, n	273	132	142
Comorbid Anxiety and Depression, n (%)	19 (6.96%)	10 (7.6%)	9 (6.3%)
Anxiety Disorder, n (%)	64 (23.4%)	29 (22%)	35 (24.6%)
Depressive Disorder, n (%)	4 (1.47%)	3 (2.3%)	1 (0.7%)

NU, Northwestern University; SCID-5, Structured Clinical Interview for DSM-5; UCLA, University of California Los Angeles.

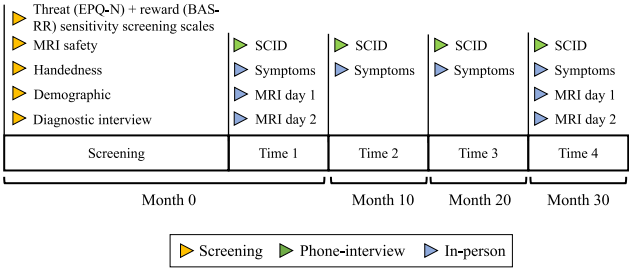

Figure 1. Timeline of longitudinal examination. After the screening stage, participants were measured across four time points. Multidimensional self-reported symptoms of the trilevel model were assessed at baseline and 10, 20, and 30 months. Functional magnetic resonance imaging (MRI) scans during a Pavlovian fear conditioning task were collected at baseline and 30 months. BAS-RR, Behavioral Action Scale-Reward Responsiveness; EPQ-N, Eynsenck Personality Questionnaire-Neuroticism; SCID, Structured Clinical Interview for DSM-5.

during a Pavlovian fear conditioning task were assessed only at baseline and 30 months. For details of screening, inclusion and exclusion criteria, numbers of participants included at each stage, handling of missing data, and comparisons between sample characteristics of participants who participated at T4 and those who did not, see the Supplement.

Trilevel Symptom Assessment and Factor Analysis. Symptom assessments included a subset of items from the following self-report measures of anxiety and depression (26,31): Fear Survey Schedule-II (38), Albany Panic and Phobia Questionnaire (39), Self-Consciousness Subscale of the Social Phobia Scale (40,41), Inventory to Diagnose Depression (42), Mood and Anxiety Symptom Questionnaire (43), Penn State Worry Questionnaire (44), and Obsessive-Compulsive Inventory Revised (45). The subset of items from these questionnaires was selected to derive empirically established factor scores of symptom dimensions (15,17,26). Confirmatory factor analyses demonstrated the goodness of fit of the trilevel model to the baseline data collected in this study (33). The factor estimates from this model were saved and used to represent the trilevel model symptom dimensions of general distress, fears, and anhedonia-apprehension. Changes in symptom dimensions over the 30 months were estimated through linear regression models, where for each subject, a linear model was fit on the four time points of trilevel model estimates for each symptom dimension, and slope values were extracted for use in subsequent regression analyses. For distributions of slopes, see Figure S4.

Fear Conditioning Paradigm in Functional Magnetic Resonance Imaging. At baseline and 30 months, participants completed a differential Pavlovian fear conditioning task following a previous protocol (20). Three phases were conducted over two functional magnetic resonance imaging (fMRI) scanning sessions: acquisition, extinction (session 1), and extinction recall (session 2). See the Supplement for details of the task. Galvanic skin conductance was recorded throughout the task. At the end of acquisition and extinction, contingency awareness assessments examined whether participants had correctly formed conditioned stimulus (CS)–unconditioned stimulus (US) associations [see the Supplement for details and recommendations for optimizing the task as proposed in (46)].

Data Processing and Analysis

fMRI Acquisition and Analysis. High-resolution structural (T1-weighted) images and blood oxygenation level–dependent (T2*-weighted) functional images were acquired and preprocessing procedures were applied (see the Supplement). First-level analyses included regressors of interest (acquisition: context, CS+, CS−, and shock; extinction: context, CS+extinguished [E], CS−; recall: context, CS+E, CS+unextinguished [U], CS−), temporal derivatives, six motion regressors, and regressors to censor outlying volumes.

Region-of-interest (ROI) analyses were conducted on anatomical ROIs (Harvard-Oxford atlas), including the amygdala, hippocampus, anterior insula, dorsal ACC, subgenual ACC (sgACC), and bed nucleus of the stria terminalis (as used in prior research) (47), and functional vmPFC ROIs, defined as spheres (5-mm radius) around peak activations reported in a meta-analysis of human fear conditioning (13). These were the same ROIs used in the previous study of the current baseline data (32); they were selected to target neural regions that have been previously associated with fear acquisition and extinction (7–9,11,18–20).

Hierarchical Mixed-Effect Models. Hierarchical mixed-effect models were carried out in R to examine the associations between symptom dimensions and activations of threat circuitry. Analyses were conducted separately for each phase of fear conditioning with the following contrasts: acquisition, CS+ versus CS− (all trials); extinction, late CS+E versus late CS− (last four trials); and recall, early CS+E versus early CS+U (first four trials of each type). These contrasts were chosen to match those used in the previous study of the current baseline data (32) and are the same as those used in 18 of 27 studies in a meta-analysis on fear acquisition (13) (Table 2). The extinction and recall contrasts are the same as those used in 13 and 5 of 31 studies in another meta-analysis on fear extinction (15) (Table 2), respectively.

For each phase, we implemented two hierarchical regression models, one for baseline neural responses and the second for the difference between baseline and 30 months. In each model, seven threat neurocircuitry ROIs were treated as repeated measures nested within each subject. The slopes of general distress, fears, and anhedonia-apprehension were used as independent variables. Age at baseline, gender, race, ethnicity, and scanning site were entered as covariates. To reduce imbalances in category sample sizes, race and ethnicity were combined into one variable with the following four categories: Hispanic/Latino, non-Hispanic Asian, non-Hispanic White, and other. To further investigate the contribution of each individual ROI, Fisher's protected *t* tests (48) were used to establish multivariate linear regression models for each ROI with normalized data (see Supplemental Results for details). The Fisher's protected *t* test approach minimizes familywise error by requiring a significant omnibus analysis of variance *F* test before proceeding to ROI-specific analyses.

Table 2. Summary of Results From Hierarchical Linear Regression Models

	Baseline (T1)			Longitudinal Change (T4 − T1)		
	Parameter Estimate	SD	*p* Value	Parameter Estimate	SD	*p* Value
Fear Acquisition (CS+ vs. CS−)						
Intercept	3.52	1.14	.002	−4.12	1.96	.037
GD	0.14	0.13	.284	−0.14	0.22	.520
Fears	0.33	0.14	.018[a]	−0.48	0.23	.040[a]
Anhedonia	0.14	0.11	.180	−0.31	0.19	.110
Fear Extinction (Late CS+E vs. Late CS−)						
Intercept	2.78	1.33	.038	−2.49	2.22	.265
GD	0.16	0.16	.300	−0.20	0.25	.432
Fears	−0.51	0.16	.753	0.12	0.26	.658
Anhedonia	−1.78	1.25	.156	0.21	0.22	.330
Extinction Recall (Early CS+E vs. Early CS+U)						
Intercept	−1.00	1.11	.370	−0.19	1.67	.910
GD	0.07	0.13	.600	−0.44	0.19	.021[a]
Fears	0.09	0.13	.481	−0.23	0.20	.240
Anhedonia	0.00	0.10	.977	0.09	0.16	.580

CS, conditioned stimulus; CS+E, extinguished CS+; CS+U, unextinguished CS+; GD, general distress; T, time.
[a]$p < .05$.

We also conducted whole-brain analyses for each task phase using a permutation-based thresholding procedure with 10,000 permutations [FSL "randomise" (49)]. Furthermore, to examine potential associations between slopes of trilevel factors, neural responses, and subjective versus physiological fear responses, we conducted post hoc pairwise correlation analyses for neural responses, subjective US (shock) contingency ratings, and skin conductance during the fear acquisition phase (baseline) and extinction recall phase (30 months minus baseline).

RESULTS

Skin conductance responses (SCRs) and parameter estimates of ROIs suggest that at both time points, participants underwent successful fear conditioning. See the Supplement for detailed results.

Hierarchical linear models revealed two major findings (Table 2). First, an omnibus effect across all ROIs showed that neural responses during fear acquisition at baseline were positively associated with increases in fears ($r = 0.33$, $p = .018$) over the 30 months over and above changes in general distress and anhedonia-apprehension (Figure 2A). A greater increase in fears was associated with a more positive contrast between CS+ and CS− during fear acquisition at baseline. Additonally, the difference between neural responses at baseline and 30 months (T4 [CS+ vs. CS−] − T1 [CS+ vs. CS−]) showed a significant inverse association with changes in fears symptoms (T4 − T1; $r = −0.48$, $p = .040$) (Figure S7). This second effect was mainly driven by baseline neural responses, as mentioned above, and was not further investigated. The two models above did not reveal any significant effects related to general distress or anhedonia-apprehension.

Post hoc linear regression models featuring each ROI showed that effects were largest in the vmPFC ($r = 0.22$, $p = .024$). Examining the directionality of this effect, greater increases in fears were associated with weaker vmPFC deactivation to CS+ relative to CS− at baseline. For illustrative

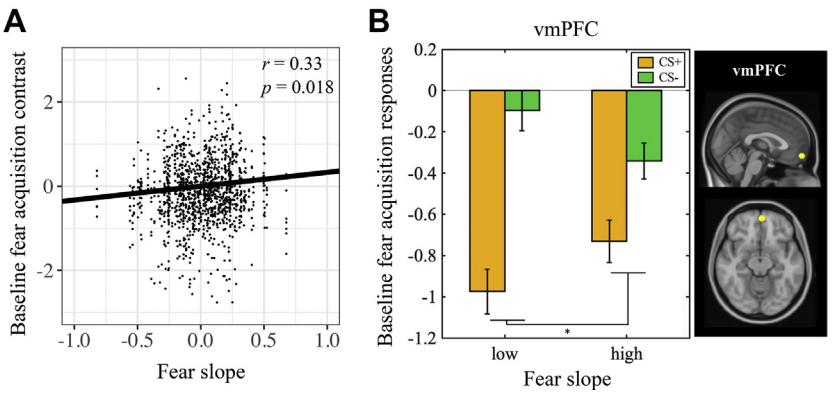

Figure 2. **(A)** Hierarchical linear models revealed a significant positive association between the fear acquisition contrast (CS+ > CS−) at baseline and the development of fears over 30 months (change in fear symptoms) ($n = 151$). Changes in symptoms are shown on the x-axis according to convention because they were used as independent variables in the model. **(B)** Left: A median split analysis revealed greater discrimination between ventromedial prefrontal cortex (vmPFC) responses to CS+ and CS− for participants with lower changes in fear symptoms compared with those with higher changes in fear symptoms during baseline fear acquisition. The difference in the deactivation of the vmPFC in response to CS+ and CS− was significantly greater in the low fears slopes group. Error bars represent standard errors. *$p < .05$. Right: Sagittal and axial views of the vmPFC spherical region of interest. CS, conditioned stimulus.

purposes, we compared vmPFC activity during fear acquisition between individuals with low versus high slope values for change in fear symptoms (median split; N_{low} = 88, N_{high} = 95); an independent *t* test showed that the low-slope group showed greater deactivation of vmPFC to CS+ relative to CS− (t_{179} = 3.312, p = .001) (Figure 2B). All other ROIs revealed the same trend of a positive association between fear acquisition neural responses and the slope of fears, but none reached statistical significance. Pairwise correlation analysis showed a significant negative correlation between vmPFC responses and US contingency ratings during baseline fear acquisition ($r = -0.18$, $p < .01$) but no correlation with SCR (Figure S9). To examine the effect of contingency over and above SCR, we ran a regression model with both US contingency and SCR as independent variables and vmPFC responses as the dependent variable. Results showed that the association between vmPFC responses and US contingency was significant over and above the relationship between vmPFC responses and SCR ($r = -0.27$, p = .009). No pairwise correlations between neural response difference scores (30 months vs. baseline difference scores) and either SCR or US contingency responses reached statistical significance ($p < .05$, uncorrected) (Figure S9, two rightmost columns). Qualitatively similar results were found when restricting this analysis to the 30-month data.

The second major finding was that changes in neural responses to extinguished stimuli from baseline to 30 month (i.e., T4 − T1) during extinction recall were negatively associated with changes in general distress over the same interval ($r = -0.48$, p = .040) (Figure 3). To better understand this effect, we ran the same hierarchical mixed-effect model on both the baseline and 30-month data. Results showed that the effect was mainly driven by a negative association between 30-month neural responses (contrast between CS+E and CS+U) and changes in general distress that approached conventional significance levels ($r = -0.26$, p = .056), indicating that greater increases in general distress are associated with a weaker contrast in neural responses between CS+U and CS+E at 30 months. To reveal the contribution of each ROI, we again ran linear regression models on each ROI. We found that the effect was largest in the sgACC ($r = -0.11$, p = .081). A median split analysis showed that individuals with more positive general distress slope estimates showed greater neural deactivation to early CS+E (N_{low} = 88, N_{high} = 95, t_{135} = 3.074, p = .003) (Figure 3B) but did not show a significant difference on early CS+U (p = .986) or on the contrast between early CS+E and early CS+U.

To examine the robustness of these findings, we ran analogous structural equation modeling analyses using the Lavaan package in R (Figures S11 and S12) and replicated the findings above. See the Supplement for details.

Our models did not reveal any significant association between slopes of trilevel factors and fear extinction neural responses. To further understand the second major finding, we ran an additional regression model to investigate the association between general distress and both fear extinction and extinction recall. Change in general distress was entered as the dependent variable. Changes in neural responses to extinguished stimuli from baseline to 30 months (i.e., T4 − T1) during fear extinction and during extinction recall were simultaneously entered as independent variables. Age at baseline, gender, race, site, and ethnicity were included as covariates as before. We found a significant omnibus effect of extinction recall neural responses ($r = -0.03$, p = .003) over and above fear extinction ($r = -0.02$, p = .019), replicating the second major finding (Figure 3A) when controlling for neural responses during fear extinction.

The explorative whole-brain analyses did not reveal any brain clusters that were significantly associated with changes in trilevel factors for any fear conditioning phase (see the Supplement for details and discussion). Post hoc pairwise correlation analyses (Figure S1) revealed strong correlations among measurements of trilevel factors across time points. In contrast, correlations between baseline and 30-month neural responses at each phase were low. These findings are in line with previous evidence that self-report measures have higher test-retest reliability than biological measures such as fMRI (30).

DISCUSSION

This study investigated longitudinal relationships between threat-related brain function and fears, anhedonia-apprehension, and general distress dimensions of an empirically derived trilevel model of anxiety and depressive symptoms (26,31) over a 30-month transition period from late adolescence to early adulthood. We report two major findings.

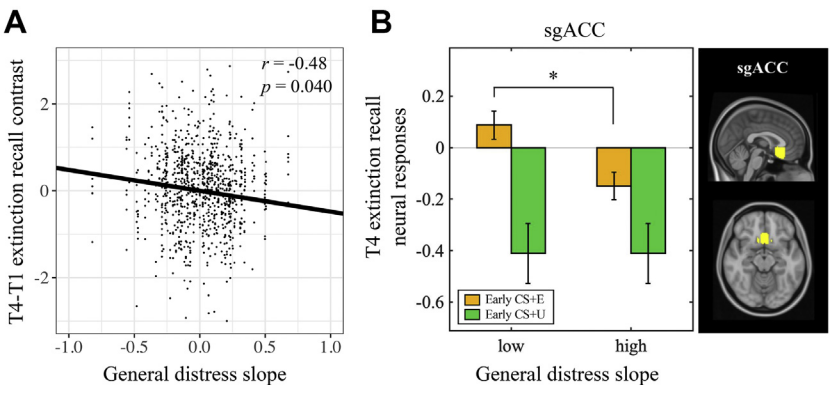

Figure 3. **(A)** Hierarchical linear models revealed a significant positive association between the difference between month 30 (T4) and baseline (T1) fear extinction recall functional magnetic resonance imaging contrasts (early CS+E − early CS+U) and change in general distress over 30 months (n = 146). **(B)** Left: Median split analysis comparing neural responses to early CS+E and early CS+U during fear extinction recall at T4 between individuals with low vs. high change in general distress. Responses to early CS+E in the subgenual anterior cingulate (sgACC) were significantly higher in the low change in the general distress group. Error bars represent the standard errors. Right: Sagittal and axial views of the sgACC region of interest. CS, conditioned stimulus; CS+E, extinguished CS+; CS+U, unextinguished CS+.

First, hierarchical mixed-effect models revealed an association between neural responses during baseline fear acquisition and changes in the trilevel fears symptom dimension, such that poorer neural discriminability between CS+ and CS− at baseline was associated with larger increases in fears over 30 months. While all threat neurocircuitry ROIs showed the same trend, we found that the effect was greatest in the vmPFC. Second, we found a negative association between changes in neural responses to extinction recall and changes in the trilevel general distress symptom dimension over 30 months; greater increases in general distress were associated with stronger deactivation to CS+E and weaker neural discriminability between CS+E and CS+U. This effect was largest in the sgACC.

The first finding suggests a long-term risk associated with a lack of reliable deactivation of the vmPFC to novel threatening stimuli. While vmPFC deactivation is more commonly acknowledged as an inhibitory signal associated with extinction (11,20), these results support other evidence for its role in fear acquisition (14–17,20,32). One interpretation is that vmPFC suppression aids fear acquisition by allowing the expression of conditional fear responses (12,20). In a related effect, large-scale decreases in connectivity (including in the vmPFC) were observed during fear acquisition (50). Furthermore, translational evidence from rats shows that prefrontal neurons are inhibited in the presence of conditional fear (51).

Successful expression of conditional fear during acquisition (via vmPFC deactivation) may protect against the development of fears by limiting overgeneralization of fear (2,4). In other words, greater discrimination between threatening (CS+) and nonthreatening (CS−) stimuli, as suggested by the pattern of greater vmPFC deactivation to CS+ versus CS− in the low change in fears symptoms group (Figure 2), may decrease overgeneralization of fear to nonthreatening stimuli. Indirect support comes from evidence of poorer discrimination between CS+ and CS− for individuals with anxiety disorders, who also show stronger fear generalization than nonanxious individuals (3). It is of note, however, that no significant association was found between fear acquisition CS+/CS− discriminability in the vmPFC and fears symptom dimension scores in the previous cross-sectional analysis of baseline data from this study (32). Together, the two sets of findings suggest that impairments in CS+/CS− discriminability in the vmPFC may reflect a neurobiological vulnerability that is not associated with concurrent symptom profiles but rather precedes symptom worsening.

We did not find a strong contribution of the amygdala to this effect on fear acquisition. Despite earlier evidence showing amygdala hyperactivity during symptom provocation or negative emotional processing in patients with anxiety disorders (8,52), other studies have shown decreased responses (32,53) or insufficient evidence for amygdala perturbations in patients with posttraumatic stress disorder or social anxiety (54). A recent meta-analysis also showed inconsistent evidence for amygdala activation in human fear acquisition (13). LeDoux and Pine (55) suggested that the conscious experience of fear is associated with cortical regions to which the amygdala provides only indirect input (55). In line with this suggestion, reduction of amygdala hemodynamic activity toward fearful targets was associated with decreasing skin conductance but not with subjective fear ratings (56). In the current study, vmPFC responses correlated with US contingency ratings but not SCR during fear acquisition, suggesting that vmPFC fear suppression is expressed on a conscious subjective level but not necessarily on a physiological level.

In contrast, fMRI approaches, which involve extensive averaging over CS presentations and conditioning phases, lack the sensitivity of other techniques such as single-cell recordings. Hence, our null results should not be taken to negate the involvement of the amygdala in fear conditioning per se. Indeed, single-cell recordings in rats (57–59) show responses of single neurons in the amygdala to conditional cues only during early trials of acquisition and only within short windows (e.g., 15–100 ms) relative to onset of the conditional cue.

The second major finding was an inverse association between changes in general distress and changes in neural responses during extinction recall. This was driven primarily by stronger deactivation to CS+E and weaker neural discriminability between the extinguished and unextinguished CS in the sgACC for participants who showed larger increases in general distress over time. Based on previous evidence that sgACC activation may facilitate extinction recall (19,60), this result suggests that individuals who show worsening of general distress—negative affect shared across anxiety and depression—over 30 months show worse recall of safety or extinction learning. However, because we did not find direct evidence for an association between sgACC activity during extinction recall and SCR or US contingency ratings, the role of sgACC activity remains unclear.

We did not find a significant association between trilevel symptoms and neural responses during fear extinction. Furthermore, in post hoc examination, the association between extinction recall and general distress was over and above that between fear extinction and general distress, suggesting that neural dysregulation associated with changes in depression and anxiety may not be specifically tied to the encoding of safety or extinction of threatening information (61) but rather to long-term retrieval of safety information during extinction recall. One possibility is that contextual specificity on extinction (e.g., environment, time) may be stronger (62) as general distress increases, resulting in less retrieval of extinction when tested in a new context (24–48 hours later in a second MRI scan in this case). This hypothesis aligns with the possibility that greater general distress is associated with stronger negative interpretive bias, which further leads to greater fragility of extinction across different contexts (63).

Impaired extinction recall is consistent with evidence for a bias toward negative memories in patients with depression (64) for both real-life events (65,66) and laboratory tasks (67,68). An example is superior recall for faces with negative expressions over faces with positive expressions (69). Such negative recall biases may lead to poorer recall of previously extinguished cue-threat associations rather than impaired learning of safety during fear extinction itself (70).

The associations between symptom dimensions of fears or general distress and activation of threat neural circuitry during fear acquisition and extinction recall reported herein are consistent with the expectation that emotional distress may be associated with brain regions that process threatening information and retain safety information. In addition, this investigation distinguishes between longitudinal changes in symptom

dimensions that are uniquely associated with anxiety and depression (i.e., fears and anhedonia-apprehension) and those shared by both (i.e., general distress). These data suggest a potential dissociation between neural processes underlying fear acquisition that are associated with the development of fears over and above symptoms that are specific to depression or that generalize to both anxiety and depression and neural processes underlying extinction recall that are associated with the development of general distress over and above fear or depression-specific symptoms (Figure S8). In line with the Research Domain Criteria initiative, these findings validate the neurobiological foundation of the trilevel symptom model and show the promise of using neural data to sort individuals into empirically established symptom profiles.

Finally, our findings should be considered in the context of study limitations. First, the dominance of female participants in this sample is consistent with the higher rate of depression and anxiety among females. Although participant gender was included as a covariate, future studies should examine generalization to male-dominant populations. Second, the data were collected across two scanning sites, which may introduce nuanced differences between subjects. However, to minimize this concern, we matched the scanner type and acquisition parameters across the two sites and found that site did not significantly affect the results when included as a covariate in our analyses. Third, this study was a macrolevel multiyear longitudinal investigation, with four time points over 30 months; future studies may provide a more microlevel picture of symptom fluctuations over a shorter timescale using mobile applications to collect daily or weekly self-report and behavioral measures (71). Fourth, while we used an ROI-based neural activation approach, future functional connectivity analyses (72,73) or multivoxel pattern analysis (56) may account for additional components of threat-response network functionality. Nonetheless, our results provide an initial understanding of how the brain and latent symptom dimensions interact over long timescales—with some preliminary evidence for causality in neural changes preceding symptom changes—within a critical stage of development.

ACKNOWLEDGMENTS AND DISCLOSURES

This research was supported by the National Institute of Mental Health (Grant No. R01MH100117 [to MGC, SYB, RN, and REZ]).

YP, JK, KY, SB, RN, RZ, and MC contributed to study conception, experimental design, data acquisition and analysis, and drafting/revising the work. NK and AME-C contributed substantially to the acquisition and analysis of the data and revision of the paper.

We thank Annelise Murillo and Brooke Feinstein for helping with data collection and data analysis. We thank Cody Cushing for helpful discussions regarding implementation of whole-brain analyses.

The authors report no biomedical financial interests or potential conflicts of interest.

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Received Jul 12, 2021; revised Dec 17, 2021; accepted Dec 27, 2021.

Supplementary material cited in this article is available online at https://doi.org/10.1016/j.bpsc.2021.12.013.

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