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Fear conditioning and extinction in anxious youth, offspring at-risk for anxiety and healthy comparisons: An fMRI study

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ABSTRACT

Dysfunctions in fronto-amygdala circuitry have been linked to anxiety. Questions remain regarding the impact of familial-risk and ongoing anxiety on such circuitry function, especially in youth. Using fMRI fear conditioning and extinction paradigms, we examined these relationships in 10–17 year-olds: 22 youth with an anxiety disorder, 22 healthy youth born to parents with past or current anxiety disorders (at risk), and 32 healthy comparisons. Skin conductance responses and subjective fear ratings were also assessed. During conditioning, healthy comparisons showed differential activation (CS + > CS–) in regions of the fronto-amygdala circuitry. In comparison, the at-risk group showed greater activation to the safety cue (CS – > CS +) in the amygdala and dorsolateral prefrontal cortex. Failure to show differential fear conditioning in the fronto-amygdala circuitry and impairment in extinction learning was specific to anxious youth. These findings expand our ability to track anxiety-related alterations and potential resilience markers to anxiety.

1. Introduction

Offspring of parents with anxiety disorders are nearly four times more likely to develop anxiety disorders than offspring of non-anxious parents (Hirshfeld-Becker, Micco, Simoes, & Henin, 2008; Micco et al., 2009). Studying both affected adolescents and unaffected adolescents at high familial risk for anxiety may differentiate factors associated with ongoing anxiety from those associated with familial risk. The neural processing of threat is known to engage a fronto-amygdala circuit, and dysfunction in this circuit occurs in anxiety. Altered fronto-amygdala function is a hypothesized risk factor for anxiety disorders (Blackford & Pine, 2012). Using functional magnetic resonance imaging (fMRI), the current study examines neural circuit function in anxious youth, unaffected offspring of parents with anxiety disorders, and healthy comparisons. The study uses a well-validated discriminative fear conditioning and extinction task – the "screaming lady" paradigm (Chauret et al., 2014; Den, Graham, Newall, & Richardson, 2015; Glenn et al., 2012; Haddad, Bilderbeck, James, & Lau, 2015; Lau et al., 2008, 2011; McGuire, Orr, Wu et al., 2016; Schiele et al., 2016).

Since anxiety disorders involve excessive, persistent fear, fear conditioning and extinction tasks are among the best-studied paradigms in anxious populations (e.g. Duits et al., 2015; McGuire, Orr, Essoe et al., 2016; Milad & Quirk, 2012; Shechner, Hong, Britton, Pine, & Fox, 2014). One commonly noted finding in anxiety patients on these tasks involves deficits in inhibitory processes, expressed as difficulty inhibiting autonomic or behavioral fear responses to both threat and safety cues during conditioning, and difficulty inhibiting conditioned fear responses following extinction trials. Such inhibitory deficits may explain patterns of findings on conditioning paradigms (Duits et al., 2015; McGuire, Orr, Essoe et al., 2016). Similar deficits have been observed on skin conductance responses (SCR) of offspring at risk for anxiety (Craske et al., 2008; Waters, Peters, Forrest, & Zimmer-Gembeck, 2014).

Amygdala engagement in the learning and expression of fear is well recognized (Phelps, 2006), and its hyperactivity is frequently reported in anxious patients (Blackford & Pine, 2012; Lissek, 2012; McClure et al., 2007). Involvement of the prefrontal cortex (PFC) – including the ventral, medial and dorsolateral subregions – also occurs, particularly in the context of conscious fear processing and threat-safety discrimination (Fullana et al., 2016; Lau et al., 2011). During extinction, these PFC regions are thought to modulate emotional reactions through their direct and indirect connections with the amygdala (Delgado, Nearing, LeDoux, & Phelps, 2008; Delgado, Olsson, & Phelps, 2006; Lissek, 2012; Shechner et al., 2014). Patients with anxiety disorders

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exhibit impaired capacity to deploy the PFC to downregulate amygdala reactivity (Duits et al., 2015; Milad & Quirk, 2012). Also included in the fear circuit, the insular cortex is involved in discriminative fear conditioning and extinction (Fullana et al., 2016, 2018) and participates in fear expression by integrating perception of introspective states with high-level cognitive representations (Simmons et al., 2013). Much like patterns of amygdala function, anxious patients also display greater activity in the insular cortex during fear conditioning and threat processing (Hofmann, Ellard, & Siegle, 2012; Marin et al., 2017).

Due to the ethical challenges associated with delivering an unconditioned stimulus (US) to youth (Lau & Waters, 2016; Pine, Helfinstein, Bar-Haim, Nelson, & Fox, 2009), few studies use fear conditioning paradigms to investigate the neural correlates of pediatric anxiety. Some prior studies find hypo-activation throughout the fear circuit in pediatric anxiety during fear conditioning and extinction recall (Britton et al., 2013; Haddad et al., 2015). These studies also link recruitment of the dorsolateral and ventromedial PFC to threat-safety discrimination (Britton et al., 2013; Haddad et al., 2015). Hypoactivation in PFC regions during fear conditioning and extinction may represent compromised capacity to engage downregulation mechanisms among anxious youth.

While these existing findings are promising, additional research is needed comparing affected youth, at-risk youth, and non-affected low-risk comparison youth. In fact, to our knowledge, no study has examined neural correlates of conditioning or extinction in these groups. The current study employs a discriminative fear conditioning and extinction paradigm to assess neural circuit function in affected youth, at-risk youth, and non-affected low-risk comparison youth. This design may differentiate neural correlates of manifest anxiety from risk or resilience factors for the disorder. Doing this work in youth, when the potential for change is the greatest, is crucial as better management of anxious and at-risk teens may effectively influence a behavioural trajectory early in its course, in the hopes of either preventing anxiety from becoming chronic or potentializing resilience to anxiety.

During fear conditioning, we hypothesized that anxious and at-risk groups would show greater amygdala and insular cortex differential activation between the threat conditioned and safe stimuli (CS+ unpaired vs. CS– contrast) compared to healthy comparisons. Lower recruitment of ventral, medial and dorsolateral PFC would be expected in the anxious group only. Regarding extinction, we hypothesized that the at-risk and healthy groups would differ from the anxious group in their ability to efficiently inhibit amygdala and insular cortex activity and to recruit the PFC. However, the current literature did not allow us to establish clear hypotheses regarding the direction of alterations within the PFC in anxious and at-risk groups.

2. Materials and methods

2.1. Participants

Three groups were recruited: (1) 22 youth with a current diagnosis of anxiety disorder, (2) 22 youth without past or current anxiety disorders, but at risk for anxiety due to their parents' past or current anxiety disorders, and (3) 32 psychiatrically healthy youth of psychiatrically healthy parents. Recruited participants were between 10 and 17 years of age, all the biological offspring of their parents. The lower age of our sample was established at 10 years to ensure participants' ability to acquire a differential fear conditioning (Glenn et al., 2012; Jovanovic et al., 2014). Groups were similar in sex, age, puberty status, socioeconomic status (SES), and estimated IQ. Socioeconomic status (SES) based on parental occupational and educational factors was assessed using the Hollingshead Two-Factor Index Scale (Hollingshead & Redlich, 1958; Miller & Salkind, 2002), and IQ was assessed using the Vocabulary, Similarities, Block Design, and Matrix Reasoning subtests of the Wechsler Intelligence Scale for Children (WISC-IV; Wechsler, 2003). Because puberty changes may influence cognitive and neurological development, both structurally and functionally (Blakemore, Burnett, & Dahl, 2010; Forbes, Phillips, Silk, Ryan, & Dahl, 2011), the pubertal status was assessed using the self-administered Tanner Puberty Stage Scale (Duke, Litt, & Gross, 1980; Tanner & Whitehouse, 1976). The picture-based questionnaire is a validated method for assessing the pubertal stage (Morris & Udry, 1980; Neinstein, 1982). The mean for pubic hair and genital/breast development index was compiled for all participants. Demographic characteristics of participants are presented in Table 1.

Anxious youth were recruited through the Anxiety Disorders Clinic of the Sainte-Justine University Hospital where they were treated for an anxiety disorder for the first time. At-risk youth were recruited through a non-profit organization (Phobies-Zéro), and flyers were distributed in medical clinics and mental health hospitals. Healthy comparisons were recruited in the community. In anxious youth, 10 participants met criteria for generalized anxiety disorder (GAD), three for panic disorder (PD), three for separation anxiety disorder (SAD), three for social phobia (SP), and three presented comorbidity among these disorders based on DSM-5 criteria. Youth presenting comorbidity between these disorders were included as these disorders commonly occur together; restricting inclusion to only one of the illnesses would have severely limited the potential subject pool and the generalization of findings. Healthy comparisons and at-risk youth were free from any past or current psychiatric illness. In the non-affected youth at risk for anxiety, only the mother was affected in 17 cases, whereas in four cases it was the father, and in one case it was both parents. Sixty-four percent of the anxious youth's parents presented a past or current anxiety disorder.

To assess inclusion and exclusion criteria, psychiatric interviews were conducted in all participants and their parents by two well-trained graduate students in psychology and all diagnostic criteria were reviewed by a licensed clinical psychologist (FSM). The semi-structured Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS; Kaufman et al., 1997 interview was assessed separately with youth and parent(s). To ensure that symptoms were not transient, anxious youth also had to present a significant level of anxiety and associated impairments on the Pediatric Anxiety Rating Scale (PARS; Ginsburg, Keeton, Drazdowski, & Riddle, 2011; The Research Units On Pediatric Psychopharmacology Anxiety Study, 2002), which persisted over a 14-day-period (scores > 9 in both testing sessions).

As assessed via the semi-structured psychiatric evaluation conducted with the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I; First, Spitzer, Gibbon, & Williams, 2002), parents of anxious youth either were free from any psychiatric disorders or met criteria for GAD, SP, PD, or comorbidity among these disorders. In offspring at risk for anxiety, one or both parents met criteria for a past or current GAD, SP, PD, or comorbidity between these disorders based on DSM-IV-R criteria. We elected to include either biological parent presenting an anxiety disorder given that paternal anxiety confers the same risk as maternal anxiety (Connell & Goodman, 2002). Parents of healthy comparisons were free from any past or current psychiatric illness.

Youth's anxiety symptoms were measured using the State-Trait Anxiety Inventory for Children (STAIC; Bergeron, Landry, & Bélanger, 1976; Spielberger, Edwards, Lushene, Montuori, & Platzek, 1973). We also measured current anxiety and depression levels using the youth and parent versions (parent's responses regarding their child) of the Screen for Child Anxiety Related Emotional Disorders–Revised (SCARED-R; Martin & Gosselin, 2012) and the Children Depression Inventory (CDI; Kovacs, 1985; Saint-Laurent, 1990), respectively. Parents' current anxiety symptoms were assessed using the State-Trait Anxiety Inventory (STAI; Bergeron et al., 1976; Gauthier & Bouchard, 1993; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). Self-report questionnaires used in this study are all French validated versions.

Participants were excluded from the study if they presented: (a) MRI contraindications (e.g., braces, pregnancy); (b) IQ score < 70; (c) any serious or chronic medical illness; (d) past head trauma with loss of

Table 1
Demographic and clinical characteristics of participants.

	Healthy (n = 32)		At-risk (n = 22)		Anxious (n = 22)		
Characteristics	*M*	(*SD*)	*M*	(*SD*)	*M*	(*SD*)	*p* [a]
Age	13.50	(2.08)	13.32	(2.34)	13.26	(2.47)	.919
Sex (% male)	40.6		48		47.8		.816
Tanner[b]	3.63	(0.98)	3.48	(1.19)	3.32	(1.25)	.616
SES	26.59	(13.09)	34.26	(13.92)	27.91	(11.71)	.077
Ethnicity (% Caucasian)	78.1		92		82.6		.370
IQ							
Verbal	110.42	(16.86)	108.68	(13.24)	108.29	(16.18)	.866
Performance	104.71	(11.59)	107.60	(17.08)	101.81	(14.10)	.393
C-GAS	86.72	(5.36)	80.42 [f]	(8.38)	57.22 [g j]	(9.13)	< .001
SCARED-R							
Children	18.10	(8.92)	25.21 [e]	(11.15)	32.21 [g]	(13.89)	< .001
Parent about child	9.65	(6.86)	16.35 [e]	(10.23)	32.34 [g j]	(11.37)	< .001
STAIC [c]							
State	48.34	(5.46)	49.84	(5.57)	54.95 [e g]	(8.55)	.002
Trait	48.19	(7.96)	54.44 [e]	(10.11)	61.77 [g h]	(9.28)	< .001
CDI							
Children	43.44	(4.91)	44.67	(4.57)	50.90 [g i]	(9.53)	< .001
Parent about child	44.32	(4.84)	51.42 [e]	(9.18)	58.28 [g i]	(7.00)	< .001
PARS (children)							
Time 1					21.27	(4.38)	
Time 2					19.76	(5.48)	
STAI (parent) [c d]							
Mothers							
State	39.22	(9.96)	43.92	(7.93)	46.45 [e]	(8.84)	.014
Trait	37.63	(8.16)	48.00 [g]	(12.67)	48.27 [j]	(9.89)	< .001
Fathers							
State	42.00	(7.90)	47.31	(12.87)	43.56	(10.16)	.323
Trait	40.64	(6.21)	44.69	(10.98)	42.33	(7.74)	.399

Note. SES = Socioeconomic status; C-GAS = Children's Global Assessment Scale; SCARED-R = Screen for Child Anxiety Related Emotional Disorders-Revised; STAIC = State-Trait Anxiety Inventory for Children; CDI = Child Depression Inventory; PARS = Pediatric Anxiety Rating Scale; [a] Group analyses regarding sex and ethnicity of participants were performed using chi squares for quantitative measures, and other variables were compared using one-way analysis of variance (ANOVA); [b] Positive correlation between age and puberty status in all participants (r = .83, p < .001); [c] Mean scores of standardised T scores; [d] to assess parents' current anxiety symptoms; [e] p < .05 *vs.* healthy; [f] p < .01 vs. healthy; [g] p < .001 *vs.* healthy; [h] p < .05 vs. at-risk; [i] p < .01 vs. at-risk; j p < .001 vs. at-risk.

consciousness; (e) use of medication that affect brain function; (f) past or present treatment for a psychiatric illness (pharmacological or behavioral); and (g) any other past or current psychiatric disorders in youth and their parents (e.g., major depressive disorder, bipolar disorder, obsessive-compulsive disorder, post-traumatic stress disorder). This study protocol was approved by the Research Ethics Boards of the Sainte-Justine University Hospital Research Center, Montreal, Canada, and of the *Unité de Neuroimagerie Fonctionnelle* (UNF) of the *Centre de recherche de l'Institut universitaire de gériatrie de Montréal* (CRIUGM), Canada. Participants and their parents respectively gave their informed assent and consent and were compensated for their participation.

2.2. Experimental design

The fMRI fear conditioning and extinction tasks were conducted in a 17-min single run (Fig. 1). This run comprises three phases: a habituation run, a fear conditioning phase and a fear extinction phase. During each phase, participants saw head shots of two actresses presenting neutral emotional expressions. During conditioning, one actress was randomly selected to serve as the threat conditioned stimulus (CS +) for each participant, whereas the other served as the safe stimulus (CS −). The CS+ was paired with the US in 50% of trials. The US was constituted of a photo of the same actress selected for the CS+ but depicting a fearful expression, and simultaneous presentation of a 90 dB shrieking female scream. Prior work finds this paradigm to trigger fear responses that are more consistent with findings in adults compared to other, less aversive US (Glenn et al., 2012; Schmitz et al., 2011). A partial reinforcement contingency ratio was used to prevent habituation to the US (Mackintosh, 1974), and participants were not informed regarding the CS+ – US association prior to the experiment. The CS− was never paired with the aversive US. During extinction, task procedures were identical to that of the conditioning phase with one exception: no US were presented. Overall, 96 stimuli were presented. Events were presented for the duration of 6 s with subjective fear rating occurring from 3 to 6 s. The habituation run comprised two event types: CS+ unpaired (n = 6) and CS– (n = 6). The conditioning phase comprised three event types: CS+ paired (n = 14), CS+ unpaired (n = 14) and CS– (n = 28), and the extinction phase comprised two event types: CS+ unpaired (n = 14) and CS– (n = 14). For the CS+ paired events, the US was presented after the fear rating for a duration of 1.1-s. Interstimulus intervals were of 3, 4, 5, 6, 8, 10, or 12 s. Trials were presented in a pseudo-random order.

2.2.1. fMRI acquisition and preprocessing

Scanning was performed within a 14–30-day delay following the psychiatric assessment. The fMRI session took place at the UNF of the CRIUGM, Canada. Scans were performed on a 3 T MRI scanner (Magnetom Tim Trio, Siemens). Before the fMRI session, participants underwent a training session in an MRI simulator to assure their comfort in the environment and to practice manipulating the button box for ratings. Participants were told they would see two different images and hear sounds, but no details were given on the images or sounds. The pictures and sound presented were different from the experimental tasks to prevent habituation to the CS+, CS– and US. Also, all female participants provided a urine sample to confirm non-pregnancy.

The 12-channel head coil was equipped with a mirror for presentation of the visual stimuli on a back projection screen and head movement was restricted by placing a comfortable foam padding around the head. The US female scream was presented through MR-compatible headphones. For functional imaging, a total of 495 volumes were registered using a single shot gradient echo T2* weighting with 32 contiguous ascending 3.3 mm axial slices, parallel to the AC-PC plane,

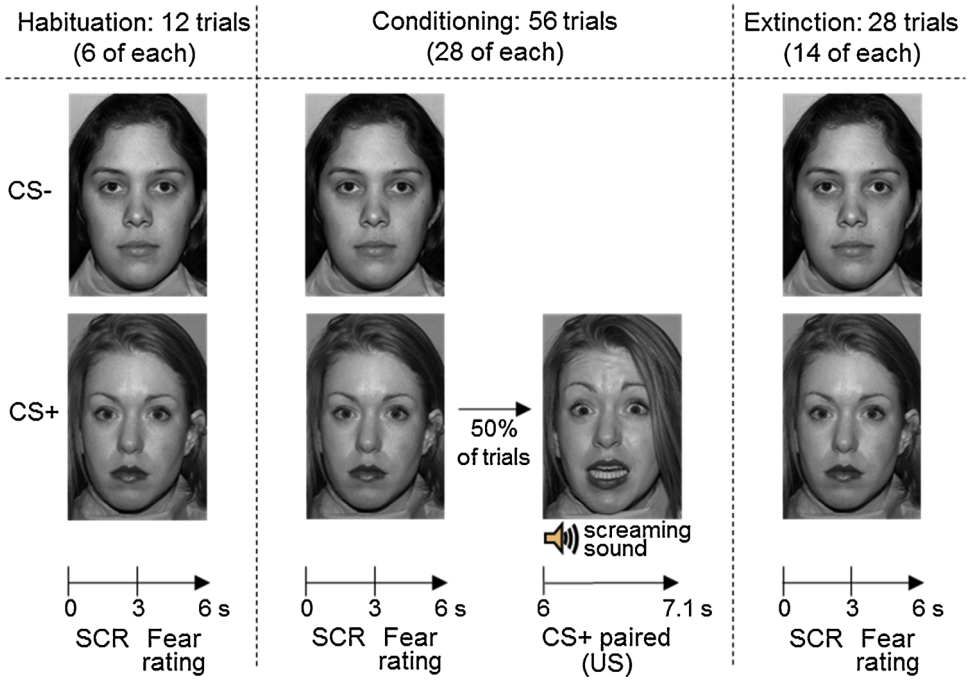

Fig. 1. A schematic depiction of the fear conditioning and extinction tasks. SCR: Skin conductance responses; CS+: conditioned stimulus; CS–: safety cue; US: unconditioned stimulus.

covering the whole brain (TR = 2300 ms, TE = 30 ms, flip angle = 90°, voxel size: 3.8 × 3.8 × 3.3 mm, matrix size: 64 × 64 mm and field of view [FOV]: 24 cm). For anatomical reference, a MPRAGE sequence was performed to acquire high-resolution T1-weighted anatomical images (TR = 2300 ms, TE = 2.98 ms, TI: 900 ms, flip angle = 9°, matrix size = 256 × 256 mm, voxel size = 1 × 1 × 1 mm^3, FOV = 256 mm, 176 slices). Estimated rotation and translation movements were less than 5 degrees or 3 mm for all subjects.

Image preprocessing and analysis procedures were conducted using Statistical Parametric Mapping (SPM) version 8 software (http://www.fil.ion.ucl.ac.uk/spm/) implemented in MatLab 7.10 release 2010a (MathWorks Inc., Natick, MA). Preprocessing procedures performed on raw functional images included realignment (4^{th} degree b-spline interpolation) to correct head movement, slice timing correction to the first volume, co-registration to their respective high-resolution structural images, segmentation, normalization to the standard space of the Montreal Neurological Institute (MNI) brain, and spatial smoothing using a 8-mm FWHM isotropic Gaussian kernel.

2.2.2. Skin conductance responses

Skin conductance responses (SCR) were recorded during fMRI acquisition using two 10-mm EDA isotonic gel radio-translucent electrodes placed on the plantar surface of participants' right foot. SCR were recorded using AcqKnowledge software (version 4.2) while being amplified, digitized, and recorded at 1000 Hz using a computerized data acquisition system (MP150-BIOPAC System). First, a smoothing of 500 ms was made using AcqKnowledge to eliminate high frequency noise. Then, SCR were analyzed with the freely available software SCRalyze 2.1.8 (scralyze.sourceforge.net), which employs a general linear convolution model for rapid event-related evoked SCR (Bach, Flandin, Friston, & Dolan, 2010; Bach, Flandin, Friston, & Dolan, 2009). Events of interest included in the GLM were the 3-s-windows following cue onset of all CS+ unpaired and CS– during conditioning and extinction phases. For a proper baseline, modeled events of no interest included the last 3-s-segment of each stimulus presentation to eliminate SCR triggered by the motor responses associated with subjective ratings, the 1.1-s of US presentation to eliminate the startle responses, and the 3-s-windows following each event to control SCR triggered by the anticipation of a possible US. Data obtained from the canonical SCR function, with time and dispersion derivatives, were band pass filtered using a 1^{st} order Butterworth filter and cut-off frequencies of 0.0159 and 5 Hz, and down sampled to 10 Hz. Because high variability characterizes SCR from one individual to another, amplitude of SCR to CS+ and CS– was standardized using Z-transformations within each subject, separately for the 56 events of the conditioning phase and for the 28 events of the extinction phases. This allowed for statistical analysis comparing SCR to the CS+ vs. CS– within each group during conditioning and extinction. Means were then calculated for CS+ and CS–.

2.2.3. Subjective fear ratings and contingency awareness

For each event in the conditioning and extinction phases, participants were asked to rate the degree of fear they felt on a 5-point Likert scale while viewing the actress (Are you afraid?; 1 = not at all, 5 = extremely). Fear ratings were recorded with a right hand-held button response box developed to allow a graded range of responses (Current designs, Philadelphia).

During a post-experiment interview, participants were asked to rate their fear levels for the two actresses on the 5-point Likert scale one last time and were debriefed to ensure deontologically that participants did not leave the session with high stress levels. Moreover, they were asked about their contingency awareness of the CS–US relationship (i.e., if the blond- and/or brown-haired actress screamed). Contingency awareness was granted if participants correctly identified which actress had been paired with the scream (CS+), and which one represented the safe signal (CS–).

2.3. Statistical analyses

Demographic, behavioral, and fMRI data analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL). All data met statistical assumptions, or were square root transformed to meet normality.

2.3.1. Imaging data

At the first level, six conditions were defined: CS+ unpaired and CS– during the habituation run; CS+ unpaired, CS+ paired, and CS– during conditioning; and CS+ unpaired and CS– during extinction. The

six movement parameters of the rigid body transformation, obtained from the realignment procedure, were included as regressors of no interest, and a high-pass filter of 128 s was used to remove low-frequency noise. For each participant, a statistical image for the contrast of interest (CS+ unpaired *vs.* CS–) was then obtained during conditioning and extinction separately. Because US were not separated by jittering intertrial intervals (ITI), only CS+ unpaired events were examined in order to avoid contrasts including changes in neural responding related to US contamination. Time of cue presentation was not included as a within-subjects factor in fMRI analysis as the number of trials per condition was too small to provide a stable hemodynamic response (Huettel & McCarthy, 2001).

To assess the main effects of group, analysis of variance (ANOVA) was performed on contrast images for conditioning and extinction separately, as conditioning is expected to reflect fear learning processes, while extinction learning is expected to reflect inhibition processes of conditioned fear. Based on our a priori hypothesis, second-level general linear model (Friston et al., 1995) compared BOLD activation between groups across the whole brain and for five region of interest (ROI), i.e., in the amygdalas, insular cortex, vPFC (BA10, 11, and 47), dlPFC (BA9 and 46), and ACC (BA24, and 32), using small volume corrections. Masks were created using the Wake Forest University (WFU) PickAtlas software (http://www.fmri.wfubmc.edu/download.htm). Although there were no significant group differences in terms of age, this variable was included as a covariate of no interest since the age range within participants was large (10–17 years old) and that maturation is known to impact neural fear circuit function. Analyses were corrected for multiple comparisons (FWE-corrected voxelwise) with Gaussian random field threshold set at $\alpha < 0.05$. For post hoc analysis, beta values within individual peak activation were extracted for all conditions of interest (i.e. CS+ unpaired and CS– during conditioning and extinction). Post hoc repeated-measures ANOVAs were then conducted in SPSS; where group (anxious, at-risk, and healthy comparisons) served as between-subjects factor, and CS-type (CS+ unpaired and CS–) served as within-subjects factors. Post hoc Tukey group comparison tests set at an alpha level of 0.05 were further performed on significant ANOVA findings. Finally, some suggest that anxiety related alterations in excitatory and inhibitory association learning could be underestimated by our contrast of interest (CS+ unpaired vs. CS–) (Lissek et al., 2005). Hence, group comparisons during extinction were performed both on the CS+ vs. CS– contrasts as well as on participants' mean activation to both CS + unpaired and CS–, relative to the low-level baseline.

2.3.2. Autonomic and behavioral measures

Regarding SCR and subjective fear ratings, repeated-measures analysis of variance (ANOVAs) were conducted separately for conditioning and extinction phases. Group (anxious, at-risk, and healthy comparisons) served as between-subjects factor, and CS-type (CS+ and CS–) and time of cue presentation (early and late; for conditioning, early: 14 first cues, late: 14 last cues; for extinction, early: 7 first cues, late: 7 last cues) served as within-subjects factors. Post hoc comparisons performed on significant ANOVA findings were done using Tukey group comparisons. Significance was defined at an alpha level of 0.05.

3. Results

3.1. Sample characteristics

A total of 85 youth met inclusion criteria and were invited to the scanning session. After removing the nine participants who ended their participation for lack of interest, technical problems, or cerebral abnormalities), 76 youth were included in the analyses (32 healthy comparisons, 22 offspring at risk for anxiety, and 22 anxious youth). No participants were lost to movement during the scanning protocol. Clinical characteristics of groups are presented in Table 1. Correlations between scores obtained on youth and parental versions of the SCARED-R ($r = .50$, $p < .001$) and the CDI ($r = .38$, $p = .001$) were consistent with expected correlation for cross-informant agreement in child and adolescent behavioral and emotional problems assessment (Achenbach, McConaughy, & Howell, 1987). The youth's scores on self-reported questionnaires of current anxiety and depression symptoms were not correlated with age, sex, SES or IQ (rs < .21, ps > .08). Severity of anxiety symptoms and associated impairments in anxious youth remained stable throughout both visits ($r = .77$, $p < .001$; PARS).

Table 2
MNI coordinates and statistics for peak of clusters from the ROI analysis during conditioning (CS+ unpaired vs. CS−) and extinction (mean BOLD activation to CS+ unpaired and CS−).

Regions	Side	BA	Voxels	x [a]	y	z	*F*	*p*
Conditioning								
Amygdala	L		29	−16	0	−24	7.43	.03
dACC	L	24	31	−4	−10	28	9.24	.03
vlPFC	L	47	138	−46	50	−8	8.33	.04
dlPFC	R	9/46	94	58	30	18	9.05	.03
Extinction								
Amygdala	L		44	−28	−6	−18	7.53	.03
Amygdala	R		66	28	−8	−14	6.43	.06
				32	−2	−16	5.88	.09

Note. [a]Coordinates for peak of clusters; L: Left; R: Right; dACC: dorsal anterior cingulate cortex; vlPFC: ventrolateral prefrontal cortex; dlPFC: dorsolateral prefrontal cortex; p at α = .05 corrected for multiple comparisons in each region.

3.2. Imaging data

3.2.1. Conditioning

There were no significant group differences for the CS+ unpaired *vs.* CS– contrast in the whole brain analysis during conditioning. ROI analyses revealed a main effect of group to the contrast CS+ unpaired *vs.* CS– in the left amygdala, dorsal portion of left anterior cingulate cortex (dACC), left ventrolateral PFC (vlPFC), and right dorsolateral PFC (dlPFC) (see Table 2 and Fig. 2). Post-hoc analyses suggested that some of these findings reflected similar differences between both the healthy and at-risk groups with the anxious group. Specifically, healthy and at-risk groups showed greater activation in both left dACC (BA24) and left vlPFC (BA47) relative to anxious youth (*ps* < .01). This result was due to a pattern of deactivation (CS– > CS + unpaired) in left dACC ($p = .001$) and left vlPFC ($p < .001$) in the anxious group. Other post hoc findings reflected a unique response in the at-risk group regarding the left amygdala and right dlPFC, differing from both the healthy and anxious groups. Specifically, at-risk youth showed patterns of deactivation (CS– > CS+ unpaired) in the amygdala ($p = .007$) and dlPFC ($p = .03$). In comparison, healthy comparisons showed greater differential activation (CS+ unpaired > CS–) in both the left amygdala ($p = .04$) and right dlPFC ($p < .001$; BA9/46), whereas anxious youth manifested absence of differential activation in both structures ($p = .10$ and $p = .42$, respectively). Regarding the amygdala, pattern of differential activation observed in healthy comparisons interestingly occurred during its deactivation, and the pattern of deactivation observed in the at-risk group occurred in the context of its deactivation to the threat cue only (see Fig. 2).

3.2.2. Extinction

BOLD activation to our contrast of interest (CS+ unpaired *vs.* CS–) did not show a main effect of group in the whole brain analysis or in ROI of the fronto-amygdala fear circuit during extinction. Based on the recommendation of Lissek et al. (2005), we also examined the main effect of group in response to both CSs (average activation to CS + unpaired and CS–, relative to the low-level baseline). A main

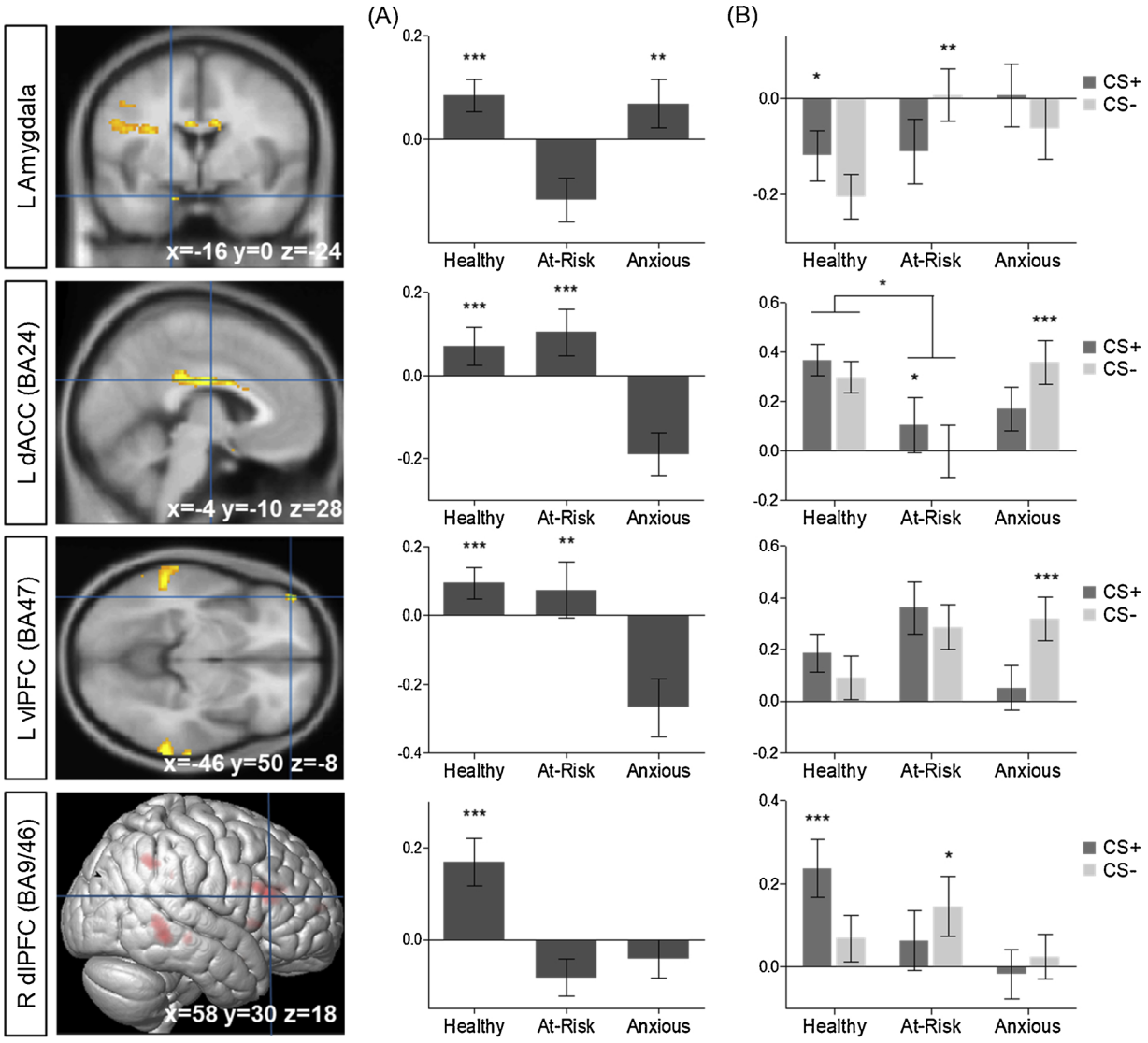

Fig. 2. Statistical maps of significant functional activation in ROIs during fear conditioning on the contrast CS+ unpaired *vs.* CS–. Images presented at an uncorrected p = .005 threshold for illustrative purpose. Bar graphs depicting mean percent signal change (A) to the contrast CS+ unpaired vs. CS– and (B) for CS+ unpaired and CS– separately by group. Error bars represent the standard error of the mean. *$p < .05$, **$p < .01$.

effect of group was found in the left amygdala (see Table 2 and Fig. 3). Post hoc analysis reflected similar differences between both the healthy and at-risk groups and the anxious group. Specifically, anxious youth showed greater activation to both CSs in the left amygdala relative to healthy comparisons ($p < .001$) and youth at risk for anxiety ($p = .002$). A trend effect of group was also observed in the right amygdala, with post hoc analysis showing greater activation in anxious youth relative to healthy comparisons ($p = .001$).

3.3. Autonomic and behavioral measures

As expected, robust evidence of discriminative conditioning was found in all three groups for both autonomic and behavioral measures. Between-group differences are described below. Differential fear responses (CS+ *vs.* CS–) in BOLD activation, SCR, and subjective fear ratings were unrelated across the whole sample and within every group (all $r < .26$, all $p > .05$). Autonomic and behavioral analyses were carried out on 59 and 72 participants, respectively (see Supplement for details, Table S1).

3.3.1. Skin conductance responses

Eleven participants were excluded from the skin conductance responses (SCR) analyses because they showed no SCR or because of low data quality (e.g. noise), and five participants presented multivariate outlier data to both the CS+ and CS– during conditioning and extinction. Hence, analyses were carried out on 25 healthy comparisons, 17 at-risk youth, and 17 anxious youth.

During conditioning, a main effect of CS-type ($F_{1,57} = 14.02$, $p < .001$; $\eta^2 = .20$; Fig. 4A) was found, with greater SCR triggered by the CS+ vs. CS– ($p < .001$). Moreover, we observed a main effect of time of cue presentation ($F_{1,57} = 13.89$, $p < .001$; $\eta^2 = .20$), with a significant increase in SCR from early to late conditioning ($p < .001$). No other two- or three-way interactions were found ($Fs_{2,57} < 3.88$; $ps > .05$).

During extinction, a two-way group x CS-type interaction ($F_{2,57} = 5.51$, $p = .006$; $\eta^2 = .16$) was found. Post hoc analyses revealed greater SCR triggered by the CS– relative to the CS+ in at-risk ($p = .03$) and anxious youth ($p = .03$), whereas healthy comparisons showed similar SCR to CS+ and CS– ($p = .09$). Moreover, a main effect of time of cue presentation ($F_{1,57} = 6.41$, $p < .01$; $\eta^2 = .10$) was subsumed by a group × time of cue presentation interaction ($F_{1,57} = 4.57$, $p = .04$;

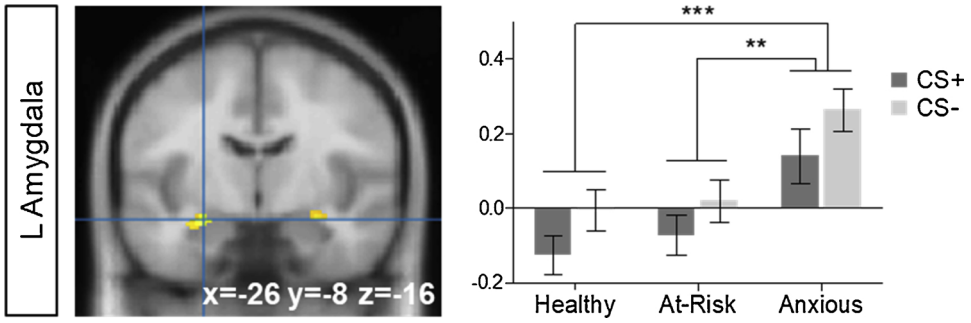

Fig. 3. Statistical maps of significant functional activation in left amygdala during the extinction phase (average activation to both CS+ unpaired and CS–), presented at an uncorrected p = .005 threshold for illustrative purpose. Bar graphs showing greater mean percent signal change to both CSs in anxious youth relative to healthy comparison and at-risk groups (*ps* < .01). Error bars represent the standard error of the mean. $^{**}p < .01$, $^{***}p < .001$.

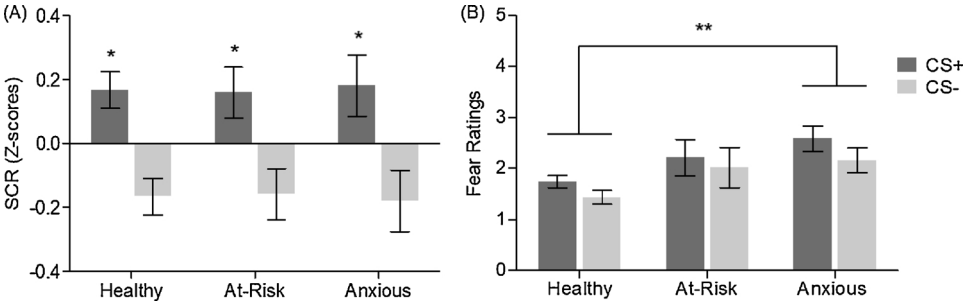

Fig. 4. Successful acquisition of discriminative conditioning. (A) Mean skin conductance responses during conditioning for CS+ and CS– in groups. Main effect of CS-type showing discriminative fear responses (CS+ > CS–) in all participants; (B) Mean subjective fear rating during conditioning for CS+ and CS– in groups. Main effect of CS-type showing discriminative fear responses (CS+ > CS–) in all participants, and a main effect of group showing greater fear ratings to both CSs in anxious youth relative to healthy comparisons (p = .004). Error bars represent the standard error of the mean. $^{**}p < .01$.

$\eta^2 = .06$). Post hoc analyses showed that, relative to healthy comparisons, at-risk and anxious youth showed greater SCR to both CSs during early extinction and lower SCR to both CSs during late extinction (*ps* < .05; Fig. 5A). However, whereas at-risk youth showed significant decrease in their SCR from early to late extinction ($p = .004$), anxious youth failed to significantly decreased their SCR over the extinction phase ($p = .06$). No other main effect nor two- or three-way interaction was observed (*Fs* < 2; *ps* > .16).

3.3.2. Subjective fear ratings

Four participants were rejected from the subjective fear ratings analysis because they did not select their ratings in ≥20% of cue presentations; hence, analyses were performed on 31 healthy comparisons, 20 at-risk youth, and 21 anxious youth.

During conditioning, results showed a main effect of group ($F_{2,70} = 4.45$, $p = .02$; $\eta^2 = .11$), with greater fear ratings to both CS+ and CS– in anxious youth relative to healthy comparisons (p = .004; Fig. 4B). The at-risk group did not differ from either group. Moreover, a main effect of CS-type ($F_{1,70} = 31.94$, $p < .001$; $\eta^2 = .31$) was observed, with CS + evaluated as more threatening compared to the CS– (p < .001), as well as a main effect of time of cue presentation ($F_{1,70} = 30.37$, $p < .001$; $\eta^2 = .30$), with greater fear ratings observed during early relative to late conditioning (p < .001). No two- or three-way interaction was observed (all *Fs* < 3.1; *ps* > .08).

During extinction, results showed a main effect of group ($F_{2,70} = 3.78$, $p = .03$; $\eta^2 = .10$), with greater fear ratings to both CS+ and CS– in anxious youth relative to healthy comparisons (p = .007; Fig. 5B). The at-risk group did not differ from either group. Moreover, we observed a main effect of CS-type ($F_{1,70} = 19.64$, $p < .001$; $\eta^2 = .22$), which was subsumed by a CS-type × time of cue presentation interaction ($F_{1,70} = 4.57$, $p = .04$; $\eta^2 = .06$). Post hoc analyses showed greater fear ratings to CS+ vs. CS– during both early and late extinction (*ps* < .001), with greater fear ratings to CS+ during early relative to late extinction ($p = .02$) and similar fear ratings to CS– during both early and late extinction ($p > .9$). No other main effect, two- or three-way interaction was observed (*Fs* < 2; *ps* > .16).

3.3.3. Post-experiment questionnaire

Over 95% of participants showed contingency awareness of the CS–US relationship. The chi-squared analysis of participants showing correct *vs.* incorrect contingency awareness did not differ between groups ($\chi 2 = 5.12$, $p = .08$). Moreover, excluding data from the 3 unaware participants did not affect the pattern of results for fMRI, SCR, and fear ratings during conditioning. Ratings obtained on the post-experiment questionnaire led to similar conclusions as those observed with ratings collected during the conditioning task. Results showed a main effect of group ($F_{2,72} = 5.04$, $p = .009$; $\eta^2 = .12$), with greater fear ratings to both CSs in anxious youth relative to healthy comparisons ($p = .004$) and no difference between the at-risk group and either group. Moreover, a main effect of CS-type ($F_{1,72} = 55.03$, $p < .001$;

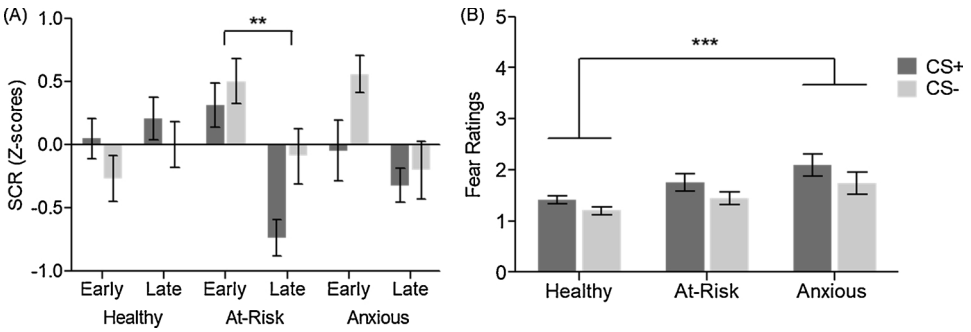

Fig. 5. (A) Mean skin conductance responses during early and late extinction for CS+ and CS– in groups. Group × Time of cue presentation interaction showing significant decreasing in SCR over time in the at-risk group only; (B) Mean subjective fear rating during extinction for CS+ and CS– in groups. Main effect of group showing greater fear ratings to both CS + and CS– in anxious youth relative to healthy comparisons, and a main effect of CS-type showing differential fear responses (CS + > CS–) in all participants. Error bars represent the standard error of the mean. $^{**}p < .01$, $^{***}p < .001$.

η^2 = .43) was found, with greater fear levels manifested to the CS+ *vs.* CS– in all participants.

4. Discussion

Three main imaging findings emerged from this study. First, during fear conditioning, the anxious group showed lower activation in the left dACC and left vlPFC (CS– > CS + unpaired) compared to both the healthy and at-risk group. Second, the at-risk group manifested a unique response in the left amygdala and right dlPFC during fear conditioning (CS– > CS + unpaired), differing from both the healthy and anxious group. Third, during extinction, the anxious group showed greater activation in the amygdala compared to both the healthy and at-risk group. Whereas differences during conditioning manifested for the contrast CS + unpaired *vs.* CS–, differences during extinction were for both stimulus types relative to a low-level baseline.

Similar recruitment of dACC and vlPFC was observed in healthy comparisons and offspring at risk for anxiety. A recent meta-analysis highlighted that co-involvement of the dACC and vlPFC during fear conditioning reflects a discriminative anticipation for threat cues (Fullana et al., 2016). Lower activation of dACC and vlPFC to the threat cue (CS– > CS + unpaired) in anxious youth suggests a neural correlate of manifest anxiety. In combination with greater fear expression to both stimulus type in the anxious group relative to the healthy comparisons during conditioning (see Supplement), our results hence support excessive fear expression to safety cues in anxiety disorders (Duits et al., 2015). Although not emphasized as key nodes in conditioning studies among anxious youth, considerable research also implicates both structures in cognitively-driven emotional regulation (Blackford & Pine, 2012; Blair et al., 2012; Frank et al., 2014). Moreover, prior studies in pediatric anxiety reported that recruitment of the vlPFC in response to threat predicts lower severity of anxiety symptoms and amygdala activity (Monk et al., 2006, 2008; Telzer et al., 2008). The present data add to prior work linking dACC and vlPFC function to various clinical aspects of pediatric anxiety (Burkhouse et al., 2017; Kujawa et al., 2016).

The fear conditioning task also highlighted greater activation to the safety cue (CS– > CS + unpaired) in the amygdala and dlPFC of offspring at risk for anxiety, differing from both the healthy comparison and anxious groups. Healthy comparisons showed expected patterns of differential activation in the amygdala and dlPFC (CS + unpaired > CS–), and anxious youth showed similar degrees of activation to both stimulus type. The finding of expected discriminative activation in healthy comparisons' amygdala is consistent with prior imaging studies in youth (Haddad et al., 2015; Lau et al., 2011); this suggests intact ability to inhibit fear responses to the safety cues (CS–) compared to anxious patients (Duits et al., 2015). Interestingly, for at-risk youth, pattern of differential activation observed in the amygdala involved deactivation to the threat cue (Fig. 2), which has been previously reported in healthy compared to anxious youth (McClure et al., 2007; Thomas et al., 2001). A pattern of increased PFC activation in tandem with reduced activation in the amygdala occurs when individuals successfully use an emotion regulation strategy (Frank et al., 2014). The current findings suggest that potential resilience in the face of familial risk for anxiety may reflect youths' ability to appropriately deactivate the amygdala in response to threat cues.

Unique engagement of the dlPFC in response to the safety cue (CS– > CS + unpaired) during conditioning may also represent a resilience marker in unaffected youth at high familial risk. This is particularly relevant in the context where our participants have been asked to rate their internal fear state to each stimulus. Indeed, involvement of the dlPFC occurs when high-level cognitive processes regulate emotion (Ochsner & Gross, 2005). Consistent with findings in healthy comparisons, emotional regulation during fear conditioning elicited greater activation to the CS + unpaired in the dlPFC of healthy adults (Delgado et al., 2008). In addition, Lau et al. (2011) reported that greater dlPFC activation to the safety cue (*vs.* CS + unpaired) predicted less fear to the CS− in adolescents. Greater dlPFC activation to the CS– in at-risk youth may reflect a more challenging but efficient regulation of emotional state in the safety condition. Longitudinal studies among youth will be required to more definitely identify brain-based resilience markers.

In anxious youth, prior studies find altered recruitment of the amygdala and dlPFC to the safety cue during conditioning, viewed is a sign of perturbed development in pediatric anxiety (Haddad et al., 2015). These findings support the involvement of the dlPFC in down-regulation mechanisms and reinforce the hypothesis of altered or immature dlPFC functioning among anxious youth.

During extinction, impaired ability to inhibit amygdala hyperactivity was observed in anxious youth compared to healthy comparison and at-risk youth. This impairment manifested as greater activation to both the CS + unpaired and the CS–, relative to the low-level baseline. Sustained amygdala activation during extinction is consistent with a neural correlate of anxiety (Barrett & Armony, 2009; Milad et al., 2009; Sehlmeyer et al., 2011). Moreover, absence of threat-safety discrimination in anxious youth's amygdala may reflect fear generalization (Lissek, 2012), suggested as one of the more robust conditioning markers of clinical anxiety.

Regarding autonomic and behavioral measures, expected anxiety-related deficits in inhibitory processes on behavioral and autonomic fear indexes (Duits et al., 2015; McGuire, Orr, Essoe et al., 2016) was supported. Indeed, the anxious youth showed greater fear ratings to both threat and safety cues during conditioning and extinction relative to healthy comparisons, whereas the at-risk group did not differ from either group (Fig. 5B). Consistent with prior work in youth (Lau et al., 2008), this result occurred in the context of similar discriminative fear levels across groups. Furthermore, greater SCR arousal to the safety cue (*vs.* CS+) was observed for both the at-risk and the anxious groups relative to healthy comparisons. Impaired extinction on SCR was previously reported in both anxious youth and offspring at risk for anxiety (Craske et al., 2008; Waters et al., 2014). However, the at-risk group differed from both the healthy and anxious groups in their ability to decrease SCR from early to late conditioning (Fig. 5A), suggesting successful but delayed fear extinction in offspring at risk for anxiety (Waters et al., 2014). This finding suggests a distinct autonomic resilience marker in offspring at risk for anxiety. Since successful extinction of psychophysiological fear responses was identified as predictors of outcomes from cognitive behavioral therapy in anxious children (Waters & Pine, 2016), orienting youth presenting risk markers for anxiety to a prevention program holds great potential.

Some limitations should be addressed. First, the number of participants per group was relatively small (n = 22–32); further imaging studies in pediatric population is important to replicate these results. Second, as maturation is associated with greater recruitment of the insular cortex and PFC regions during fear conditioning and threat processing (Blackford & Pine, 2012; Haddad et al., 2015; Lau et al., 2011; Yurgelun-Todd & Killgore, 2006) and that conditioning and extinction undergo large changes over this age range, age range within participants (10–17 years old) may have influenced data gathered in this study, despite statistical control for age in fMRI analysis. Third, absence of group difference on our contrast of interest (CS+ unpaired *vs.* CS–) during extinction may reflect an early extinction process during conditioning. Using a shorter conditioning phase may have partly countered this effect (e.g., see Schiele et al., 2016). In the same vein, further research will be needed to characterize the influence of online fear ratings on emotional regulation processes. Finally, we acknowledge that our interpretations regarding the fear circuit function are inferences based on our a-priori hypothesis. Future studies using functional connectivity, such as psychophysiological interaction (PPI; McLaren, Ries, Xu, & Johnson, 2012) or dynamic causal modelling (DCM; Friston, Harrison, & Penny, 2003), will be needed to determine the functional role of PFC regions over the amygdala or behavioral measures.

The current study investigated the neural fear circuit in relation to pediatric anxiety and the familial risk of developing an anxiety disorder using fear conditioning and extinction tasks. Findings during fear conditioning provides support for proper functioning of the fronto-amygdala circuit in healthy youth. Of clinical relevance, potential resilience markers for anxiety disorders were highlighted in the amygdala and dlPFC during fear conditioning. Moreover, successful extinction of fear responses on neural, autonomic, and behavioral fear indexes appears to differentiate offspring at risk for anxiety from affected youth. Nevertheless, this work should draw attention to compromised down-regulation mechanisms observed in this high risk pediatric population. Our data further suggest that increasing involvement of dACC, vlPFC, and dlPFC in response to threat, as well as lowering persistence of amygdala activation, could represent potential targets for future clinical research in pediatric anxiety.

Disclosures

No disclosures were reported.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopsycho.2019.107744.

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