Published in final edited form as:

Am J Psychiatry. 2013 October 1; 170(10): 1195–1204. doi:10.1176/appi.ajp.2013.12050651.

Response to learned threat: an fMRI study in adolescent and adult anxiety

Jennifer C. Britton, Ph.D.^{1,*}, Christian Grillon, Ph.D.¹, Shmuel Lissek, Ph.D.^{1,2}, Maxine A. Norcross, B.S.¹, Kristin L. Szuhany, B.S.¹, Gang Chen, Ph.D.¹, Monique Ernst, M.D., Ph.D.¹, Eric E. Nelson, Ph.D.¹, Ellen Leibenluft, M.D.¹, Tomer Shechner, Ph.D.¹, and Daniel S. Pine, M.D.¹

¹National Institute of Mental Health, Bethesda, MD

²Department of Psychology, University of Minnesota

Abstract

Objective—Poor threat-safety discrimination reflects prefrontal cortex dysfunction in adult anxiety disorders. While adolescent anxiety disorders are impairing and predict high risk for adult anxiety disorders, no prior study examines neural correlates of threat-safety discrimination in this group. The current study compares prefrontal cortex function in anxious and healthy adolescents and adults following conditioning and extinction, processes requiring threat-safety learning.

Method—Anxious and healthy adolescents and adults (n=114) completed fear conditioning and extinction in the clinic. Conditioned stimuli (CS+) were neutral faces, paired with an aversive scream. Physiological and subjective data were acquired. Several weeks later, 82 participants viewed the CS+ and morphed images resembling the CS+ in a magnetic resonance imaging (MRI) scanner. During scanning, participants made difficult threat-safety discriminations while appraising threat and explicit memory of the CS+.

Results—During conditioning and extinction, anxious groups reported more fear than healthy groups, but patient groups did not differ on physiology. During imaging, both anxious adolescents and adults exhibited lower sub-genual anterior cingulate (sgACC) activation than healthy peers, specifically when appraising threat. In ventromedial prefrontal cortex (vmPFC), relative to their age-matched peer groups, anxious adults exhibited reduced activation when appraising threat, whereas anxious adolescents exhibited a U-shaped pattern of activation, with greater activation to the most extreme CS and CS−.

Conclusions—Two regions of the prefrontal cortex are involved in anxiety disorders. Reduced sgACC engagement is a shared feature in adult and adolescent anxiety disorders, but vmPFC dysfunction is age-specific. The unique U-shaped pattern of vmPFC activation in many anxious adolescents could reflect heightened sensitivity to threat and safety conditions. How variations in the pattern relate to later risk for adult illness remains to be determined.

*Correspondence: Jennifer C. Britton, Ph.D., University of Miami, Department of Psychology, 5665 Ponce de Leon Blvd., Flipse Building 354, Coral Gables, FL 33146, 305-284-4943, Fax: 305-284-4795, j.britton@miami.edu.

Previous versions of these findings were presented at annual conferences of the American College of Neuropsychopharmacology (ACNP) in Miami, Florida in December 2009 and in Waikoloa, Hawaii in December 2010 and of the Anxiety Disorders Association of America (ADAA) in Baltimore, Maryland in March 2010 and in New Orleans, Louisiana in March 2011. In addition, the skin conductance results during fear acquisition partially overlaps with data published in Lau et al., Proceedings of the National Academy of Sciences, 2011. Our data are presented as an applied example of the methodology described in Chen et al., 2013.

Disclosures

All authors report no competing interests.

INTRODUCTION

In adult anxiety disorders, poor threat-safety discrimination is thought to arise from prefrontal cortex dysfunction that can be ameliorated by exposure therapy (1). Adolescent anxiety disorders are impairing, predict high risk for adult anxiety disorders, and, like adult anxiety disorders, respond to exposure therapy (2, 3). However, while research has identified the neural correlates of threat-safety discrimination in adult anxiety disorders (4), there are no parallel studies in adolescents. The current study uses conditioning, extinction, and a threat-safety discrimination paradigm to compare prefrontal cortex function in anxious and healthy adolescents and adults (5).

Conditioning and extinction are often used in animals to study fear and safety learning (6, 7). In fear conditioning, a previously-neutral conditioned stimulus (CS+) acquires threat value after it is paired with an aversive, unconditioned stimulus (UCS). In extinction learning, the CS+ is presented in the absence of the aversive UCS, typically resulting in a new CS+-safe association. The level of fear experienced in response to subsequent encounters with the CS+ is thought to reflect competition between memory for the original threat and subsequent safety associations.

Cross-species work maps circuits supporting memory for an extinguished CS+ to the ventral prefrontal cortex (8). When humans encounter an extinguished CS+, they engage the subgenual anterior cingulate (sgACC) and ventromedial PFC (vmPFC), two medial prefrontal cortical subregions. Moreover, levels of activation are lower in anxious than in non-anxious adults (4, 9). However, no research examines prefrontal cortex responding to an CS+ following fear conditioning and extinction in healthy or anxious adolescents. To do so, we adapted previously-used paradigms in which neutral faces served as the CS(10, 11), and a scream served as the UCS (12, 13). While prior fMRI studies in healthy youth used our paradigm to study conditioning (13), we instead compared neural responses to a threat following conditioning and extinction in healthy and anxious adolescents and adults.

First, we studied participants in the clinic to compare responses during fear acquisition and extinction. Based on prior work, we hypothesized that, compared to healthy individuals, anxious individuals would condition similarly, measured by relative differences between the CS+ and CS-, but rather would show greater subjective anxiety to both CS+ and CS-. We further expected both anxious groups to show lower levels of extinction than healthy groups (14).

Next, we used functional magnetic resonance imaging (fMRI) to map brain regions engaged during exposure to an array of CS+/CS- stimuli three weeks after extinction. This array contained images created by morphing the previously-extinguished CS+ with the CS- in incremental levels. Research participants were asked to make fine-grained distinctions among these stimuli during scanning. Specifically, the paradigm required subjects to appraise the level of threat conveyed by each stimulus, a process highly relevant to clinical anxiety (5). This task was expected to engage ventral prefrontal cortex, given the role of this region in threat-safety discrimination.

Prior literature suggested two sets of hypotheses. Adolescent anxiety disorders predict high risk for adult anxiety disorders, and exposure therapy appears similarly beneficial in the two age groups. Therefore, we hypothesized that anxiety disorders in the two age groups would manifest overlapping areas of prefrontal cortex dysfunction. However, prior work on the neural circuitry mediating normal types of fear finds age-related differences in prefrontal cortex function (12). Moreover, only a subset of adolescent anxiety disorder persists into adulthood (2). As a result, we also expected to observe age-related differences in prefrontal cortex function in both the healthy and anxious groups.

METHODS

Participants

Youth and adults participated as paid volunteers. After describing the study fully, written informed consent from adult participants and parents and written assent from youth participants were obtained. Procedures were approved by the National Institute of Mental Health Institutional Review Board.

Individuals were studied if they were medication-free, physically healthy, and had an IQ > 70. All participants received a comprehensive psychiatric assessment, Structured Clinical Interview for DSM-IV-TR (SCID) for adults or Kiddie Schedule for Affective Disorders and Schizophrenia (KSADS-PL) for youth under 18 years old (15, 16). Parents and youth were interviewed separately and discrepancies were reconciled with both individuals. Healthy participants were free from any current Axis I disorder. Anxious groups met DSM-IV criteria for a primary diagnosis of either generalized anxiety disorder (GAD) or social phobia. To be consistent with prior work, additional anxiety disorders and major depressive disorder (MDD) were permitted; however, current or past diagnoses of post-traumatic stress disorder or obsessive-compulsive disorder or other co-morbid disorders were exclusionary.

Procedures

This study adapts a differential fear conditioning and extinction paradigm used in earlier work (13). During fear acquisition, individuals passively viewed neutral faces of two women, the conditioned stimuli (CS). One woman, the CS+, predicted the UCS, a fearful face coterminating with an aversive scream, while the other woman, the CS-, did not. Participants were instructed that they could learn to predict when the UCS would occur but were not informed of the CS/UCS contingency. During extinction, the CS+ and CS- were presented repeatedly in the absence of the UCS (Figure 1). Skin conductance response (SCR), startle response, and self-reported anxiety were used to assess fear acquisition and extinction. Responses were measured to the CS+, CS-, UCS and the inter-stimulus interval (ISI) (Supplemental Information).

Three weeks after fear conditioning and extinction (19.7 ± 9.7 days), participants underwent MRI scanning. The MRI sample included 14 anxious youth, 15 anxious adults, 25 healthy youth, and 28 healthy adults (Table 1 and Supplemental Information).

The current fMRI paradigm was explicitly designed to capture precisely the transitional boundary between threat and safety through the use of morphed images (17–19) (Figure 1). In blocks of trials, participants viewed the CS+ and CS- and nine morphed images consisting of different blends of the CS- and CS+. This stimulus array was used to measure response gradients (e.g., linear or quadratic trends) along a continuum (19, 20). Additionally, prior research on recall in clinical anxiety used passive viewing to mimic animal work; however, passive viewing fails to engage psychological processes which are particularly relevant to clinical anxiety, such as the internal focus on subjective fear levels (i.e., threat appraisal) (5). Threat appraisal can be assessed and compared to other psychological processes by directing participants to focus on a particular question in regard to each face. Therefore, for each block, participants used a button press to answer one of three yes/no questions in response to each face: 1) Are you afraid now? (threat appraisal); 2) Did she scream in the past? (explicit memory); and 3) Is her hair jet black? (perceptual discrimination).

Using linear mixed-effects, random-effects analysis in AFNI (21), statistical interactions were tested among between-group factors [diagnosis (anxious, healthy), age-group (adult, youth)], within-group factors [cognitive instruction (threat appraisal, explicit memory,

physical discrimination) and covariates [linear (Morph-Level), quadratic (Morph²-Level) trends across morphed images]. The linear and quadratic trend regressors consisted of the morphed level for each image and its square, respectively. In addition, scanner and days between fear conditioning and scanning also were included as covariates of non-interest. Interactions were tested at the whole-brain level and in the ventral region of medial prefrontal cortex, an *a priori* region. Due to their relevance to fear conditioning and extinction, the amygdala and hippocampus were also examined. In our omnibus analysis, we corrected for multiple comparisons by performing a family-wise error (FWE) approach at the cluster level through Monte Carlo simulations. The cluster threshold probability was set at $\alpha = 0.05$ with a voxel-wise p-value of 0.005. Statistical significance was defined as 90 voxels (1406 mm³) for whole brain. To restrict the search territory, regional masks of ventral regions of the medial prefrontal cortex, encompassing both sgACC and vmPFC, (11453 mm³, effectively a 14 mm radius sphere) and amygdala/hippocampus (1828 mm³, effectively a 7 mm radius sphere) were used to determine the cluster threshold for *a priori* regions. Based on calculations, the cluster threshold was 15 voxels (234 mm³) for medial prefrontal cortex and 3 voxels (47 mm³) for the amygdala and hippocampus. These thresholds were determined using AFNI's AlphaSim program using methods described in Supplemental Materials.

Post-hoc tests were conducted within each significant cluster identified by higher level interactions (i.e., diagnosis $\times$ age-group $\times$ instruction $\times$ Morph²-Level). Percent-signal change values, relative to baseline, were averaged across voxels within each significant cluster for each of the 33 effects of interest for all individual subjects. Using these extracted values in SPSS, linear mixed model analysis and post-hoc tests with Bonferroni correction decomposed group differences that had emerged from the omnibus analysis. These post-hoc tests determined group differences in activation levels and patterns of response (i.e., significant linear and quadratic trends) when participants were directed to different cognitive states (i.e., threat appraisal, explicit memory). Behavioral data were analyzed in a similar manner (Supplemental Information).

RESULTS

Several noteworthy outcomes emerged following fear acquisition and extinction. We briefly summarize these effects here, and provide further details in Supplemental Information. First, more anxious than healthy individuals discontinued participation [diagnosis odds-ratio: 4.2, $p < 0.02$], as did more youth than adults [odds-ratio for a five-year increment in age: 10.3, $p < 0.001$]. Second, both anxious groups reported higher subjective ratings for both CS+ and CS- than healthy groups [$F(1,109) = 19.3$, $p < 0.001$]. During fear acquisition, both physiological and subjective measures showed robust fear conditioning (CS+ > CS-) with similar levels in all groups [SCR: mean difference between CS+ and CS- = 0.1, $t(99) = 4.8$, $p < 0.001$, EMG: mean difference between CS+ and CS- = 2.0, $t(113) = 4.3$, $p < 0.001$, subjective fear: mean difference between CS+ and CS- = 1.9, $t(113) = 7.9$, $p < 0.001$; all group effects $p > 0.1$]. Finally, during extinction, no diagnostic or age-group differences in conditioning levels were demonstrated using SCR [all $p > 0.1$]. However, anxiety-related differences in subjective response [diagnosis $\times$ age-group $\times$ phase $\times$ CS-type interaction: $F(2,218) = 4.5$, $p < 0.02$] and age-related differences in EMG response of conditioned fear were detected [age-group $\times$ phase $\times$ CS-type interaction: $F(4,440) = 2.6$, $p < 0.04$].

Behavioral data were collected during scanning several weeks later. Across all groups, a quadratic pattern of on-line ratings across stimulus was noted for threat appraisal [$\beta = 0.003$, SE = 0.001, $t(899) = 2.6$, $p < 0.001$] and explicit memory [$\beta = 0.008$, SE = 0.001, $t(899) = 7.5$, $p < 0.001$], albeit weaker for threat appraisal [instruction $\times$ Morph²-Level interaction: $F(2,1931) = 4.2$, $p < 0.01$]. Although behavior differed based on cognitive instruction, there

were no effects of diagnosis or age-group on these ratings during scanning [all $p > 0.2$] (Supplemental Figure 2). Additional behavioral data are included in the Supplemental Information.

For the fMRI data, whole-brain, random-effects analyses indicate four-way interactions (diagnosis $\times$ age-group $\times$ instruction $\times$ Morph²-Level) in two clusters within the medial prefrontal cortex: sgACC/BA25/BA32 [(-9, 26, -9), 29 voxels (453 mm³), $F(2,2592)=8.5$] and vmPFC/BA32/BA11 [(4, 49, -6), 43 voxels (672 mm³), $F(2,2592)=9.2$] (Figure 2). No other regions (i.e., amygdala or hippocampus) survived *a priori* cluster-level or whole-brain cluster-level correction.

To examine higher-level interactions in the significant clusters, the average percent signal change values, relative to baseline, from voxels within these clusters were extracted from individual subjects. Data for each cognitive instruction were examined separately (i.e., threat appraisal, explicit memory). See Supplemental Information.

Threat Appraisal

The sgACC and vmPFC patterns differed when participants were instructed to indicate whether or not they were afraid (i.e., threat appraisal). Relative to healthy subjects, anxious patients showed sgACC hypo-activation during threat appraisal [$F(1,207)=12.1$, $p < 0.001$ in full sample; adults: $F(1,207)=9.1$, $p < 0.003$, adolescents: $F(1,207)=3.7$, $p < 0.06$], with no diagnosis group $\times$ age-group interaction [$p > 0.5$]. The sgACC activation also did not vary across morphed images [$p > 0.5$] (Figures 3A and 4A).

In contrast, vmPFC activation during threat appraisal varied with both clinical anxiety and development. First, anxious adults exhibited a unique pattern [diagnosis $\times$ age-group: $F(1, 479)=6.1$, $p < 0.02$], showing vmPFC hypo-activation relative to the other three groups [$F(3, 188)=4.9$, $p < 0.003$; all $p < 0.04$]. Thus, for anxious adults, patterns of activation for vmPFC in threat appraisal resembled the pattern for sgACC. Second, anxious youth exhibited a unique pattern across morphed images [diagnosis $\times$ age-group $\times$ Morph²-Level: $F(1,540)=4.3$, $p < 0.04$]. Unlike the sgACC, the pattern in anxious youth for the vmPFC differed from the pattern for anxious adults in the vmPFC. Specifically, anxious youth manifested greater activation to the morphed images at the extremes of the continuum relative to the blended images both relative to the healthy youth and the anxious adults [both $t(2536) > 2.2$, $p < 0.03$]. (Figures 3B and 4B). Thus, anxious youth exhibited a stronger “U-shaped” response across the morphed images than these groups.

Explicit Memory

The main purpose of the analyses for explicit memory was to demonstrate that findings in threat-appraisal, the more clinically-relevant state, were task-specific. Results suggested that patterns described above were specific to the threat appraisal task. Namely, when participants were instructed to remember the UCS (i.e., the explicit memory task), patterns of activation in the sgACC differed from those found in the threat-appraisal task. Specifically, anxious youth had sgACC hyper-activation compared to both healthy groups [diagnosis $\times$ age-group: $F(1,504)=5.2$, $p < 0.02$; $F(3,226)=5.2$, $p < 0.002$, both $p < 0.005$]. Moreover, unlike during the threat appraisal task, morph status affected sgACC activation during the memory task [diagnosis $\times$ age-group $\times$ Morph²-Level: $F(1,542)=6.4$, $p < 0.02$]. Specifically, quadratic effects of stimulus class differed in anxious and healthy youth groups, [$t(2667)=3.1$, $p < 0.002$] and between healthy adults and healthy youth [$t(2667)=2.2$, $p < 0.03$] (Figure 3C and Figure 4C).

Task demands also affected the vmPFC, albeit with somewhat more evidence of similarities between the threat appraisal and explicit memory tasks than for the sgACC. Thus, both age

and diagnosis influenced vmPFC responding on explicit memory task, as they had for the threat appraisal task; nevertheless, the nature of findings in explicit memory differed from those in appraisal. Specifically, anxious youth had signs of overall vmPFC hyper-activation, relative to other groups [$F(3,203)=10.2$, $p<0.001$; all $p<0.02$]. Additionally, healthy youth had significantly less deactivation than anxious adults [$p<0.05$]. Finally, unlike youth, both adult groups had a quadratic pattern of response across the morphed images [both $t(162)>2.4$, $p<0.02$]. This generated a significant difference in patterns across morphed images between healthy youth and both adult groups [both $t(2536)>2.3$, $p<0.03$] (Figures 3D and 4D).

DISCUSSION

In this study, clinically anxious and healthy adults and adolescents underwent fear conditioning and extinction in the clinic, followed by an fMRI scan while CS+ and similar-appearing stimuli were viewed in different attention states. In the clinic, healthy and anxious groups differed on overall self-reported fear and on changes in self-reported fear between conditioning and extinction. In the MRI scanner, two key findings emerged during events that required participants make difficult threat-safety discriminations when appraising threat. First, relative to healthy individuals, anxious adults and adolescents both exhibited hypo-activation in the sgACC, suggesting the presence of a shared feature in adult and adolescent anxiety disorders. In contrast, there were age-specific findings in the vmPFC. Specifically, relative to age-matched subjects, anxious adults showed less vmPFC activation, whereas anxious adolescents showed greater vmPFC activation to the most extreme CS+ and CS- (i.e., greater quadratic response).

Fear Conditioning and Extinction

Data on discontinuation rates and self-reported fear address the ecological validity of the paradigm. We observed high discontinuation rates in anxious youth, demonstrating the difficulty of studying fear in youth. While no adults discontinued, 49% of anxious and 14% of healthy youth did. Moreover, youth who discontinued tended to be more anxious than those who did not, suggesting that the paradigm evokes clinically-relevant avoidance. Prior research using fear conditioning (22, 23) and similarly aversive procedures in youth also finds high discontinuation rates (24, 25). Both anxious adults and youth reported more fear than their respective healthy peers to both the CS+ and CS-, across both conditioning and extinction. Moreover, relative to the healthy groups, the anxious groups reported less change in fear to the CS+ between conditioning and extinction. These findings also address the ecological validity. That is, had similarly low levels of fear been reported by both the anxious and healthy groups then the clinical relevance of the paradigm would be questioned. Alternatively, had consistently higher fear been reported in one patient group than the other then these differences could have impacted our fMRI findings.

During both conditioning and extinction, the pattern of results differed between the physiological data and self-reported fear. Indeed, such discrepant findings have appeared in prior studies (12, 14). While anxious and healthy groups differed on self-reported fear in the current study, these groups showed no such differences on measures of physiology, either during conditioning or extinction. This fear-conditioning study is the first to include both anxious youth and anxious adults in a single study, but previous research in each age group alone finds results similar to ours (4, 13). That is, prior studies report both between-group differences in overall levels of self-reported fear and the absence of differences in SCR or EMG (26, 27). Moreover, this discrepancy between self-reported fear and physiology is consistent with a wealth of other research documenting that individual differences in reported fear correlate weakly, if at all, with individual differences in SCR or EMG (28, 29).

Shared Features

Another result in the current study demonstrated sgACC hypo-activation during threat appraisal in both adult and adolescent anxiety-disorder patients, relative to their peer groups. Such findings are consistent with data implicating the sgACC in emotion regulation and signs of deficient emotion regulation in PTSD. For instance, other work in healthy adults linked sgACC engagement to effective emotion regulation (30, 31), and studies in post-traumatic distress disorder (PTSD) found lower sgACC function in patients than healthy adults during exposure to an extinguished CS+ (4).

Observations of sgACC hypo-activation in both the current study and in prior studies of anxious adults are notable, given cross-study differences in methodology. For example, shock was used as the UCS in the adult studies, but not here due to concerns about potential harm in adolescents. Moreover, prior studies contrasted sgACC activation to extinguished versus non-extinguished CS+ (4, 32). Rather than contrasting such distinct types of CS+, the current study used stimuli with various degrees of resemblance to a single CS+. Finally, whereas prior studies in adults used passive viewing, the current study employed an explicit task adapted from prior studies, which was designed to demonstrate engagement of a disorder-relevant process. This decision followed best-practice recommendations for fMRI research in difficult-to-study populations, such as adolescent anxiety disorders (33). In any case, the observation of sgACC hypo-activation across methodologically-different studies demonstrates the presence of a replicable association in adult anxiety disorders. Our work now extends this observation to adolescents.

By comparing adolescents and adults, the current study bridges longitudinal findings on the outcome of adolescent anxiety disorders with fMRI findings on prefrontal cortical dysfunction in adult anxiety disorders. In longitudinal research, most adults with anxiety disorders also met criteria for an anxiety disorder in adolescence. Such findings suggest similar neural correlates in adolescent and adult anxiety disorders. As noted above, fMRI research in adults reports reduced sgACC function in the context of deficient fear learning, and the current study is the first to compare directly fear learning among anxious and healthy adolescents and adults. It is important to do so, since healthy adolescents often differ from healthy adults in fMRI studies of emotional processes. For example, a recent report compared healthy adults and adolescents using fMRI with fear conditioning procedures employed in the current study. This prior report found that functioning differed among healthy adults and adolescents in both the amygdala and prefrontal cortex (12).

Our work showing sgACC dysfunction in both adolescents and adults with anxiety disorders is also relevant to research in both animal models and novel therapeutics. For example, rodent studies find that lesions to the infra-limbic cortex, a possible rodent homolog of the sgACC, interfere with extinction (7, 34). Such findings have generated interest in novel treatments for adult anxiety disorders that might reduce anxiety through increasing sgACC function; our findings suggest that such treatments might also be effective in adolescent anxiety disorders.

Age-Specific Features

Another result in this study concerned age-specific dysfunction in vmPFC. Whereas anxious adults exhibited vmPFC hypo-activation during threat appraisal across the range of stimuli, anxious adolescents exhibited enhanced vmPFC response to the most extreme CS+ and CS−. This pattern could reflect heightened sensitivity in anxious adolescents to novelty, predictability, safety (26) or dissimilarity (19), especially when appraising threat.

These data raise questions about other differences between adolescent and adult anxiety disorders. As noted above, adolescent anxiety disorders predict high risk for adult anxiety

disorders. Nevertheless, there is also discontinuity in longitudinal outcome. While the overwhelming majority of adults with an anxiety disorder suffered from an anxiety disorder in adolescence, only about half of adolescents with an anxiety disorder continue to manifest anxiety as adults (2). This divergence suggests that adolescent anxiety disorders are heterogeneous, including sub-groups that will mature to account for the majority of adult anxiety disorders and other subgroups that will remit. Differences in vmPFC function among anxious adolescents and adults could relate to these varying longitudinal trajectories. For example, changes in vmPFC function from adolescence through adulthood could influence anxiety symptom trajectories, thus mediating the adult outcome of adolescent anxiety disorders. Alternatively, the level of vmPFC dysfunction present in adolescence could define distinct subgroups of anxiety disorders and predict outcome. For example, the subgroup of anxious adolescents with vmPFC hypo-activation, a deficit characteristic of adult disorders, may be at particularly high risk for persistence. Other adolescents who show elevated vmPFC activation may experience a better long-term outcome. As noted in Figure 3B, vmPFC activation in anxious youth showed a quadratic pattern during the threat appraisal task, albeit with marked variability. The subgroup of anxious youth in Figure 3B with particularly low vmPFC activation, i.e., whose activation overlaps with that of anxious adults, might face high risk for adulthood anxiety.

Limitations

First, a broad age range in the current study may obscure developmentally-sensitive processes (35). For example, evidence suggests that it may be more difficult to extinguish conditioned fear in early- relative to late-adolescence (34). Second, the youngest subjects were the least likely to complete the procedures, raising questions on the degree to which comparable findings would occur with more tolerable procedures for younger patients. However, to generate comparable findings, it would be important for future work with alternative procedures to elicit robust group differences in avoidance and subjective fear, as did the current paradigm. Third, while group differences emerged in prefrontal cortical function, corresponding differences in task-elicited behavior were not found. Some investigators view the absence of such differences as a limitation, whereas others argue that important experimental confounds are removed (33). Fourth, data on peripheral physiology were acquired only during the first visit. Future studies should acquire physiological and neural data simultaneously, though including physiological data collection would lengthen the fMRI procedures and potentially reduce their tolerability. Finally, while anxious and healthy groups were matched on key variables, IQ differed in adolescents and adults. Nevertheless, IQ showed no associations with any dependent measure, and group differences in brain function persisted in sub-groups matched on both age and IQ.

Conclusions

No previous fMRI study compares healthy and anxious adolescents and adults in the context of a fear-learning paradigm. The current study specifically examines threat appraisal and explicit memory during exposure to CS+ in adolescents and adults following conditioning and extinction. Using a paradigm that evokes elevated fear in adolescents and adults with an anxiety disorder, we found sgACC hypo-activation in anxious adolescents and adults, compared to healthy adolescents and adults. Such hypo-activation occurred in both age groups, specifically when appraising the fear response. In contrast, vmPFC activation differentiated anxious adults and adolescents both from each other and from their age-matched healthy peers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported in part by the Intramural Research Program of the National Institute of Mental Health, National Institutes of Health (K99-MH-091183-01 awarded to JCB). We thank Marilla Geraci for assistance with recruitment and David Luckenbaugh for statistical assistance.

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Figure 1. Experimental Procedures

During fear acquisition, one female face (CS+) was paired with a fearful face co-terminating with a scream (UCS). The other female face (CS-) was never paired with the UCS. During extinction, the two female faces were presented without the UCS. Several weeks later, participants viewed morphed images continuously varying in similarity from the CS- to CS+. Participants reported whether they were afraid (threat appraisal), whether the CS screamed in the past (explicit memory), or whether the CS had jet black hair (physical discrimination).

CS=conditioned stimulus, UCS=unconditioned stimulus.

Figure 2. Whole-brain analyses indicate four-way interactions (diagnosis×age-group×cognitive instruction×quadratic trend)
 The subgenual anterior cingulate (−9, 26, −9) and the ventromedial prefrontal cortex (4, 49, −6) survive 15 voxel cluster threshold, defined using $p < 0.005$ voxel-wise probability, which yields a $p < 0.05$ corrected threshold for medial prefrontal cortical regions. Images are displayed in radiological convention (left=right). Green lines in the axial slice indicate position of sagittal slices.

Figure 3. Response patterns during threat appraisal and explicit memory

Group differences are plotted in the subgenual anterior cingulate cortex (sgACC) and ventromedial prefrontal cortex (vmPFC) during threat appraisal (Panels A and B, respectively) and during the explicit memory task (C and D, respectively). Panel A plots percent signal change values relative to baseline. Panel B–D plots the beta coefficient across morphed images for the quadratic response. *Group difference in neural activation.

^Significant quadratic trend across morphs #Group difference in the quadratic pattern.

Significance is defined as $p = 0.05$.

Figure 4. Response patterns across morphed images

To further illustrate effects in Figure 3, the neural patterns across morphed images are plotted in the subgenual anterior cingulate cortex (sgACC) and ventromedial prefrontal cortex (vmPFC) during threat appraisal (Panels A and B, respectively) and during the explicit memory task (C and D, respectively). For illustrative purposes, the percent signal change values, relative to baseline, for every two morphed images other than 0%, 50%, and 100% are averaged together. Only group differences in neural activation are reflected here (*). Significance is defined as $p = 0.05$.

Table 1

Group Characteristics

			N		Age (years) ^a				IQ ^a		Diagnoses (total)					Days Between Visits	
			Total	# males	Mean	SD	Range		Mean	SD	GAD	Social Phobia	Separation Anxiety	Panic	MDD ^a	Mean	SD
Fear Acquisition and Extinction	Anxious	Youth	23	12	13.3	3.3	8–19		108.4	12.6	18	11	6	2	4	-	-
		Adults	18	6	32.0	6.8	23–48		121.3	10.0	13	9	NA	2	0	-	-
	Healthy	Youth	42	19	14.2	2.6	8–17		112.4	11.7	-	-	-	-	-	-	-
		Adults	31	14	28.8	7.2	21–45		119.4	11.3	-	-	-	-	-	-	-
Recall	Anxious	Youth	14	7	14.8	2.8	11–19		108.0	10.9	11	9	2	1	4	18.5	6.8
		Adults	15	6	32.9	7.0	24–48		122.3	10.0	11	7	NA	1	0	20.9	6.6
	Healthy	Youth	25	10	14.4	2.6	9–17		111.7	10.5	-	-	-	-	-	22.1	12.6
		Adults	28	12	29.1	7.5	21–45		120.4	10.4	-	-	-	-	-	17.6	9.1

SD=Standard deviation. GAD = generalized anxiety disorder; MDD = major depressive disorder.

^aSignificant age-group difference, $p < .05$. In all initial analyses, IQ was included as a covariate of non-interest and results indicated IQ did not alter the significant findings.