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Altered Prefrontal Cortex Function Marks Heightened Anxiety Risk in Children

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Abstract

Objective—Anxiety disorders are prevalent and cause substantial disability. An important risk factor for anxiety disorders is inhibited temperament, the tendency to be shy and avoid new situations. Inhibited adults have heightened amygdala activation and less flexible engagement of the prefrontal cortex (PFC); however, it remains unknown if these brain alterations are present in inhibited children prior to the onset of anxiety disorders.

Method—Thirty-seven children (18 inhibited, 19 uninhibited), ages 8–10 years, completed a task testing anticipation and viewing of threat stimuli and social stimuli in the magnetic resonance imaging (MRI) scanner. Brain activation and functional connectivity were measured.

Results—During the anticipation of threat stimuli, inhibited children failed to show the robust PFC engagement observed in the uninhibited children. In contrast, when viewing social stimuli, inhibited children had increased medial PFC and dorsolateral PFC activation. Connectivity analyses revealed a pattern of reduced connectivity between prefrontal and limbic regions and among distinct PFC regions in the inhibited group. The medial PFC emerged as a key hub of the altered PFC circuitry in inhibited children.

Conclusion—This study provides new evidence of a neural signature of vulnerability to anxiety disorders. By investigating both anticipation and response to images, we identified that high-risk, inhibited children have widespread alterations in PFC function and connectivity, characterized by an inability to proactively prepare for social threat combined with heightened reactivity to social

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stimuli. Thus, children at high risk for anxiety show significantly altered prefrontal cortical function and connectivity before the onset of anxiety disorders.

Keywords

inhibited temperament; behavioral inhibition; fMRI; functional connectivity; anticipation

INTRODUCTION

Anxiety disorders are highly prevalent and cause substantial disability and economic burden. One in three Americans will suffer from an anxiety disorder,[1] the second leading mental health cause of global disease burden.[2] The long-term impact of anxiety disorders is especially pernicious because they have an early onset,[1] produce a long course of suffering, and often lead to comorbid depressive and substance use disorders.[3–5] Thus, prevention of anxiety disorders has the potential to substantially reduce the overall burden of disease.

For psychiatric disorders, prevention is considered one of the most important, yet still unsolved, problems. Neuroimaging studies of children at risk for developing psychiatric disorders—siblings of children with autism[6] or individuals with a schizophrenia prodrome[7]—show promise for identifying early brain differences associated with risk. For anxiety disorders, a risk phenotype has been well described; however, remarkably little is known about whether there are associated brain alterations. The risk phenotype—inhibited temperament (or behavioral inhibition)—is characterized by shy and cautious responses to novel situations and stimuli. Inhibited children have a significantly increased risk for developing anxiety disorders[8–15] and subsequent depression.[3] Identifying the neural substrates of inhibited temperament in young children holds promise for elucidating the neurobiological basis of anxiety risk.

To date, neuroimaging studies of inhibited temperament have largely focused on inhibited young adults or older adolescents who were inhibited as children, some of whom have an anxiety disorder (for a review, see[16]). In these studies, alterations in amygdala function have been well-established, including increased activation,[17,18] faster and sustained responses,[18–21] and abnormal modulation by attention state.[22,23] Inhibited adolescents and young adults show both increased and decreased prefrontal cortex (PFC) function across studies: decreased dorsal anterior cingulate cortex (dACC) and dorsolateral PFC (dlPFC) activation when viewing expected negative social stimuli;[22] increased rostral ACC (rACC) and dACC activation when anticipating fear faces;[24] and increased dorsomedial PFC, dlPFC, and rACC activity when performing a task that requires cognitive control.[25,26] A recent study in inhibited children found heightened dlPFC response during a threat executive-attention task.[26] Studies examining risk versus resilience in inhibited temperament have highlighted that greater PFC activity during emotional tasks that require cognitive control predicts resilience to anxiety disorders, suggesting that lack of PFC activity may be associated with anxiety risk.[16,24,27] During non-emotional tasks, over-control has also been associated with anxiety risk.[28] Finally, in patients with social anxiety disorder, a meta-analysis of functional magnetic resonance imaging (fMRI) studies shows hyperactivity of the amygdala, insula, rACC, mPFC and dlPFC, although it should be noted that the PFC

findings are mixed across individual studies and likely reflect task differences.[29] Thus, the amygdala and multiple prefrontal cortical regions have been implicated in inhibited temperament, anxiety risk, and social anxiety disorder.

While prior studies have made important contributions to our understanding of the neural basis of inhibited temperament in late adolescents and adults, the age groups studied were largely past the average age of onset of anxiety disorders and thus represented a heterogeneous sample of resilient individuals and individuals with past or current anxiety disorders. Studies in young high-risk children, prior to the onset of anxiety disorders, are critical for disentangling the neural markers of anxiety risk from the neural consequences (i.e., scar markers) of anxiety disorders. In the present study, we examine, for the first time, the neural correlates of anticipatory processing and stimulus viewing in 8- to 10-year-old inhibited children, prior to the development of anxiety disorders. We examined anticipatory processing since anticipatory anxiety is a hallmark of anxiety disorders[30] and is associated with activation of the prefrontal cortex, amygdala, and insula.[31] During anticipation of negative emotional events, patients with anxiety disorders fail to activate the PFC and have hyperactivity of the amygdala and insula.[30–32] Our working model is that during anticipation of fear faces, high-risk inhibited children will have decreased activity in brain regions involved in emotional regulation (ACC, mPFC, dlPFC) and increased activity in limbic-related regions (e.g., amygdala and insula).

METHOD

Participants

Forty children initially participated in this study (20 children with inhibited temperament and a comparison group of 20 children with an uninhibited temperament) and 37 children were in the final analytic sample (18 inhibited, 19 uninhibited). Consistent with the extreme discordant phenotypes approach,[33] we compared inhibited children and uninhibited children at the extreme ends to maximize our chances of identifying differences. To obtain pure risk groups (not confounded by existing disorders), children were excluded from the study for having any current or past psychiatric diagnoses, as measured by the Kiddie-Schedule for Affective Disorders and Schizophrenia - Present and Lifetime Version (KSADS-PL)[34] or having received treatment for anxiety symptoms. Children were also excluded if they had cognitive deficits that might affect task performance (developmental delay, repeating a grade, or receiving special assistance in school), contraindications to MRI scanning, or factors that might affect blood oxygen level-dependent (BOLD) signal (psychotropic medications, history of head injury, major medical or neurological conditions). Intelligence quotient (IQ) was assessed using the Kaufman Brief Intelligence Test.[35] Handedness was assessed using the Edinburgh Handedness Inventory.[36]

There were no significant differences between groups in age, gender, ethnicity, handedness, or IQ (see Table 1). Parents provided informed consent, and children provided informed assent for participation in the study. The Vanderbilt University Institutional Review Board approved this study. Financial compensation was provided.

Recruitment and Screening—Participants were recruited from the Vanderbilt University Medical Center and surrounding community using flyers, emails, and research recruitment databases. Advertisements were for children who were "quiet," "cautious," "shy," "outgoing," and general recruitment for a study on "temperament and brain function." Prior to the first study visit, parents completed a brief online screening, including the Behavioral Inhibition Questionnaire-Parent (BIQ-P),[37] a validated measure of childhood inhibited temperament, which has been used for screening in a recent neuroimaging study[26] and shows convergent validity with behavioral measures and other measures of social inhibition.[37–39] Although four questions in the questionnaire refer to younger age groups, these questions were highly correlated with other items in the scale and therefore were retained as written. Children were selected based on a temperament score ± one standard deviation from the mean based on published norms (inhibited > 123; uninhibited < 59);[37] these norms were similar to those identified in children ages 4–15[39] and those used in a recent similar neuroimaging study.[26]

Temperament Measures—During the first study visit, behavioral interaction with an unfamiliar adult was measured, based on prior studies of inhibited temperament (for examples, see [40,41]). Children were brought into a room and were told that a "new experimenter was going to come in soon and ask them some questions." The unknown female experimenter asked a set of standard questions (~15 minutes). Following the interview, the experimenter rated global inhibited temperament and seven other measures (latency to respond, amount of speech, tense or uncomfortable behaviors, positive affect, negative affect, trust, and volume and tone of voice) on a 1–5 Likert scale, based on Ballespí et al.[42] Children also completed a self-report of temperament, the Behavioral Inhibition Questionnaire-Child (BIQ-C).[39]

Psychiatric Symptom Measures—To further characterize participants, both parents and children reported on a number of psychiatric symptom measures, including the Screen for Child Anxiety-Related Disorders,[43] Social Phobia and Anxiety Inventory for Children,[44] Retrospective Infant Behavioral Inhibition Scale,[45] Social Communication Questionnaire,[46] Children's Depression Inventory,[47] and Conners' 3 to measure symptoms of attention-deficit/hyperactivity disorder (ADHD).[48]

Experimental Design

Cued Anticipation Task—Anticipatory processing was assessed using a cued anticipation task. Children were trained to associate each of three cues (colored shapes) with specific image types (fear face, neutral face, neutral object). Successful learning was confirmed verbally. The test period consisted of four runs (Figure 1). Each trial included: cue (1s); anticipation period (jittered, 3–8s); image (1s); and blank screen (jittered, 3–8s) before the next trial. Each run consisted of eight trials of each type (fear face, neutral face, neutral object) for a total of 24 trials per run and 32 of each trial type across the entire task. Child faces from the National Institute of Mental Health Child Emotional Faces Dataset[49] were used. For the control stimuli, neutral objects were used—round non-social objects, the approximate size and shape of faces (i.e., a patterned bowl, a clock)—obtained from several

sources, including the International Affective Picture System image set,[50] iStockPhoto.com, and publically available images.

Task Accuracy—To provide a measure of attention to the task, children were asked to press one button during each of the 1s cue and image events. Across all cues and images, children accurately pressed the button within the 1s window 89.7% of the time. To ensure only events where children were paying attention to the task were included, we used a three-step method: 1) participant level: each participant was checked for greater than 50% button press accuracy for each event type across the entire task (fear face cue, neutral face cue, neutral object cue, fear face, neutral face, neutral object); 2 participants with lower than 50% accuracy for any one event type were excluded from the analyses (5% of all participants; 1 inhibited, 1 uninhibited); 2) run level: individual functional runs were excluded for less than 50% button press accuracy across all events (3.3% of total runs); and 3) event level: individual events were excluded if the button was not pressed during the 1s event (7.2% of all remaining events). Thus, only data with an accurate button press were included in the final analyses.

MRI Acquisition—Each child completed a mock MRI scan during the second study visit to acclimate the child to the scanner and improve data quality.[51] Data were collected using a 32-channel headcoil on a Philips 3 Tesla scanner. T1-weighted structural data were acquired using the following parameters: 256 mm FOV, 170 slices, 1 mm slice thickness, 0 mm gap, 2s TR, 22ms TE, 90° flip angle, 1.8 SENSE factor, 240 mm F OV, 3×3 mm in plane resolution. Functional (echo-planar imaging [EPI]) data were acquired using a sequence optimized for the temporal lobe and orbitofrontal cortex with the following parameters: 40 slices, 2.5 mm slice thickness, 0.25 mm gap, and an axial oblique acquisition, tilted 15 degrees, anterior higher than posterior, relative to the intercommisural plane.

MRI Preprocessing—Data preprocessing was performed in Statistical Parametric Mapping – Version 8 (SPM8; http://www.fil.ion.ucl.ac.uk/) implemented in Matlab 2010a software (Version 7.10.0, Mathworks, Inc., Natick, MA). Preprocessing steps included: slice time correction to the middle slice; realignment of functional volumes to mean volume; coregistration of functional and structural scans; normalization of functional scans to EPI template; and smoothing with a 6mm full width at half maximum (FWHM) kernel. For each participant, scans were checked for data quality; functional and structural data were visually inspected for artifacts, coverage of brain regions, and signal dropout. One inhibited participant was excluded for functional data artifacts. Thus the final analytic sample included 18 inhibited and 19 uninhibited participants. Among those included in the analyses, certain functional runs were also excluded for: artifact on visual inspection (1.1%), or an incomplete run in the scanner (2.2%). All participants had at least two runs of data included in the analyses. To control for motion, we used the Robust Weighted Least Squares (rWLS) toolbox.[52] rWLS uses standard robust methods to weight the contribution of each volume using the inverse of the variance, thereby reducing the statistical influence of motion outliers without removing data or disrupting the temporal sequence of the data. Overall average maximum displacement due to motion per run was low and there were no significant differences between groups in motion (inhibited [IT]: 1.34 mm translation, .024 radians

rotation; uninhibited [UT]: 1.11 mm translation, .019 radians rotation; translation: p = .62; rotation: p = .30; see Supplement 1 for additional details, available online). For all participants with >10mm maximum displacement, the performance of rWLS was reviewed by visually comparing the inverse variance maps across time for each participant.

FMRI Data Modeling—For each participant, a general linear model was created in SPM8 with seven regressors: fear face cue, neutral face cue, neutral object cue, fear face image, neutral face image, neutral object image, and errors. Six contrasts were created for each participant (each condition vs. baseline): fear face anticipation; neutral face anticipation; neutral object anticipation; fear face viewing; neutral face viewing; and neutral object viewing.

Regions of Interest—Analyses were restricted to five regions of interest (ROIs) to focus the analyses on hypothesized regions and reduce the number of overall comparisons. The ROIs were the amygdala, insula, dorsolateral prefrontal cortex (dlPFC), medial prefrontal cortex (mPFC), and anterior cingulate cortex (ACC; see Supplement 1, Method, for definitions, available online). Within each region, data were tested for group voxel-wise differences. Data were cluster-corrected ($\alpha < .05$) using the AFNI 3dClust function with actual smoothing and 5,000 iterations. Cluster thresholds included (averaged across left and right sides, midline regions were considered bilaterally) with a voxel p-value of .05: amygdala (k = 20; 540 mL), insula (k = 69; 1,863 mL), dlPFC (k = 164; 4,428 mL), mPFC (k = 111; 2,997 mL), and ACC (k = 79; 2,133 mL).

Whole Brain Analyses—To determine if there were any additional regions that showed a temperament x emotion interaction, an exploratory voxel-wise whole brain analysis was conducted. The cluster threshold was calculated using 3dClust using the whole-brain mask image created by SPM, 3x3x3mm voxels, intrinsic smoothing, an FWE rate of .05 and 5,000 iterations. For this analysis, a voxel p-value of .005 and a cluster size of k = 99 (2,673mL) provided an FWE-corrected p-value of .05.

Functional Connectivity—In order to further elucidate key neural networks, follow-up analyses were performed to identify patterns of connectivity with the brain regions that showed significant temperament effects in activation (entire cluster of temperament difference was used as the seed). A general psychophysiological interaction (gPPI) analysis[53] was performed with three regressors: the psychological regressor, which was the difference between activation during task, relative to baseline; the physiological regressor, the time series extracted from the seed region; and the interaction of the task and the time series. Since the interaction term in the gPPI is statistically independent from the main effect of task, this analysis is statistically independent from the task findings. The interpretation of the functional connectivity results focused on the inhibited group, based on evidence that the beta estimates do not have an absolute value meaning[54] and can only be discussed in relative terms.

Image Rating—Following the MRI scans, children rated the valence of the cues and of a subset of images (10 fear faces, 10 neutral faces, 10 neutral objects). Half of the selected faces were female and half were male. Children were instructed to rate how “happy or sad

the pictures made them feel" (1 = very happy; 5 = very sad). Image ratings were accompanied by schematics of each rating (i.e., "1" was presented above a smiley face). The cues and images were presented using Eprime software outside of the scanner. Valence rating data were missing for one uninhibited child due to technical problems.

Data Analysis

Behavioral Data—The behavioral data—button press hit rate (i.e., completed button press during the 1s stimulus), button press reaction time, image valence rating—were analyzed using a general linear model with type (cue/image) and condition (fear face/neutral face/neutral object) as within-subject variables and temperament group as the between-subject variable. As a manipulation check, the image valence rating data were analyzed using a general linear model with type and condition. SPSS (Version 21.0.0.0, IBM Corporation) was used for data analysis with $\alpha = .05$.

Statistical Analysis—Within-subjects effects of condition were modeled using a flexible factorial model with the within-subjects effects explicitly modeled in SPM8.[55] Analyses of variance (ANOVAs) were conducted to test for an interaction of temperament x condition during anticipation and face viewing. To understand how each condition contributed to each interaction, post-hoc analyses were conducted. Effect estimates (percent signal change for fMRI and beta values for gPPI) were extracted from the significant clusters for the fear face, neutral face, and neutral object contrasts using EasyROI toolbox (http://www.sbirc.ed.ac.uk/LCL/LCL_M1.html).

RESULTS

Temperament and Behavioral Data

The inhibited children were significantly more inhibited than uninhibited children on parent report, behavioral assessment, and self-report ($p < .001$; Table 1). The three measures of temperament—parent report, child report, and behavioral ratings—were significantly correlated (Table S1, available online). Parent and child reports of anxiety symptoms were significantly correlated and were also correlated with temperament measures (Table S1, available online). Inhibited children had more anxiety symptoms; however, anxiety levels were below the clinical cut-offs/norms (Table S2, available online). There were no significant group differences in social communication skills, hyperactivity, or depression (Table 1).

Inhibited and uninhibited children showed similar ratings on reaction times and valence ratings overall and by stimulus type, condition, and type x condition (Table S3, available online). There was an interaction of temperament x condition (fear face versus neutral face) on the hit rate, whereby inhibited children were significantly more likely to miss a neutral face cue than a fear face cue (neutral face cue: 8.0% ± 10.1%; fear face cue: 4.1% ± 5.9% missed; $p = .04$).

Task Analyses

Region of Interest Analyses—During anticipatory processing, there were significant temperament x condition interactions in two prefrontal cortical regions—the rostral portion of the anterior cingulate cortex (ACC) and the medial prefrontal cortex (mPFC; $p < .05$, corrected; Figure 2; see Table S4, available online). Post hoc analyses revealed that uninhibited children showed a robust pattern of prefrontal engagement during anticipation of fear faces that was absent in inhibited children (see Figure S1, available online).

During face viewing, temperament x condition interactions were observed in the mPFC and bilateral dorsolateral PFC ($p < .05$, corrected; Figure 2; see Table S4, available online). Post hoc analyses showed that inhibited children had heightened prefrontal cortical reactivity to faces (both fear and neutral); in contrast, uninhibited children showed relatively little neural response to viewing faces.

Whole-Brain Analyses—In the whole-brain analyses, significant temperament x condition interactions were found in the mPFC, right dlPFC, frontal pole, and precuneus during face viewing ($p < .05$, corrected; see Table S4, available online). There were no whole-brain temperament x condition differences during anticipatory processing.

Functional Connectivity Analyses

In the follow-up functional connectivity analyses, we examined connectivity with the key regions identified in the task analyses. During anticipation of faces (both fear and neutral), inhibited children had reduced mPFC-amygdala connectivity compared to uninhibited children (both $p < .05$, Figure 3, see Table S5 and Figure S2, available online). During anticipation of fear faces specifically, inhibited children had increased mPFC-insula and ACC-insula connectivity compared to the uninhibited children.

During viewing of faces (fear and neutral), inhibited children had reduced connectivity between dlPFC-ACC and mPFC-insula relative to uninhibited children. During fear face viewing specifically, inhibited children had decreased connectivity between the dlPFC-ACC and dlPFC-mPFC but increased connectivity between the mPFC-ACC ($p < .05$, Figure 3; see Table S5 and Figure S2, available online). Thus, widespread alterations in connectivity during anticipation and face viewing were observed between prefrontal regions and limbic regions as well as among prefrontal regions.

DISCUSSION

Here, we provide the first report, to our knowledge, of the neural correlates of anticipatory processing and face viewing in young children at high risk for developing anxiety disorders. We identified an altered prefrontal cortex (PFC) response associated with anxiety risk. Uninhibited children demonstrated a proactive pattern, with increased PFC engagement during fear anticipation followed by disengagement during fear face viewing, suggesting that they had successfully prepared to view fear faces during the anticipation period. In contrast, the inhibited children had a similar pattern across all event types during anticipation—they failed to engage PFC regions and instead had heightened PFC activation when viewing all social stimuli, regardless of threat. This pattern suggests that inhibited

(high-risk) children were both unable to effectively prepare for threat and showed a delayed PFC response, emerging only in the context of social stimuli. The connectivity findings highlight the medial PFC as a key hub in the circuits underlying alterations in connectivity across all conditions. Thus, findings from this study provide evidence for a neural signature of anxiety risk in children that is characterized by delayed PFC engagement and reduced PFC-limbic connectivity.

The medial PFC region where both activation and connectivity differences were observed is similar to our previous study of inhibited adults;[24] however, the pattern of findings in children was unique, suggesting important developmental considerations. In the current study, uninhibited children showed significant prefrontal cortical activation during threat anticipation, whereas in our previous study, uninhibited adults showed relatively little brain activation during anticipation.[24] This age difference is consistent with findings in healthy individuals[56] and suggests a developmental shift, whereby uninhibited children respond to the anticipation of mild threat by proactive preparation, whereas for young adults, this preparation is either very rapid (and so undetected), or unnecessary. In the current study, inhibited children failed to engage the PFC during threat anticipation, whereas in the previous study, inhibited adults had heightened PFC activation during threat anticipation. Critically, the previous study included inhibited adults with and without anxiety disorders, and higher prefrontal cortex activation predicted lower anxiety symptoms and better coping skills.[24] The PFC undergoes protracted development[57–59] that parallels the development of cognitive control, and in neuroimaging studies, increased mPFC and dlPFC activity correlates with enhanced cognitive control and emotion regulation.[60,61] Thus, across development, differences in prefrontal cortical function likely emerge and the high-risk children who are able to engage the PFC in preparation for threat are likely to be the most resilient to developing anxiety. In this case, therapies that target the PFC may provide a novel approach to preventing and treating anxiety disorders in children.

While increased amygdala activation is a relatively consistent finding in anxious children with anxiety[62] and has been shown in anticipation tasks in children and adolescents with anxiety,[63–65] in this study inhibited children did not have hyperactive amygdala responses. Importantly, findings may depend on the task used to probe brain function. For example, two previous studies of anticipatory processing in inhibited adolescents and young adults failed to find temperament differences in amygdala function.[24,65] However, amygdala hyperactivity was observed in inhibited children using an executive-attention task.[26] Studies that include multiple different tasks will be instrumental in elucidating task effects. A second possibility is that amygdala hyperactivity is specific to anxiety[62] and is not a neural correlate of anxiety risk. Future studies are needed to examine the neural circuitry in inhibited children with and without anxiety disorders to isolate their unique effects.

These findings should be interpreted in the context of the study limitations. First, we used an extreme discordant phenotypes approach,[33] which maximizes the ability to find group differences in initial investigations. One limitation of this approach is that the full range of temperament values was not included; future studies should include children with average temperament scores. Another limitation is that the uninhibited children—while at low risk for developing anxiety—may be at increased risk for developing ADHD[66] or other

externalizing symptoms.[67] While children in this study would never meet criteria for ADHD (given that ADHD symptoms must be present by age 7), they may still be at heightened risk for other disorders. In order to isolate risk for disease, inhibited children with psychiatric disorders were also excluded from the study. One limitation of this approach is that the highest-risk children—those who had an early-onset anxiety disorder—were not represented in this sample. Studies of younger children and longitudinal studies will be necessary to disentangle the unique contributions of inhibited temperament and anxiety disorders to brain function. Finally, the connectivity analyses performed here focused on the observed differences from the task; although the connectivity findings are statistically independent from the task findings, it is important to acknowledge that the interpretations should be limited to this task and that replication in an independent sample is needed.

These findings point to a neural signature of anxiety risk characterized by prefrontal cortex hypoactivity during threat anticipation with a shift to prefrontal cortex hyperactivity during face viewing. High-risk children show specific alterations in engaging prefrontal cortical resources to prepare for an upcoming aversive event and hyper-reactivity to social stimuli. Studies in adults with anxiety disorders have shown alterations in prefrontal cortex function,[68] and here we show that alterations in prefrontal cortex activation may be a neural signature of anxiety risk. Following these children longitudinally will help us understand whether these altered PFC responses in the inhibited group increase vulnerability for anxiety disorders. Importantly, these findings highlight the need to focus on the PFC. Specifically, strategies that focus on rapid engagement of the prefrontal cortex during anticipation of threat may be beneficial for these high-risk children and may be a critical component of preventive interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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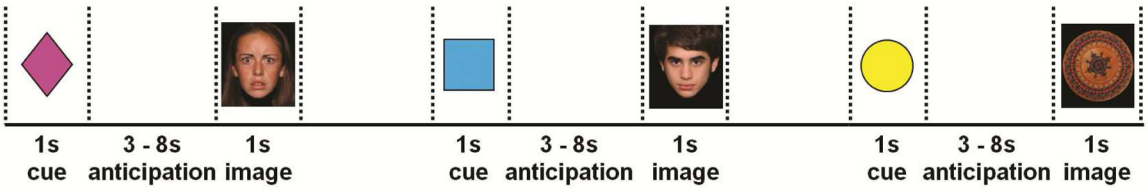

Figure 1.

Study design. Note: The task consisted of four runs of 8 fear face, 8 neutral face, and 8 neutral object trials for a total of 24 trials of each type. Each trial consisted of a cue (1s), an anticipation period (3–8s, jittered), and an image (1s). Following each trial, a 3–8s blank screen (jittered) was shown to allow for return to baseline. Fear face, neutral face, and neutral object trials were randomized within runs.

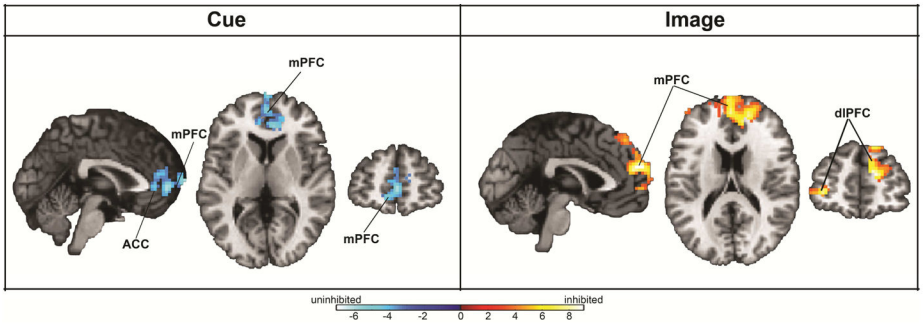

Figure 2.

Group differences in brain activation during anticipation and viewing of faces. Note: During anticipation there was an interaction of temperament x condition in the anterior cingulate cortex (ACC) and medial prefrontal cortex (mPFC) (all $p < .05$, corrected). When viewing faces, there was an interaction of temperament x condition in the mPFC and bilateral dorsolateral prefrontal cortex (dlPFC; $p < .05$, corrected).

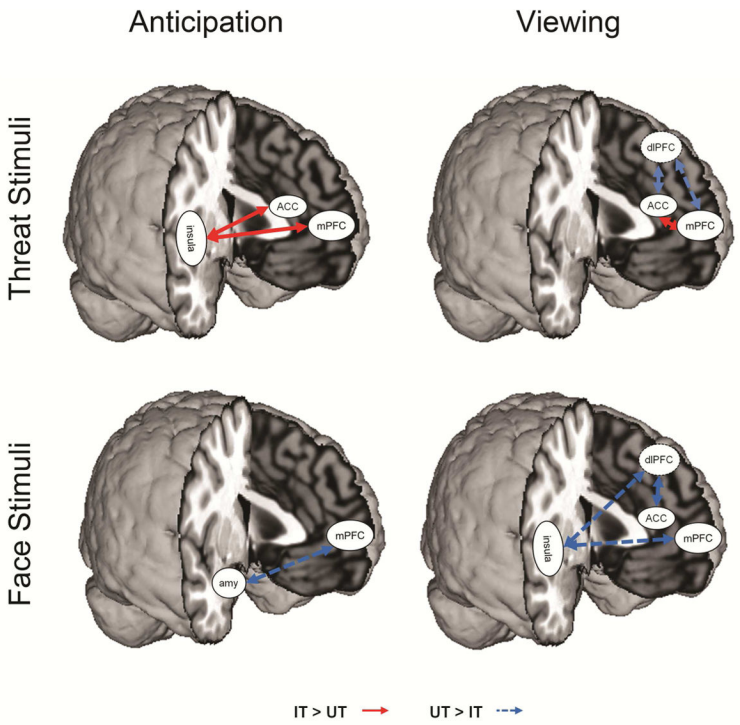

Figure 3.

Differences in connectivity during threat and face anticipation and viewing. Note: During anticipation of viewing threat, the inhibited group had stronger connectivity between the insula and anterior cingulate cortex (ACC) and medial prefrontal cortex (mPFC). When viewing threat, the inhibited group had less connectivity between the dorsolateral prefrontal cortex (dlPFC) and the ACC and mPFC. Connectivity between the ACC and mPFC was increased during threat viewing in the inhibited group. When anticipating faces overall, the inhibited group had less connectivity between the mPFC and amygdala (amy). When viewing faces overall, the inhibited group also had less connectivity among a prefrontal-insular network, including the dlPFC, ACC, mPFC, and insula.

Table 1

Characteristics of the Sample

	IT		UT		
	Mean	**SD**	**Mean**	**SD**	**p-value**
Demographics					
Age (years)	9.5	1.0	9.8	.9	ns
Temperament score (parent report)	153.8	20.2	44.7	8.5	< .001
Temperament score (child report)	122.8	19.8	91.7	42.3	.005
Temperament score (interviewer rating)	3.4	1.2	2.3	1.1	.002
IQ	116.8	9.9	116.5	12.3	ns
	n	**(%)**	**n**	**(%)**	**p-value**
Ethnicity					ns
Caucasian	18	81	18	84	
African-American	2	13	2	11	
Asian	1	6	1	5	
Female gender	10	62	9	47	ns
Right handed	14	88	18	95	ns
Clinical Characteristics	**Mean**	**SD**	**Mean**	**SD**	**p-value**
Anxiety (parent report)	20.9	8.8	3.9	3.4	< .001
Anxiety (child report)	19.5	11.1	11.8	10.8	.03
Depression (child report)	5.7	3.2	5.4	7.4	ns
Hyperactivity (parent report)	2.9	3.2	4.0	3.1	ns
Social communication skills (parent report)	3.8	3.6	2.7	1.9	ns

Note: IT = inhibited temperament; UT = uninhibited temperament.

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