HHS Public Access

Author manuscript

Psychol Med. Author manuscript; available in PMC 2020 December 03.

Published in final edited form as:

Psychol Med. 2020 January ; 50(1): 96–106. doi:10.1017/S0033291718003999.

Levels of early-childhood behavioral inhibition predict distinct neurodevelopmental pathways to pediatric anxiety

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Abstract

Background.—Anxiety symptoms gradually emerge during childhood and adolescence. Individual differences in behavioral inhibition (BI), an early-childhood temperament, may shape developmental paths through which these symptoms arise. Cross-sectional research suggests that level of early-childhood BI moderates associations between later anxiety symptoms and threat-related amygdala–prefrontal cortex (PFC) circuitry function. However, no study has characterized these associations longitudinally. Here, we tested whether level of early-childhood BI predicts distinct evolving associations between amygdala–PFC function and anxiety symptoms across development.

Methods.—Eighty-seven children previously assessed for BI level in early childhood provided data at ages 10 and/or 13 years, consisting of assessments of anxiety and an fMRI-based dot-probe task (including threat, happy, and neutral stimuli). Using linear-mixed-effects models, we investigated longitudinal changes in associations between anxiety symptoms and threat-related amygdala–PFC connectivity, as a function of early-childhood BI.

Results.—In children with a history of high early-childhood BI, anxiety symptoms became, with age, more *negatively* associated with right amygdala–left dorsolateral-PFC connectivity when

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Conflict of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0033291718003999.

attention was to be maintained on threat. In contrast, with age, low-BI children showed an increasingly *positive* anxiety–connectivity association during the same task condition. Behaviorally, at age 10, anxiety symptoms did not relate to fluctuations in attention bias (attention bias variability, ABV) in either group; by age 13, low-BI children showed a negative anxiety–ABV association, whereas high-BI children showed a positive anxiety–ABV association.

Conclusions.—Early-childhood BI levels predict distinct neurodevelopmental pathways to pediatric anxiety symptoms. These pathways involve distinct relations among brain function, behavior, and anxiety symptoms, which may inform diagnosis and treatment.

Keywords

Amygdala; anxiety; attention; behavioral inhibition; children; connectivity; developmental; fMRI; prefrontal cortex

Introduction

Anxiety symptoms gradually emerge during childhood and adolescence (Kessler *et al.*, 2005a). Research on behavioral inhibition (BI), an early-childhood fearful temperament, suggests distinct neurodevelopmental paths through which these symptoms arise (Fox *et al.*, 2001; Fox and Kalin, 2014; Pine and Fox, 2015). Specifically, in children with a history of high *v.* low BI, anxiety symptoms exhibit distinct associations with attention and associated function in amygdala–prefrontal cortex (PFC) circuitry (Hardee *et al.*, 2013; Pine and Fox, 2015; White *et al.*, 2017a). However, no brain imaging study tracks longitudinal associations between attention-related fronto-limbic circuitry function and anxiety symptoms as a function of early-childhood BI, precluding strong inferences on developmental trajectories. Here, we investigated longitudinal associations across peri-adolescence between anxiety symptoms and amygdala–PFC function during a threat-related attention task, in children assessed for BI in early childhood.

High relative to low BI manifests differently with age. In infancy and early childhood, BI manifests as increased reactivity to novelty and threat; in later childhood, it presents as reticence in social circumstances; and, in later life, it confers high risk for anxiety disorders (Fox *et al.*, 2001; Hane *et al.*, 2008; Pine and Fox, 2015). However, the association between early-childhood BI levels and later anxiety symptoms is modest, mixed at times (Caspi *et al.*, 1996; Prior *et al.*, 2000; Chronis-Tuscano *et al.*, 2009; Lewis-Morrarty *et al.*, 2012; Frenkel *et al.*, 2015), and with evidence of moderation by cognitive factors (e.g. Barker *et al.*, 2015; Frenkel *et al.*, 2015; Buzzell *et al.*, 2017). Thus, early-childhood BI levels may evolve into later anxiety symptomatology; however, the nature of this link is not yet understood.

Behavioral studies indicate that preferential allocation of attention to threat stimuli is one factor moderating this link (McDermott *et al.*, 2009; White *et al.*, 2011; Fox and Pine, 2012; Fox and Kalin, 2014; Perez-Edgar *et al.*, 2014). Attention biases to threat relate to anxiety symptoms in youth and adults (Bar-Haim *et al.*, 2007; Abend *et al.*, 2018). In addition, threat biases moderate the association between early-childhood BI and later anxiety-related symptoms; specifically, children with a history of high *v.* low BI who display a threat bias also exhibit increased symptoms of anxiety (Perez-Edgar *et al.*, 2010; Perez-Edgar *et al.*,

2011; Cole *et al.*, 2016; Morales *et al.*, 2017; White *et al.*, 2017a). Thus, while behaviorally inhibited temperament and attention biases to threat each predict anxiety symptoms independently, the interaction between these two factors further qualifies the conditions contributing to the development of anxiety. Moreover, these behavioral data suggest that attention biases may define two developmental pathways into later anxiety symptoms. In children with early BI, a bias toward threat identifies a particularly elevated risk, while in children without early BI, anxiety develops in the absence of such associations. Furthermore, the nature of the association between BI and attention bias changes with age, suggesting dynamic developmental interactions among early-childhood BI, attention to threat, and anxiety symptoms (White *et al.*, 2017a). Delineating the developmental interplay among these factors informs our understanding of the emergence of pediatric anxiety symptoms (Pine and Fox, 2015).

Behavioral indices of attention allocation provide important, but limited, insight into mechanisms giving rise to anxiety, due to poor reliability of such indices and indirect relations between behavioral and neural correlates of attention (e.g. Price *et al.*, 2015; White *et al.*, 2016). Stronger insights may be gained through brain imaging, which yields reliable markers of threat-related attention processes (White *et al.*, 2016). Previous studies identify amygdala–PFC functional connectivity, primarily with ventrolateral and dorsolateral PFC (DLPFC), during threat-related attention tasks as relating to anxiety-related symptoms (Monk *et al.*, 2008; Hardee *et al.*, 2013; Fu *et al.*, 2017; White *et al.*, 2017b). The amygdala and PFC constitute key nodes in a neural circuit influencing attention allocation to threats (LeDoux, 1996; Pine and Fox, 2015; Shechner and Bar-Haim, 2016). Variations in the interplay between these nodes may influence regulation of attention allocation to threat, contributing to the emergence of anxiety symptoms (Pine and Fox, 2015; LeDoux and Pine, 2016).

Amygdala–PFC circuitry undergoes substantial developmental change across childhood and adolescence. The plasticity of this circuitry may contribute to the evolving associations between threat-related attention and anxiety symptoms. Evidence suggests that the amygdala matures early (e.g. Thomas *et al.*, 2001; Ulfig *et al.*, 2003), whereas the PFC shows a more prolonged developmental trajectory (e.g. Monk *et al.*, 2003; Gogtay *et al.*, 2004). As such, the nature of functional connectivity between these regions may change with development in ways that influence effective PFC regulation of amygdala activity and its impact on attention allocation (e.g. Perlman and Pelphrey, 2011; Gee *et al.*, 2013). In this developmental context, different levels of early-childhood BI, manifesting as a varying fearful temperament, may reflect early-onset differences in amygdala–PFC circuitry function that uniquely influence the emergence of anxiety symptoms as this circuitry matures (Perlman and Pelphrey, 2011; Birn *et al.*, 2014; Casey *et al.*, 2015; Pine and Fox, 2015).

Data from a small body of cross-sectional work using the dot-probe task support our hypotheses regarding dynamic relations among BI, anxiety, and attention-related amygdala–PFC connectivity. Two studies are particularly relevant. One examined clinically anxious and non-anxious youths aged 8–17 years. In this study, White *et al.* (2017b) observed that anxiety was related to positive amygdala–PFC connectivity when attention was maintained at a location previously occupied by a threat stimulus (so-called ‘threat-congruent’ trials).

These findings suggest that perturbed amygdala–PFC connectivity manifests in anxiety during the processing of attended threats (White *et al.*, 2017b). However, this study did not consider the influence of BI. The second study examined adults considerably older than in White *et al.* (2017b) but followed since infancy and classified with a history of high *v.* low BI (Hardee *et al.*, 2013). In high- *v.* low-BI subjects, this study found stronger *negative* amygdala–PFC connectivity when processing threat stimuli, particularly in the presence of ongoing anxiety symptoms. Taken together, these studies related anxiety symptoms to perturbed amygdala–PFC connectivity and suggest that the age of the participant and the presence of BI influence relations this association. Specifically, the studies suggest a possible age-related shift in amygdala–PFC connectivity in anxiety symptoms from early positive to later negative connectivity (Hardee *et al.*, 2013; White *et al.*, 2017b). Critically, however, no longitudinal brain imaging study examines how early-childhood BI predicts distinct patterns of evolving associations between connectivity and anxiety symptoms. Thus, our hypotheses remain tentative.

Here, we used a prospective longitudinal design to examine whether high *v.* low early-childhood BI predicts distinct neurodevelopmental pathways to anxiety symptoms. To this end, we documented longitudinal associations between anxiety symptoms and threat-related amygdala–PFC connectivity in a sample of children who were previously assessed for BI from infancy through early childhood. Anxiety symptoms and amygdala–PFC connectivity were both measured at ages 10 and 13 years, allowing us to examine associations among these factors using longitudinal data during a critical developmental period when anxiety symptoms typically increase (Kessler *et al.*, 2005a, 2005b). We hypothesized that high *v.* low early-childhood BI predicts distinct associations between anxiety symptoms and threat-related amygdala–PFC function over time. Given previous cross-sectional findings in distinct age groups, we predicted age-related changes in the relations among BI, anxiety, and amygdala–PFC connectivity. We specifically predicted that, among high-BI children, anxiety symptoms relate to positive amygdala–PFC connectivity, indicative of increased engagement by threat. However, we expected this relationship to attenuate with age, as this circuitry matures (Gee *et al.*, 2013; Hardee *et al.*, 2013; White *et al.*, 2017b).

Methods and materials

Participants

Participants were drawn from a larger community cohort of 291 developmentally healthy children selected at 4 months of age based on criteria for negative and positive reactivity to novelty (Fox *et al.*, 2001), for a longitudinal study on the temperament of BI (see details in Hane *et al.* (2008)). Individuals were assessed at ages 2 and 3 years for levels of BI (see below). See Supplementary material for information about inclusion/exclusion criteria and diagnoses. In terms of behavioral data, associations between BI, anxiety symptoms, and threat bias at ages 5 and 7 years were previously reported for this full sample (White *et al.*, 2017a). No previous study reports on the brain imaging data in the current study.

A total of 107 participants meeting inclusion criteria provided data for the current study. Data were collected at two time points: around age 10 (M = 10.51 years, S.D. = 0.43) and age 13 (M = 13.04, S.D. = 0.65) years, providing time-points before and after the median

age-of-onset of anxiety disorders in the general population (11 years; Kessler *et al.*, 2005a). Eight participants who provided data at age 10 were excluded from analyses (five performed the attention task with sub-threshold accuracy, two aborted, one had excessive head motion during scanning). Twelve participants who provided data at age 13 were excluded (two for sub-threshold accuracy, three aborted, seven for data collection technical issues). Thus, the final sample consisted of data provided by 87 participants (81%). Of those, 61 participants provided data at age 10 (36 females) and 64 provided data at age 13 (37 females), for a total of 125 scans (see Table 1 for demographic details). Thirty-eight participants provided data at both time-points. To provide more generalizable group effects, data from all 87 participants were used in analyses (see below). Study procedures were approved by the National Institute of Mental Health and University of Maryland Institutional Review Boards. Informed consent and assent were obtained from parents and youth, respectively.

Behavioral inhibition assessment

BI was assessed in the larger cohort ($N = 291$) when children were 2 and 3 years of age. Laboratory assessments and parent reports were combined into BI composite scores that were then standardized across the full cohort, and median-split into low- and high-BI samples that were used in several studies on developmental trajectories as a function of early-childhood BI (Fox *et al.*, 2001; Lahat *et al.*, 2014; Lamm *et al.*, 2014; Buzzell *et al.*, 2017; Lahat *et al.*, 2018). For comparability with these previous reports, we retained participants' original categorical group allocation in the current study (see Table 1); additional analyses using BI as a continuous measure are reported in Supplementary material. At each time-point, the low- and high-BI groups differed in mean BI composite score (the age 10 and 13 samples were not identical since not all participants contributed data at both time-points), ps < 0.001, but not in age, sex, IQ, attention bias indices (see below), or the Screen for Childhood Anxiety Related Emotional Disorders (SCARED) scores (or their change over time), ps > 0.11, ensuring that early-childhood BI differentiated the groups.

Current anxiety symptom severity

Current anxiety symptom severity was measured within 6 weeks of each scan using SCARED (Birmaher *et al.*, 1997, 1999), a 41-item child- and parent-report measure of anxiety symptomology. Cronbach's α for youth and parent measures at each time-point were >0.90. Total scores were calculated by summing all item-level scores. Total scores from each parent–youth dyad were averaged (Guyer *et al.*, 2008; Michalska *et al.*, 2017; Shechner *et al.*, 2017) to create a mean score reflecting current symptom severity for each participant.

Dot-probe task

Considerable research applies the dot-probe task to measure attention biases to threat-related stimuli (MacLeod *et al.*, 1986; Bar-Haim *et al.*, 2007; Van Bockstaele *et al.*, 2014; Abend *et al.*, 2018), yielding reliable amygdala–PFC connectivity measures (White *et al.*, 2016). To test for specificity of findings to threat and given previous findings in BI regarding bias to positive-valence stimuli (Perez-Edgar *et al.*, 2010; Shechner *et al.*, 2012), the task included angry and happy faces. At each time-point, participants completed the same version of the fMRI dot-probe task (Supplementary material). Briefly, in each trial (online Supplementary

Fig. S1), a pair of angry and neutral, happy and neutral, or two neutral faces was presented. A probe then replaced one of the faces (counterbalanced across emotions). Thus, five trial types were presented: angry-congruent (AC, 48 trials), angry-incongruent (AI, 48 trials), happy-congruent (HC, 48 trials), happy-incongruent (HI, 48 trials), and neutral (N, 96 trials; neutral–neutral trials). These conditions enabled us to isolate neural and behavioral responses to threat-related and positive stimuli.

Imaging data acquisition and analysis

fMRI acquisition and preprocessing—Neuroimaging data were collected on a 3 T General Electric scanner (Waukesha, Wisconsin, USA), using a 32-channel head coil. Blood oxygen level-dependent (BOLD) signal was measured by echoplanar imaging at 2.5 × 2.5 × 3.0 mm voxel resolution. Data were preprocessed and analyzed using AFNI (Cox, 1996). See online Supplementary material for additional details.

Analyses—Based on previous findings (Monk *et al.*, 2008; Hardee *et al.*, 2013; White *et al.*, 2016; White *et al.*, 2017b), primary analyses focused on task-related amygdala–PFC functional connectivity. Individual-level general linear models (GLMs) included regressors for correct trials across the five task conditions (AC, AI, HC, HI, N) and nuisance regressors. To identify task-specific differences in functional connectivity, we used generalized psychophysiological interaction (gPPI; McLaren *et al.*, 2012), with anatomically defined right and left amygdala seeds (Hardee *et al.*, 2013; White *et al.*, 2017b). In addition, GLMs were also created using the same regressors to test secondary analyses of changes in mean BOLD signal activation. Estimated βs, one for each regressor, were generated at the individual-subject level and submitted to group-level analyses.

Of note, some participants provided data only at one time-point (see Table 1 for breakdown by BI group). Absence of data was not associated with BI group, SCARED scores, age, or gender, all $ps > 0.27$. To provide generalizable results, prior research indicates the importance of basing longitudinal analyses on all participants providing data at any time-point (Matta *et al.*, 2017). Therefore, group-level analyses were conducted on all participants contributing at least one useable data-point ($N = 87$) using linear mixed-effects (LME) models through AFNI's 3dLME program (Chen *et al.*, 2013). LME overcomes missing data in longitudinal designs, yielding more reliable effect estimates than complete-case analyses (Donders *et al.*, 2006; Chen *et al.*, 2013; Matta *et al.*, 2017). Group (Low BI, High BI) served as a between-subjects factor. Condition (AC, AI, HC, HI, N) served as a within-subject factor, and Anxiety (SCARED score, collected around the time of each scan and mean-centered separately for each scan) served as a continuous, between-subjects factor. Data for the Condition and Anxiety factors were collected at each of the two time-points; thus, reflecting the longitudinal design of the study, a fourth variable, Time (Age 10, Age 13), was included as a within-subject factor reflecting the longitudinal design of the study. Finally, in addition to these fixed-effects variables and their interactions, random effects for intercept and SCARED scores across participants were included in the LME model.

Across all analyses, significant clusters were identified using an initial voxel-wise threshold of $p < 0.005$. Based on previous findings and our focus on amygdala–PFC connectivity

(White *et al.*, 2016; White *et al.*, 2017b), we applied a mask encompassing all gray-matter PFC voxels with $y \geqslant 0$ coordinates. Using AFNI's 3dClustSim tool, which assumes a non-Gaussian auto-correlation smoothing function (Cox *et al.*, 2017) in light of Eklund *et al.* (2016), we calculated a cluster-wise threshold size of 734 mm^3 reflecting a family-wise error rate of $\alpha = 0.05$ (based on 10 000 Monte-Carlo simulations). Group maps were also thresholded to include only voxels for which 90% of participants had valid data (White *et al.*, 2016; Stoddard *et al.*, 2017; White *et al.*, 2017b). Within each significant cluster identified in group-level analyses, estimated βs for each individual participant were extracted for further analysis as described below.

Our primary hypothesis was that the association between current anxiety symptoms and threat-related amygdala–PFC functional connectivity changes with development, and that early-childhood BI moderates this developmental effect. This hypothesis was tested via gPPI analyses, one each for the left and right amygdala seeds. In each analysis, gPPI β estimates were submitted to an LME model testing the omnibus statistics for the Group × Anxiety × Condition × Time interaction, with the Time factor representing the two time-points (ages 10 and 13) at which anxiety and fMRI task data were collected. Effects within significant clusters identified by this four-way interaction were interpreted by decomposing the interaction into lower level interactions, and then into effect estimates of two-variable associations (*b* slope coefficients) tested using χ^2 likelihood ratio, all within the omnibus LME models (online Supplementary Fig. S2). As secondary analyses, we examined the omnibus effect on mean BOLD signal activation using the same analytic plan. In addition to threat-related processing, prior behavioral studies also document existing, but weaker, associations among attention biases to positive stimuli, anxiety, and BI (Perez-Edgar *et al.*, 2010; Shechner *et al.*, 2012; White *et al.*, 2017a); as such, we also report effects on task conditions involving happy faces.

Behavioral data analysis

To complement the analyses of imaging data, two types of behavioral measures indexing attentional processes were analyzed: attention bias and attention bias variability (ABV) scores (Bar-Haim *et al.*, 2007; Shechner and Bar-Haim, 2016).

Attention bias scores—Attention biases to threat-related stimuli have been associated with anxiety, including in children and adolescents (Bar-Haim *et al.*, 2007; Van Bockstaele *et al.*, 2014; Abend *et al.*, 2018). Here, we computed attention bias scores to threat and to positive stimuli (see online supplementary material). An analytic approach similar to that used for the imaging data was applied to bias scores, with scores submitted to an LME model testing the omnibus effect for the Group (Low BI, High BI) × Anxiety (SCARED scores) × Condition (Threat, Happy) × Time (Age 10, Age 13) interaction.

ABV scores—ABV measures within-session temporal variability and fluctuation in attention biases, taken to reflect a loss of attentional control and stability, and has been shown to exhibit stronger reliability than attention bias scores (Iacoviello *et al.*, 2014; Naim *et al.*, 2015; Price *et al.*, 2015; Zvielli *et al.*, 2015; Shechner and Bar-Haim, 2016). ABV scores to threat and to positive stimuli were calculated in accord with previous studies (see

online Supplementary material), and then submitted to an LME model testing the Group × Anxiety × Condition (Threat, Happy) × Time interaction.

Statistical analyses were conducted using AFNI, the *nlme* and *phia* packages in R (Pinheiro and Bates, 2000), and SPSS 23. All statistical tests were two-sided; significance threshold was set to $\alpha \leqslant 0.05$.

Results

Imaging analyses

Right amygdala functional connectivity—For right amygdala functional connectivity, a significant omnibus Group × Anxiety × Condition × Time interaction emerged in left DLPFC [LPI peak coordinates: [−19,21,39], $k = 57$, 890 mm^3, $F_{(4,500)} = 6.26$, $p = 0.0001$; Figure 1a]. To identify the task conditions contributing to this interaction effect, we next tested the Group × Anxiety × Time interaction effect separately within each of the five conditions (online Supplementary Fig. S3). For conciseness, and in line with our primary hypothesis focusing specifically on threat-related processing, Fig. 1b presents the decomposition of this interaction effect for the AC and AI conditions, which differ by requiring attention to either be maintained on, or shifted away from, threat, respectively. The Group × Anxiety × Time interaction was significant only for the AC condition, $F_{(1,32)} = 21.04$, $p = 0.0001$, Fig. 1b (left). See online Supplementary material for non-significant results in the remaining four conditions.

Next, within the AC condition (Fig. 1b, left), we examined the longitudinal nature of changes in associations between anxiety symptoms and amygdala–DLPFC connectivity (Anxiety × Time interaction), as a function of BI group. The Anxiety × Time interaction was significant in the low-BI group, $F_{(1,15)} = 11.04$, $p = 0.005$. Follow-up correlations indicated no significant association between anxiety symptoms and amygdala–DLPFC connectivity at age 10, $b = -0.015$, $p = 0.20$. However, there was a significant *positive* association between these variables at age 13, $b = 0.027$, $p = 0.015$. In the high-BI group, the Anxiety × Time interaction effect also was significant, $F_{(1,17)} = 10.53$, $p = 0.005$. However, follow-up tests indicated a different pattern from the low-BI group, with a significant *positive* association between anxiety symptoms and amygdala–DLPFC connectivity at age 10, $b = 0.022$, $p = 0.024$, but a *negative* association at age 13, $b = -0.034$, $p = 0.001$. Thus, children with a history of low *v.* high BI exhibited distinct longitudinal patterns of association between anxiety symptoms and amygdala–DLPFC connectivity.

A series of separate auxiliary analyses testing the omnibus effect are reported in online Supplementary material. The first analysis aimed to verify the validity of the LME model (Matta *et al.*, 2017) by considering only participants who provided data at both time-points ($n = 38$). A second analysis used BI as a continuous variable. A third exploratory analysis added sex as a fifth factor which was considered as a nuisance variable in the model. These analyses yielded left DLPFC clusters in full or partial overlap with the DLPFC cluster reported for the primary analysis, albeit with a smaller cluster extent. A fourth auxiliary analysis contrasted brain function between participants who provided data at one time-point

v. those who provided data at both time-points, but found no significant differences between these groups.

Additional lower order interaction effects emerged within the model tested in the primary analysis on all participants (Table 2). Of note, a significant Group × Anxiety × Condition interaction emerged in bilateral superior frontal gyrus (SFG) and right dorsomedial PFC (dmPFC). Decomposition of this interaction revealed a similar pattern across clusters (online Supplementary Fig. S4). Specifically, in the HC condition, the low-BI group exhibited a negative association between connectivity and anxiety symptoms (ps < 0.005), whereas the high-BI group showed a positive association between these factors (ps < 0.025). This suggests a time-invariant group difference in amygdala–PFC connectivity when processing happy faces.

Left amygdala functional connectivity—For left amygdala functional connectivity, the hypothesized omnibus Group × Anxiety × Condition × Time interaction did not yield significant clusters. Lower order interaction effects emerged primarily in bilateral inferior and medial frontal gyri (Table 2), but did not include a task-condition term.

Functional activation—No significant clusters emerged for the Group × Anxiety × Condition × Time interaction conducted on PFC activation β estimates. See online Supplementary Table S1 for all clusters identified in lower order effects. In addition, no significant clusters emerged for the Group × Anxiety × Condition × Time interaction in either right or left amygdala, even when cluster extent threshold for these regions was reduced to two contiguous voxels.

Behavioral analyses

Attention bias scores—There was no significant main or interaction effect when testing the Group × Anxiety × Condition × Time effect on attention bias scores, all Fs < 1.52, ps > 0.22.

ABV scores—There was no significant Group × Anxiety × Condition × Time interaction on ABV scores, $F_{(1,149)} = 0.19$, $p = 0.67$. However, the Group × Anxiety × Time interaction effect tested within the model was significant, $F_{(1,149)} = 5.34$, $p = 0.022$ (Fig. 2). This finding indicates that early-childhood BI moderated the change in anxiety–ABV association over development and across both emotional expressions. Follow-up tests indicated no difference in anxiety–ABV associations between the BI groups at age 10, $F_{(1,57)} = 0.05$, $p = 0.83$. In contrast, a significant group difference in anxiety–ABV associations emerged by age 13, $F_{(1,60)} = 6.64$, $p = 0.012$, with the low-BI group showing a trend toward a negative association between anxiety and ABV, $b = -0.0007$, $p = 0.073$, while the high-BI group exhibited a significant positive association, $b = 0.0009$, $p = 0.019$. These results suggest that with age, anxiety symptoms relate to increased attentional stability (lower ABV) among individuals with a history of low BI, and to decreased attentional stability (greater ABV) among individuals with a history of high BI.

Discussion

The current study tested the hypothesis that level of early-childhood BI predicts distinct patterns of associations between anxiety symptoms and attention-related amygdala–PFC circuitry function across development. Consistent with our hypothesis, evidence for two distinct developmental trajectories emerged from the primary neural and behavioral findings. As children with a history of high BI were getting older, anxiety symptoms became more negatively correlated with DLPFC–amygdala connectivity when processing salient, proximal threats; the opposite developmental pattern was observed in low-BI children. Furthermore, on task behavior, a history of high BI predicted a negative association between anxiety symptoms and attentional stability that emerged by age 13, while the opposite pattern was observed in low-BI children. Together, these findings suggest that different early-childhood BI levels predict distinct neurodevelopmental anxiety trajectories.

This study builds on previous work that finds amygdala–PFC connectivity to reliably index threat-related attention orienting (White *et al.*, 2016), which, in turn, relates consistently to anxiety symptoms (Monk *et al.*, 2008; Hardee *et al.*, 2013; Birn *et al.*, 2014; Price *et al.*, 2016; White *et al.*, 2017b). Indeed, the longitudinal associations in the current study emerged specifically in threat-related amygdala–DLPFC connectivity. Such findings are consistent with previous reports of associations between brain activity specifically in the AC condition and anxiety-related phenotypes (Britton *et al.*, 2012; Thai *et al.*, 2016; White *et al.*, 2017b), as well as age-related changes in patterns of amygdala–PFC connectivity (Gee *et al.*, 2013; Wu *et al.*, 2016). Taken together, the findings highlight the importance of developmental changes in brain functions engaged when processing attended, as opposed to unattended, threats (Cisler and Koster, 2010). These developmental changes unfold differently based on a child's early-life levels of BI and their level of anxiety at the time of scanning. As such, this study extends previous cross-sectional work on the association between anxiety symptoms and neural circuitry function by illuminating distinct developmental trajectories through which these associations arise.

Specifically, these two developmental pathways differ based on early-childhood BI levels and the nature of subsequent associations between amygdala–DLPFC connectivity and anxiety symptoms. PFC–amygdala circuitry has been implicated in emotion regulation and attention control, especially as related to anxiety symptoms (Bishop, 2007; Bishop, 2008; Kim *et al.*, 2011; Etkin *et al.*, 2015). Evidence indicates that this circuitry undergoes substantial maturation during childhood and adolescence (Thomas *et al.*, 2001; Ulfig *et al.*, 2003; Gogtay *et al.*, 2004), causing developmental shifts in PFC regulation of amygdala activity (e.g. Perlman and Pelphrey, 2011; Gee *et al.*, 2013; Wu *et al.*, 2016). Varying levels of BI may reflect early-onset differences in amygdala–PFC circuitry function, which become more pronounced as this circuitry matures in peri-adolescence, and differentially relate to the emergence of anxiety symptoms (Bishop, 2008). Specifically, one developmental pathway is associated with high early-childhood BI. We found that this temperamental pattern of increased fear responses (Fox *et al.*, 2000; Fox *et al.*, 2005; Hane *et al.*, 2008) predicted a pattern in which increases in anxiety symptoms unfold with age in association with decreasing threat-related DLPFC–amygdala connectivity and, at a task-behavior level, attentional stability. A high-BI temperament may therefore indicate an early-emerging

deficiency in the capacity to regulate bottom-up attention capture by threats, manifesting as increased behavioral and physiological fear responses throughout childhood (Fox *et al.*, 2005; Bishop, 2007; Birn *et al.*, 2014). As PFC–amygdala circuitry matures, increased anxiety may relate to decreased functional 'cross-talk' between these nodes and to decreased attentional stability. As high BI has been shown to confer risk for anxiety disorders later in development (e.g. Frenkel *et al.*, 2015), high-BI children in the current sample are expected to manifest steadily increasing levels of anxiety symptoms over time, coupled with decreasing DLPFC–amygdala connectivity in the AC condition.

Of note, while prior work shows that high BI confers risk for anxiety, the magnitude of this reported association is only moderate, and is not always observed (Caspi *et al.*, 1996; Lewis-Morrarty *et al.*, 2012; Frenkel *et al.*, 2015; White *et al.*, 2017a); indeed, the low- and high-BI groups here manifested equivalent levels of anxiety symptom severity. As a result, many children with low levels of BI may also develop anxiety symptoms. Our findings suggest that, in these children, anxiety symptoms arise through a second developmental pathway, distinct from that for children with a history of high BI. This second trajectory is characterized by reduced fear responses in early childhood as well as increasingly positive associations between anxiety symptoms and both threat-related DLPFC–amygdala connectivity and, at a task-behavior level, attentional stability. A history of low BI may reflect a pattern of amygdala–PFC function associated behaviorally with reduced fear reactivity, potentially reflecting early-emerging, enhanced cortical regulation of bottom-up processing of threat stimuli (Bishop, 2007). With development, however, some individuals may exhibit excessive dominance of top-down influence, and diminished phasic amygdala responses, manifesting as increased amygdala–DLPFC coupling during attention allocation to threat. Tonic and indiscriminate amygdala activation has been associated with diminished sensitivity to the associability of environmental cues, and to inefficient deployment of attentional resources toward them, potentially contributing to the emergence of anxiety (Grupe and Nitschke, 2013). Further research is needed to directly link this maladaptive processing pattern to the emergence of anxiety symptoms.

The relatively low levels of anxiety symptoms at both time-points do constrain these conclusions. However, prior research suggests that BI and sub-clinical anxiety symptoms represent two of the strongest risk factors in childhood for later anxiety disorders (Beesdo *et al.*, 2009). As such, clinical relevance arises from the demonstration of dynamic relationships between these two important risk factors. Findings suggest that these risk factors predict changes in functioning within a circuit previously linked to clinical disorders. This demonstration might shape views of risk factors and their influence on the brain. Consideration should be given to shifting emphasis from static views of childhood risk to more developmental perspectives, emphasizing dynamic interplay among risk factors as drivers of brain development.

The current results point to developmental changes in threat-related DLPFC–amygdala connectivity as a potential factor dissociating *phenocopies* of pediatric anxiety symptoms, i.e. subtypes of anxiety symptoms with distinct psychobiological mechanisms. Identifying distinct phenocopies of anxiety symptoms may potentially promote more accurate diagnosis and effective treatment. While the groups studied here manifested equivalent levels of

anxiety symptom severity, our results suggest that symptoms differentially related to neural and behavioral threat-processing profiles. Phenotyping according to pathophysiological mechanisms, rather than symptom-based diagnostic categories, guides the development of more precisely targeted novel treatments (Insel *et al.*, 2010; Cuthbert, 2015; Shanmugan *et al.*, 2016). For example, procedures aiming to enhance attention stability (Naim *et al.*, 2015; Shechner and Bar-Haim, 2016) may alleviate anxiety symptoms in children with a history of high, but not low, BI. Similarly, treatments developed to modulate neural connectivity patterns (Paret *et al.*, 2016; Nicholson *et al.*, 2017) could consider BI history in determining application parameters and which patients are most likely to respond.

This study identified developmental trajectories characterized by distinct longitudinal patterns of associations between threat-related amygdala–DLPFC connectivity and anxiety symptoms. Of note, other lower order interactions revealed associations between anxiety symptoms and amygdala–PFC connectivity that did not change with age. These associations emerged primarily in the HC condition, with low BI predicting a negative association between anxiety and amygdala–SFG and –dmPFC connectivity, in contrast to high BI, which predicted the opposite pattern. While most research on attention biases in anxiety focuses on threat processing, previous findings in BI note consistent associations between anxiety and attention biases to positive stimuli (Perez-Edgar *et al.*, 2010; Shechner *et al.*, 2012; White *et al.*, 2017a). The current results extend these findings. Thus, at age 10, BI levels predict a similar association between anxiety and connectivity when subjects maintain attention on either positive or threat stimuli, suggesting general reactivity to emotional stimuli as a function of BI. By age 13, however, these associations change for threat stimuli only, suggesting that anxiety symptoms may track more closely with maturational changes in circuitry underlying threat processing (Gee *et al.*, 2013; Casey *et al.*, 2015).

Several limitations of this study should be considered. First, the sample size was modest. Second, there were missing data due to attrition, often related to unavoidable factors such as use of dental hardware in puberty. Third, mean anxiety levels were generally sub-clinical, and did not differ between low- and high-BI groups. While our longitudinal design attempted to capture the emergence of pediatric anxiety symptoms by placing data collection points before and after the median age of anxiety symptoms onset in the general population (age 11 years; Kessler *et al.*, 2005a), symptoms typically peak later in adolescence (Beesdo *et al.*, 2009). As such, our findings suggest distinct trajectories from risk factors (Fox *et al.*, 2001; Frenkel *et al.*, 2015; White *et al.*, 2017a) to symptoms which may fully manifest clinically only later in development. Future studies may consider data collection during later adolescence when anxiety symptoms are expected to manifest more severely. Finally, a broader limitation to this field of research is the use of neutral faces as contrasts to emotional faces, as anxious individuals have been shown to process neutral faces differently than healthy controls, both behaviorally and neurally (Filkowski and Haas, 2017). Although prior research reveals attentional biases in anxiety using such faces (e.g. Abend *et al.*, 2018), the field may benefit from establishing other types of baseline emotion conditions (Filkowski and Haas, 2017).

These limitations are offset by several strengths. Because the study presents longitudinal data collected over 10 years, many key variables represent within-subject factors, which

generally possess more statistical power than between-subjects factors. Furthermore, we applied an LME statistical approach in all analyses; this approach is considered more valid than complete-case approaches which introduce selection biases. Finally, anxiety–connectivity associations emerged in terms of individual differences, i.e. continuous associations spanning the range of anxiety symptoms, and as such may be more generalizable than group mean differences. Of note, BI effects emerged more strongly when a categorical operationalization of this factor was used in line with previous work, potentially indicative of qualitative as well as quantitative differences between BI levels.

In conclusion, this longitudinal study provides evidence for two distinct neurodevelopmental pathways that relate levels of early-childhood BI to pediatric anxiety symptoms. These pathways highlight longitudinal interactions among BI and anxiety symptoms in relation to neural activity during threat-related attention allocation. As such, these findings extend our understanding of the pathophysiology of pediatric anxiety and help guide the development of targeted treatments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements.

We thank the participants and families, as well as the staff of the Intramural Research Program of the National Institute of Mental Health, National Institutes of Health.

Financial support. This research was supported (in part) by the NIMH Intramural Research Program (ZIAMH002781-15, NCT00018057; RA, CS, LWK, CF, KK, SPH, BEB, EL, DSP) and NIH grant U01MH093349 (NAF).

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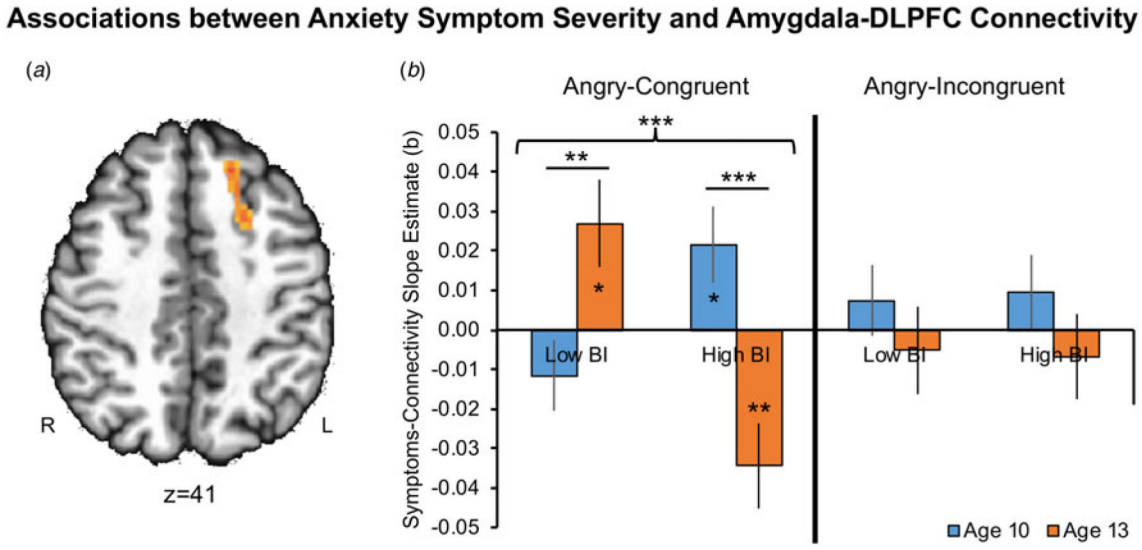

Fig. 1.

Associations between current anxiety symptoms and right amygdala–left DLPFC functional connectivity at ages 10 and 13 for the AC and AI task conditions and per BI group (Low BI, High BI). Each bar represents the slope estimate between SCARED scores and PPI β estimates for the respective group, task condition, and age. Asterisks within bars indicate slope estimates significantly different from 0; asterisks between bars indicate significant differences between slope estimates. Error bars indicate one standard error of the slope estimate. DLPFC, dorsolateral prefrontal cortex; SCARED, Screen for Child Anxiety Related Disorders; AC, angry-congruent; AI, angry-incongruent; BI, behavioral inhibition; PPI, psychophysiological interaction; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

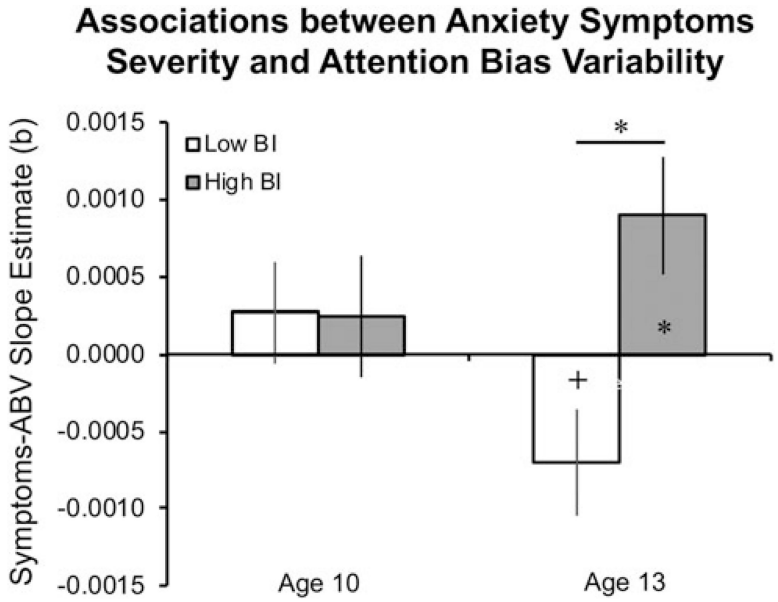

Fig. 2.

Associations between current anxiety symptom severity and ABV scores at ages 10 and 13, in the low-BI and high-BI groups. Each bar represents the slope estimate between SCARED scores and ABV scores (averaged across angry-neutral and happy-neutral trials) collected at the same age. Asterisks within bars indicate slope estimates significantly different from 0; asterisks between bars indicate significant differences between slope estimates. Error bars indicate one standard error of the slope estimate. ABV, attention bias variability; BI, behavioral inhibition; SCARED, Screen for Child Anxiety Related Disorders; $*p < 0.05$, $^{+}p < 0.10$.

Table 1.

Sample demographics, clinical indices, and performance on the dot-probe task, by group and time-point of data collection

	Age 10[a]		Age 13[b]		Total	
	Low BI *n* = 28	**High BI *n* = 33**	**Low BI *n* = 29**	**High BI *n* = 35**	**Low BI *n* = 39**	**High BI *n* = 48**
Demographics						
Sex (% female)	57.1%	60.6%	59.3%	56.8%	58.2%	58.6%
Age (years)	10.42 (0.40)	10.58 (0.45)	13.09 (0.68)	13.00 (0.64)	11.72 (1.47)	11.93 (1.42)
IQ	114.40 (12.57)	115.85 (12.40)	115.26 (13.76)	117.81 (13.35)	114.82 (13.05)	116.89 (12.86)
Clinical indices						
BI scores	−0.59 (0.42)	0.55 (0.49)	−0.56 (0.48)	0.49 (0.46)	−0.58 (0.45)	0.52 (0.47)
SCARED	18.29 (9.39)	14.67 (7.80)	11.24 (7.68)	10.32 (6.72)	14.83 (9.22)	12.37 (7.52)
Current anxiety dx (n)	1	3	5	4	6	7
Dot-probe performance						
Threat bias (ms)	0.38 (30.31)	6.54 (31.18)	9.47 (21.94)	12.00 (37.83)	4.85 (27.85)	9.42 (34.02)
Happy bias (ms)	9.50 (30.74)	−0.25 (30.74)	−1.45 (26.17)	7.73 (36.00)	4.13 (29.87)	3.97 (32.62)
ABV threat	0.047 (0.020)	0.048 (0.021)	0.046 (0.016)	0.048 (0.019)	0.046 (0.018)	0.048 (0.020)
ABV happy	0.041 (0.015)	0.050 (0.014)	0.052 (0.021)	0.048 (0.020)	0.046 (0.019)	0.049 (0.017)

BI, behavioral inhibition; SCARED, Screen for Childhood Anxiety Related Emotional Disorders (total scores); dx, diagnosis; ABV, attention bias variability.

At age 10 and 13, BI scores significantly differed between the low- and high-BI groups, whereas the other factors did not (see text).

[a] *n* = 23 provided data only at age 10.

[b] *n* = 26 provided data only at age 13.

Table 2.

Results of two functional connectivity analyses using right and left amygdala seeds

	Peak Talairach coordinates					
	x	*y*	*z*	Cluster size (mm^3; *k*)	Peak location	Brodmann area
Right amygdala seed						
Group × Anxiety × Condition × Time						
	−19	21	39	890; 57	L dorsolateral PFC	L BA8
Group × Anxiety × Condition						
	11	64	11	5671; 363	R superior frontal gyrus	R BA10
	−34	49	16	2656; 170	L superior frontal gyrus	L BA10
	1	34	36	1359; 87	R dorsomedial PFC	R BA8
Group × Anxiety × Time						
	4	36	4	1218; 78	R anterior cingulate	R BA32
Group × Anxiety						
	−51	21	16	2015; 129	L inferior frontal gyrus	L BA45
Time						
	−24	29	41	3015; 193	L medial frontal gyrus	L BA8
	29	6	−4	1296; 83	L superior frontal gyrus	L BA10
Left amygdala seed						
Group × Anxiety × Time						
	−54	26	19	984; 63	L inferior frontal gyrus	L BA45
Anxiety × Time						
	−46	31	16	3656; 234	L inferior frontal gyrus	L BA46
	36	36	9	1062; 68	R inferior frontal gyrus	R BA46
Group × Time						
	−6	24	56	1171; 75	L superior frontal gyrus	L BA6
Time						
	9	26	46	6687; 428	R medial frontal gyrus	R BA8
	49	4	19	1203; 77	R inferior frontal gyrus	R BA44
	−21	6	44	984; 63	L middle frontal gyrus	L BA24

	Peak Talairach coordinates					
	x	*y*	*z*	Cluster size (mm^3; *k*)	Peak location	Brodmann area
	34	39	9	937; 60	R middle frontal gyrus	R BA10

L, left; R, right; PFC, prefrontal cortex; BA, Brodmann area.

Presented are all significant clusters that emerged for all effects tested within the two linear mixed-effects models.

Initial voxel-wise threshold was $p = 0.005$; cluster size threshold was 734 mm^3 (reflecting a family-wise error rate of $\alpha = 0.05$; $k = 56$).