Early Childhood Behavioral Inhibition Predicts Cortical Thickness in Adulthood

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Objective: Behavioral inhibition (BI) during early childhood predicts risk for anxiety disorders and altered cognitive control in adolescence. Although BI has been linked to variation in brain function through adulthood, few studies have examined relations between early childhood BI and adult brain structure.

Method: The relation between early childhood BI and cortical thickness in adulthood was examined in a cohort of individuals followed since early childhood (N = 53, mean age 20.5 years). Analyses tested whether anxiety and/or cognitive control during adolescence moderated relations between BI and cortical thickness. Cognitive control was measured with the Eriksen Flanker Task. Initial analyses examined cortical thickness in regions of interest previously implicated in BI, anxiety disorders, and cognitive control: dorsal anterior cingulate (dACC), anterior insula (aI), and subgenual anterior cingulate (sgACC); and volumes of the amygdala and hippocampus. Exploratory analyses examined relations across the prefrontal cortex.

Results: BI during early childhood related to thinner dACC in adulthood. Neither anxiety nor cognitive control moderated this relation. A stronger congruency effect on the Eriksen Flanker Task during adolescence independently related to thinner dACC in adulthood. Higher anxiety during adolescence related to thicker cortex in the right ventrolateral prefrontal cortex (VLPFC) in adulthood among those with low BI as children.

Conclusion: Temperament in early childhood and the interaction between temperament and later anxiety relate to adult brain structure. These results are consistent with prior work associating BI and anxiety with functional brain variability in the dACC and VLPFC.

Key words: behavioral inhibition, anxiety, cortical thickness, structural MRI, cingulate

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Behavioral inhibition (BI) is a temperament defined by high negative reactivity to novelty, particularly in social contexts.[1,2] Some toddlers high in BI[3,4] exhibit social reticence through the school years and face high risk for anxiety disorders through early adulthood.[5-7] An early history of childhood BI also predicts patterns of brain function through adulthood,[8,9] especially during tasks that measure cognitive control, such as the Eriksen Flanker Task.[10-12] Interestingly, prior research suggests that anxiety symptoms in adolescence moderate relations between atypical brain activity and BI.[10,13,14] Although several studies link early childhood BI to variation in brain function during adolescence and adulthood, few studies examine the relations between early childhood BI and adult brain structure. The current study examines these relations.

Behavioral inhibition, anxiety disorders, and cognitive control have all been linked to functional variation in a common set of brain regions, including the amygdala, hippocampus, dorsal anterior cingulate (dACC), anterior insula (aI), and subgenual anterior cingulate (sgACC).[15-22] The amygdala and hippocampus, in the bilateral medial temporal lobes, play roles in evaluating the emotional significance of stimuli.[23] Variation in functioning of these structures may relate to increased emotional reactivity to specific stimuli.[24,25] The dACC and anterior insula in the prefrontal cortex both are members of a "cingulo-opercular" network putatively involved in adjusting cognitive control in response to errors.[26] Activity in the dACC in response to errors appears to be increased in both BI and anxiety disorders, and variation in the function of the dACC may explain increased error sensitively associated with these phenotypes.[10] Finally, the sgACC in the medial prefrontal cortex is thought to regulate amygdala activity, and variation in this region has been associated with poorer emotion regulation.[27]

Although there are well-established links between early childhood BI and later brain function, little is known about the relation between BI and adult brain structure. In contrast to functional brain studies, measures of brain structure do not depend on the particular task chosen by the experimenter or moment-to-moment fluctuations in mood state. Thus, structural brain studies complement functional studies by elucidating enduring, state-independent structural correlates of early childhood BI. Early childhood BI could be associated with altered adult brain structure either because such differences are present at birth or because high

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Supplemental material cited in this article is available online.

BI relates to behaviors or experiences associated with atypical brain development. In either case, linking adult brain structure to early childhood BI biologically connects early childhood BI to an adult measure that could inform outcome prediction and early intervention in children with high BI.

Prior research provides initial evidence for a link between individual differences in BI and brain structure. Schwartz *et al.*[28] used a longitudinal design and compared cortical thickness in young adults with or without histories of high reactivity at 4 months of age, an early marker of BI. Adults with high reactivity at 4 months of age had thinner left orbitofrontal cortex and thicker right ventromedial prefrontal cortex compared to adults with low reactivity at 4 months of age. Another study examining relations between brain structure during adulthood and retrospective report of BI during childhood noted increased amygdala and caudate volumes associated with BI.[29] The current study examines adult brain structure in relation to a stable measure of BI acquired prospectively over 4 assessments from 14 months to 7 years of age. Given that prospectively acquired measures of stable BI more consistently relate to later-life psychopathology than measures collected at a single time point,[5] the current study uses a phenotype relevant to functioning in adolescence and young adulthood.

The current study tests the hypothesis that BI measured during early childhood predicts cortical thickness measured during adulthood. This study further tests whether relations between early childhood BI and cortical thickness in adulthood are moderated by anxiety and/or altered cognitive control occurring during adolescence. Such moderation is expected, given that both of these conditions have been linked to BI and have been associated with an overlapping set of brain regions. To address these questions, the study uses a well-characterized, rigorously ascertained cohort containing individuals followed since 4 months of age through early adulthood.[3,30] This unique longitudinal cohort permits a rare opportunity to test prospective relations. Initial analyses test whether childhood BI predicts adult cortical thickness in regions commonly associated with BI, anxiety, and cognitive control: dACC, aI, or sgACC; or volume of the amygdala or hippocampus. These analyses test whether anxiety and/or cognitive control measured at intervening time points (in adolescence) moderate any relations detected between early childhood BI and cortical thickness in adulthood. Parallel exploratory vertexwise analyses examine these same relations across all of the prefrontal cortex.

METHOD

Participants

Participants were derived from an ongoing longitudinal study of BI in which behavioral data were available beginning at 4 months of age.[3,30] Participants were originally recruited from the suburban Washington, DC area through commercially available mailing lists. Potential participants were screened over the telephone and excluded on the basis of prematurity, low birth weight, or perinatal complications. A home or laboratory visit was then scheduled for 433 infants (from 2 separate cohorts) within 2 weeks of their 4-month birthday, and infants were assessed for motor reactivity and emotional reaction to novel stimuli. Infants at the extreme ends of these measures were invited to participate in the full longitudinal study on the basis of selecting infants at high and low risk for developing behaviorally inhibited temperament.[3,31] A small number of participants (5 in the sample used in the current study) were added to the study in early childhood to serve as unfamiliar peers in a social laboratory assessment (4 or 7 years of age).

A total of 163 were potential participants for the current study on the basis of having participated in BI assessments during childhood as well as anxiety assessments during adolescence or young adulthood. Of these 163 participants, 25 refused participation in the young adult study, and an additional 105 were either excluded or not approached on the basis of the following: contraindication to magnetic resonance imaging (MRI), psychotropic medication use, psychopathology requiring immediate clinical attention, recent completion of other studies, and/or participant request not to be contacted for a period of time after which the current study was completed.[12] Acceptable neuroimaging data were therefore available for a subset of participants (n = 53, mean age 20.5 years, 29 male and 24 female). Table 1 lists demographics for this subset and participation rates for the 7 time points where data were acquired (ages 4 months, 14 months, 24 months, 4 years, 7 years, early adolescence [mean age 14.7 years], and young adulthood [mean age 19.8 years]).

Children were observed for an assessment of BI at 14 and 24 months of age and for an assessment of social reticence at 4 and 7 years of age.[1,32,33] BI at 14 and 24 months was assessed by measuring infants' reactions to novel objects and people.[3] Mothers also reported their child's social fear at 14 and 24 months using the Toddler Behavior Assessment Questionnaire.[34] Social reticence is considered a marker of BI during the early school-age years. For the assessments of social reticence at ages 4 and 7 years, children's

TABLE 1 Demographic Characteristics and Assessment Participation Rates

Female	24	(45.3)
Age, y, mean (SD)	20.5	(1.62)
Ethnicity		
White	53	(100)
Maternal education		
High school graduate	3	(5.7)
Some college or technical school	6	(11.3)
College graduate	26	(49.1)
Graduate professional training	15	(28.3)
Other/no information	3	(5.7)
IQ, m (SD)	114.1	(10.5)
Data available for assessment		
Age 4 mo visit	43	(81.1)
Age 14 mo visit[a]	44	(83.0)
Age 24 mo visit[a]	45	(84.9)
Age 4 y visit[a]	45	(84.9)
Age 7 y visit[a]	44	(83.0)
Adolescent visit (mean age 14.7 y)[b]	49	(92.5)
Young adult visit (mean age 19.8 y)[b]	52	(98.1)
Conflict task visit (mean age 14.8 y)	29	(54.7)

Note: Data are expressed as n (%) except where noted.

[a]*Indicates assessments that were used to compute the behavioral inhibition score.*

[b]*Indicates assessments that were used to calculate the anxiety score.*

reticent behavior with 3 unfamiliar peers was measured with Rubin's Play Observation Scale.[33] Mothers also rated their child's social fear at 4 and 7 years using the shyness subscale of the Colorado Child Temperament Inventory.[35] For each of the 4 time points, observed behavioral and maternal report measures were standardized in the full cohort and then averaged to create a single measure of early childhood BI.[9,36,37] For our primary analyses, we used this continuous composite measure of early childhood BI and social reticence. This composite provides a stable measure of early childhood BI and has been used extensively in prior work.[11,12,36,37] For a secondary analysis to compare results with other studies, we categorized participants on the basis of their reactivity profiles at 4 months of age. This analysis attempts to replicate findings from the only other longitudinal structural brain study of young adults with histories of BI in infancy.[28] All 53 participants had the primary composite measure of BI available. For the secondary analysis, 43 had complete data for the 4-month reactivity phenotype available (of which 19 were classified as high negative reactive and 11 as low reactive).

In addition to examining the influence of early childhood BI, we wished to test whether anxiety and/or cognitive control during adolescence moderated the relation between early childhood BI and brain structure in young adulthood. This analysis minimized the number of statistical comparisons and derived a measure of anxiety incorporating as much data from the current study as possible. Accordingly, a single composite measure of anxiety over adolescence and young adulthood was created for each participant. This composite measure was created on the basis of 3 measures obtained during adolescence (mean age 14.7 years): parent report from Screen for Child Anxiety Related Disorders (SCARED), child report from SCARED[38]; the Anxious/Depressed raw score on the Youth Self-Report (YSR)[39]; and 3 measures obtained during young adulthood (mean age 19.8 years): Beck Anxiety Inventory,[40] Liebowitz Social Anxiety Scale,[41] and the Anxious/Depressed raw score on the YSR. Standardized scores were created for each measure for each participant on the basis of all participants with available data on these anxiety measures (n = 163), not just the subset imaged for this current study. The use of standardized scores allows an equal weighting of each measure in the composite. The composite anxiety measure was an average over these 6 standardized measures (Cronbach's alpha = 0.71). The adolescent and young adult composites were correlated (Spearman's rho = 0.48, $p < .001$), suggesting traitlike features. Data were available in more than 80% of the imaged participants for each individual scale; 92.5% of participants had at least 1 measure from adolescence, and 98.1% had at least 1 measure from adulthood. All participants had at least 2 of the 6 measures available.

The Eriksen Flanker Task was used to measure cognitive control in the current sample. A subset of the cohort with imaging data in young adulthood had completed the Eriksen Flanker Task in adolescence (n = 25, mean age 14.8 years, 9 female and 16 male). The Eriksen Flanker Task consisted of equal numbers of congruent (HHHHH and SSSSS) and incongruent (HHSHH and SSHSS) trials in which participants had to indicate the central letter with a button press.[42] Cognitive control was measured with the congruency effect, computed as the difference in reaction time for incongruent versus congruent trials, divided by the reaction time on congruent trials. Higher scores indicate poorer cognitive control.

Neuroimaging

All MRI data were acquired on the same scanner, a GE Healthcare MR750 3.0 Tesla scanner with a 32-channel head coil. Each scanning session included a single high-resolution, T1-weighted structural imaging sequence (MPRAGE; sagittal acquisition; 176 slices; 1 mm^3 isotropic voxels; 256 × 256 matrix; flip angle = 7°; repetition time [TR] = 7.7 milliseconds; echo time [TE] = 3.42 milliseconds; inversion time [TI] = 425 milliseconds).

Image Processing

FreeSurfer 5.3 (http://surfer.nmr.mgh.harvard.edu/) was used to generate subcortical segmentations,[43,44] cortical parcellations using the Destrieux *et al.* atlas,[45] and pial and white matter surfaces from the T1-weighted images.[46-48] After the parcellations and surfaces were generated, they were visually reviewed by trained research assistants and, where necessary, manually edited and regenerated. Regional volumes and cortical thicknesses were obtained from the edited parcellations and surfaces.[45,48]

Regional A Priori Analyses

Three bilateral regions of interest (ROIs) from the Destrieux *et al.* atlas[45] were selected a priori for initial cortical thickness analyses: the middle anterior part of the cingulate gyrus and sulcus (dACC), the short insular gyrus (aI), and the subcallosal gyrus (sgACC). These ROIs are depicted in Figure 1A. Bilateral volumes of the amygdalae and hippocampi were examined in a priori analyses as well. In an effort to replicate findings by Schwartz *et al.*,[28] 2 additional ROIs were explored exclusively in the secondary analysis when examining the relations between 4-month temperament and cortical thickness: left orbitofrontal and right ventromedial regions were hand-drawn on an average FreeSurfer volume based on Figures 1 and 2 from Schwartz *et al.*[28] These regions were then projected to individual participants to generate thickness values for the left orbitofrontal and right ventromedial regions for each participant.

Prefrontal Cortex Exploratory Analyses

We additionally performed exploratory analyses in the prefrontal cortex.[28] General linear modeling programs available through FreeSurfer (Qdec) were used for these vertexwise analyses. A prefrontal cortex mask was generated by combining the following parcellations from the Desikan atlas[49]: superior frontal, rostral, and caudal middle frontal, pars opercularis, pars triangularis, pars orbitalis, lateral and medial orbitofrontal, frontal pole, and rostral and caudal anterior cingulate cortices. To correct for multiple comparisons, Monte Carlo simulations available in the FreeSurfer software were used to determine that an area of cortex 68.1 mm^2 with each individual vertex $p < .001$ was required to meet prefrontal cortex-wide clusterwise significance of $p < .05$. Clusters meeting multiple comparison correction are reported in Montreal Neurological Institute (MNI) coordinates.

Statistical Analyses

All statistical tests were performed using SPSS version 20 (IBM, Armonk, NY) with the exception of the prefrontal cortex vertexwise analyses, which were carried out using FreeSurfer. We used *t* tests to compare BI and anxiety in the subset of participants that provided versus did not provide imaging data, and χ^2 tests to test for potential group differences in sex. Analogous tests compared participants in the current study that did versus did not provide Eriksen Flanker Task data in adolescence.

The relations among temperament, anxiety, and cortical thickness were examined with repeated measures analyses of variance predicting thickness. Specifically, for each a priori region, cortical thickness values from left and right hemispheres were included in a single model, with hemisphere treated as a repeated measure. Additional factors for the primary models comprised childhood BI, anxiety in adolescence/young adulthood, the interaction between

BI and anxiety, sex, and whole-brain average cortical thickness. Variables were mean centered before computing interactions for all analyses. When significant effects emerged in primary models, secondary models added IQ and maternal education as covariates. Three participants each were missing IQ and maternal education data, and these missing values were replaced with group means; excluding these participants did not change the significance of results. Only results from secondary models are reported; primary models were included to ensure that results were not driven exclusively because of the addition of multiple covariates. For amygdala and hippocampus analyses, volumes were used instead of thicknesses, and whole-brain volume was used as a covariate instead of whole-brain average thickness. Bonferroni correction was used to protect against false-positive results for the 5 models based on the a priori regions.

Prefrontal cortex vertexwise analyses were conducted separately for each hemisphere using the primary model with FreeSurfer. One participant's composite BI measure was 3 standard deviations from the mean, and another participant's composite anxiety measure was 3 standard deviations from the mean. These outliers were handled by Winsorizing for all analyses; no outliers were detected in other measures.

Analyses examining the relations between cognitive control and cortical thickness of dACC were based on Westlye *et al.*[50] The congruency effect on the Eriksen Flanker Task for each participant was computed as the difference in reaction time for incongruent versus congruent trials divided by the reaction time on congruent trials. Higher scores indicate poorer cognitive control, and the mean score across the sample was 0.10 with a standard deviation of 0.051. To attempt replication, the relation between congruency and cortical thickness was examined with a repeated-measures analysis of variance with hemisphere as a repeated measure and congruency, sex, and IQ as covariates. This model was followed up by additionally controlling for whole-hemisphere thickness. Finally, we included congruency, early childhood BI, and the interaction between BI and congruency in a single model to determine whether congruency moderated the relation between BI and cortical thickness.

Power analyses were computed using G*Power 3.1.[51] Using a single predictor in a regression model to detect a moderate effect size (partial $\eta^2 = 0.15$), power in the full sample of 53 was 0.84, power in the sample using just the 4-month phenotype (n = 30) was 0.59, and power in the sample using the Eriksen Flanker Task data (n = 25) was 0.51. The power to detect a larger effect size (partial $\eta^2 = 0.25$, on the order of the effect size of the main result in this study) was 0.98 in the full sample, 0.86 for the 4-month phenotype data, and 0.79 for the Eriksen Flanker Task data.

RESULTS

Participants

Compared to participants from the larger longitudinal study who did not undergo imaging, participants who did undergo imaging for the current study had significantly lower anxiety scores over adolescence/young adulthood (−0.18, SD = 0.57, versus 0.16, SD = 0.91, $t = 2.5$, $p = .012$, $d = 0.45$), but there were no differences in early childhood BI scores (−0.06, SD = 0.73, versus 0.012, SD = 0.60, $t = 0.71$, $p = .48$, $d = 0.11$) or sex ($\chi^2 = 1.5$, $p = .22$). Follow-up analyses revealed that imaged participants had significantly lower levels of anxiety compared to excluded participants (−0.18 versus 0.17, $t = 2.6$, $p = .012$). There was no difference in anxiety scores between the imaged participants and participants who refused participation (−0.18 versus −0.07, $t = 0.7$, $p = .49$). It should be noted, however, that the absolute difference in anxiety between imaged participants and the total cohort was less than 0.2 SDs from the cohort mean, and the imaged participants were representative of the original longitudinal cohort in terms of BI scores. Within the current study sample, there were no significant differences in anxiety, BI, or sex ($t = 1.39$, $p = .17$, $d = 0.38$; $t = 0.24$, $p = .81$, $d = 0.07$; $\chi^2 = 0.23$, $p = .63$, respectively) between the subset of individuals who participated versus

FIGURE 1 Early childhood behavioral inhibition predicts cortical thickness in the dorsal anterior cingulate (dACC) in young adulthood. Note: Panel A illustrates the 3 cortical a priori regions of interest, derived from the Destrieux atlas.[45] Panel B depicts the significant relation between early childhood behavioral inhibition (BI) and cortical thickness in the dACC in adulthood. Each dot represents a single participant, and thickness values are averaged across left (L) and right (R) dACC. The encircled participant's BI value was Winsorized because BI > 3 SD from the mean. sgACC = subgenual anterior cingulate.

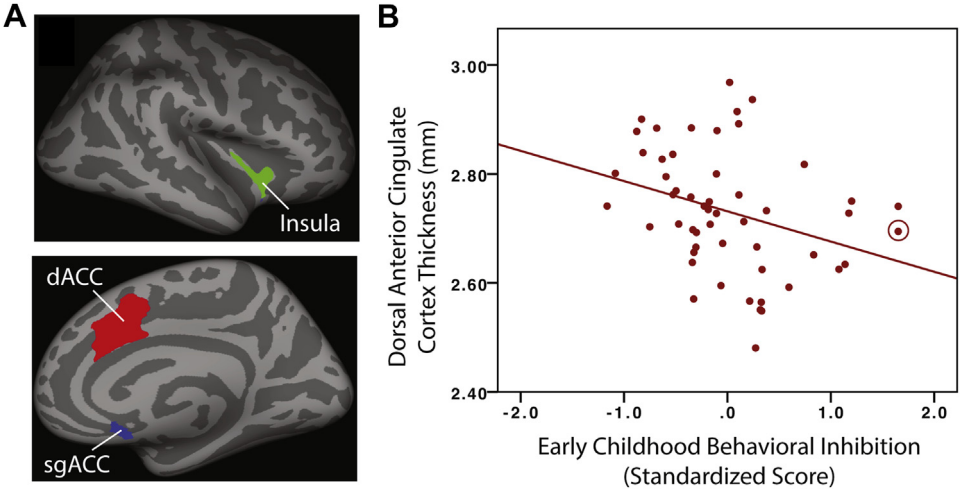
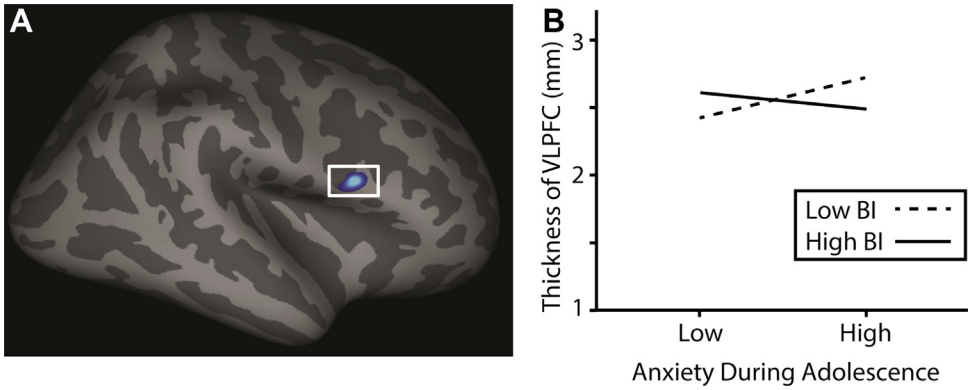


FIGURE 2 Anxiety during adolescence moderates the relation between early childhood behavioral inhibition (BI) and the thickness of the right ventrolateral prefrontal cortex (VLPFC) during adulthood. Note: Panel A depicts a z-map for the interaction between early childhood BI and anxiety in adolescence in relation to cortical thickness in adulthood. Illustrated results survive clusterwise multiple comparison correction across prefrontal cortex ($p < .05$). Panel B graphically illustrates the interaction for the right VLPFC region in panel A. Higher anxiety during adolescence is related to increased thickness of the right VLPFC during adulthood among individuals with low but not high early childhood BI.



the subset of those who did not participate in the Eriksen Flanker Task in adolescence. Early childhood BI and anxiety in adolescence/young adulthood were not significantly related in the sample used in this study ($r = -0.043$, $p = .76$).

Behavioral Inhibition, Anxiety, and Cortical Thickness: Regional Analysis

The relations among BI during early childhood, anxiety in adolescence/young adulthood, and cortical thickness in young adulthood were examined in 3 bilateral a priori cortical regions of interest: the dorsal anterior cingulate (dACC), anterior insula (aI), and subgenual anterior cingulate (sgACC). These regions are depicted in Figure 1A. Parallel analyses examined relations with hippocampus and amygdala volumes. As depicted in Figure 1B, early childhood BI predicted thinner cortex in the dACC with a large effect size ($F_{1,45} = 16.2$, $p < .001$ uncorrected, partial $\eta^2 = 0.26$, survived Bonferroni correction for 5 a priori regions). This analysis controlled for multiple potential confounding factors, including sex, maternal education, IQ, whole-brain average cortical thickness, anxiety in adolescence/young adulthood, and the interaction between BI and anxiety. Of note, similar results emerged in bivariate analyses examining only the relations between BI and dACC thickness. Early childhood BI similarly predicted thinner cortex in the sgACC ($F_{1,45} = 5.2$, $p = .027$ uncorrected, partial $\eta^2 = 0.10$), although with a smaller effect size. Unlike findings for the dACC, this result did not survive Bonferroni correction for 5 comparisons. Neither anxiety nor the interaction between BI and anxiety was significantly related to dACC or sgACC cortical thickness, suggesting that anxiety did not moderate the relation between early childhood BI and thickness of the dACC in adulthood. There were no significant relations among childhood BI, anxiety in adolescence/young adulthood, or the interaction between BI and anxiety with anterior insula thickness or hippocampus or amygdala volumes.

Behavioral Inhibition, Anxiety, and Cortical Thickness: Prefrontal Cortex Exploratory Analysis

To complement the a priori ROI analysis, a parallel analysis examined the relations among BI during early childhood, anxiety in adolescence/young adulthood, and cortical thickness in young adulthood in an exploratory fashion across prefrontal cortex. In contrast to the regional analysis, the prefrontal cortex vertexwise analysis did not detect any regions surviving multiple comparison correction that were related to early childhood BI alone. A whole-brain vertexwise map, uncorrected at $p < .05$, demonstrating the relation between early childhood BI and cortical thickness in young adulthood, is provided in Figure S1, available online. This map is consistent with the regional analysis, as the thickness of a large swath of cortex near the dACC was related to early childhood BI; as above, however, this cluster did not survive multiple comparison correction across the prefrontal cortex. The prefrontal cortex vertexwise analysis likewise did not detect any regions surviving multiple-comparison correction that were related to anxiety in adolescence/young adulthood.

The prefrontal cortex vertexwise analysis did, however, detect a region in which thickness in young adulthood was related to the interaction between early childhood BI and anxiety in adolescence/young adulthood. As illustrated in Figure 2, cortical thickness in an 83 mm^2 patch of the right ventrolateral prefrontal cortex (VLPFC; centered at +48.2 +10.0 +13.9 in MNI coordinates, within the pars triangularis) was predicted by an interaction between childhood BI and anxiety in adolescence/young adulthood, controlling for sex and whole-hemisphere thickness ($p = .024$, corrected for multiple comparisons across prefrontal cortex). To explore the source of this interaction, a median split divided participants into those with low versus high levels of early childhood BI, and a similar median split was performed for anxiety. Note that effect sizes for this median split analysis are biased by the circular

nature of the analysis, which computes statistics on a region initially identified in an exploratory test. Effect sizes are reported solely to interpret the interaction. In participants with low early childhood BI, a high level of anxiety during adolescence/young adulthood predicted thicker VLPFC compared to a low level of anxiety ($d = 1.56$). In participants with high early childhood BI, however, anxiety during adolescence/young adulthood was unrelated to VLPFC thickness in young adulthood ($d = -0.46$).

Temperament at 4 Months

The characterization of early childhood BI discussed above was a composite of measures from age 14 months to 7 years. Analyses in the only other study using a similar design, Schwartz *et al.*[28] relied on data from 4 months of age. In secondary analyses, we used phenotypes similar to this prior study in the small subset (57%) of our participants with either high reactive negative (n = 19) or low reactive (n = 11) temperament at age 4 months. This measure did not predict cortical thickness in young adulthood in any of the a priori regions tested including the left orbitofrontal and right ventromedial regions derived from Schwartz *et al.*[28] Prefrontal cortex vertexwise analyses similarly did not reveal any significant relations between 4-month temperament and regional cortical thickness. Using a less stringent threshold of $p < .05$ uncorrected also did not reveal any relations between cortical thickness near the regions derived from Schwartz *et al.*[28] and temperament at 4 months.

Cognitive Control

We examined whether congruency effects on the Eriksen Flanker Task during adolescence moderated the relations between early childhood BI and dACC thickness in young adulthood. Higher congruency effects on the Eriksen Flanker Task during adolescence (reflecting poorer cognitive control) predicted thinner dACC during young adulthood ($F_{1,23} = 4.66$, $p = .042$, partial $\eta^2 = 0.17$), controlling for sex and IQ. There was no relation, however, between BI in early childhood and the congruency effect in young adulthood among the current study sample ($r = -0.01$, $p = .94$). The interaction between BI and congruency likewise was unrelated to dACC thickness ($F_{1,21} = 2.12$, $p = .16$, partial $\eta^2 = 0.09$), indicating that the congruency effect (1 measure of cognitive control) did not moderate the relation between early childhood BI and young adult dACC thickness.

DISCUSSION

The primary goal of the current study was to test the hypothesis that BI in early childhood predicts thickness in specific cortical regions, as well as volume of amygdala and hippocampus, during adulthood. Consistent with this hypothesis, BI during early childhood predicted thinner dACC in adulthood, although no differences as a function of BI were detected in anterior insula, sgACC (after correction for multiple comparisons), amygdala, or hippocampus. Neither anxiety nor cognitive control during adolescence moderated the relation between early childhood BI and dACC thickness in adulthood, although a higher congruency effect on the Eriksen Flanker Task during adolescence was independently related to thinner dACC in adulthood. High anxiety during adolescence and young adulthood was related to thicker cortex in the right VLPFC in young adulthood, but only among those who had low BI as children.

These results are consistent with prior literature linking BI to the dACC. The dACC is part of a network of brain regions involved in identifying and signaling the need for increased cognitive control.[26] A series of studies in the current and other samples use event-related potentials[42,52,53] and functional MRI (fMRI)[11,12] to measure brain activity during tasks that require trial-by-trial changes in levels of cognitive control. This series of studies demonstrates consistently larger increases in neuronal activity in or near the dACC among children and adolescents with high BI relative to those with low BI.

These data are consistent with a model in which the dACC of individuals high in BI is highly sensitive to conflict, signaling the need for cognitive control.[10] Notably, research has shown this BI-related pattern of brain function does not generate behavioral benefits but, rather, relates to risk for anxiety.[10] In light of this model, the current findings of thinner dACC among individuals with higher BI suggests the hypotheses that either a thinner dACC generates a larger neural signal or that repeatedly high activity in the dACC over development results in thinner cortex. Longitudinal studies incorporating neuroimaging early in life are needed to adjudicate these possibilities. In either case, these data may provide a biological explanation for the link between early childhood BI and adult outcomes. Moreover, by linking early temperament to adult brain structure, the current findings raise questions about the types of impairing behaviors expressed in adulthood that could relate to both early childhood temperament and dACC structure. For example, adults with a childhood history of high BI may have subtle abnormalities of cognitive control associated with decreased dACC thickness. If so, interventions targeting impairment in children with high BI could influence these or other behaviors in adulthood.

Importantly, variation in neither adolescent anxiety nor cognitive control on the Eriksen Flanker Task moderated the relation between early childhood BI and adult dACC thickness. These results suggest that the association between early childhood BI and adult dACC thickness manifests independently of anxiety and/or this 1 particular measure of cognitive control. One possibility is that although thinner dACC in individuals with a history of BI may increase risk for pathological anxiety and alterations in cognitive control, some individuals compensate for this risk. Further studies could test whether this compensation occurs functionally within the dACC (i.e., activity is normal even though structure is abnormal) or through functional and/or structural changes in other brain areas.

In the current study, adolescent/young adult anxiety moderated the relations between early childhood BI and cortical thickness in a portion of the right VLPFC. High anxiety in adolescence and adulthood related to increased VLPFC thickness in young adults with a history of low BI in

early childhood. No such relation occurred in adults with high BI in early childhood. These results suggest that the neurodevelopmental pathway to high anxiety could differ depending on underlying temperament,[10] with variation in the VLPFC related to anxiety only in individuals low in BI. Previous work consistently relates variation in the structure and function of the right VLFPC to both anxiety disorders and orienting to threat.[54-56] The right VLPFC may be a portion of the ventral attention network, involved in stimulus-driven attention, and increased thickness may be related to increased orienting to threat and subsequently anxiety.[27,57,58] Because VLPFC thickness is only relevant to individuals low in BI during early childhood, a speculative possibility is that the mechanisms of orienting to threat and the pathway to anxiety differ depending on levels of BI.

In a secondary analysis that was only able to use a small percentage (57%) of our participants, we did not replicate Schwartz *et al.*,[28] who reported thinner left orbitofrontal cortex and thicker right ventromedial prefrontal cortex in young adults with a history of high reactivity as 4-month-old infants. This difference could reflect low power associated with small sample size, as well as methodological factors. Nevertheless, the current study, Schwartz *et al.*,[28] and other prior work[7] suggest that early childhood BI predicts aspects of brain structure or function later in adulthood. Moreover, additional analyses replicated prior work linking dACC thickness to congruency effects on the Eriksen Flanker Task.[50,59] Thus, the current findings replicate some brain–behavior associations seen in prior work.

The results of this study should be interpreted in light of several limitations. We had a moderate sample size for the primary analysis and a small sample for our secondary analyses. As a result, failure to detect associations could reflect a type II error. Small sample sizes reflect the difficulty of acquiring brain imaging data on participants followed prospectively for more than 20 years. Demonstrating large effect sizes in some analyses, however, speaks to the value of such work, even with limited sample size. Another consideration is that the sample used in this study had a small (less than 0.2 SDs) but significant difference in the amount of anxiety experienced during adolescence and young adulthood relative to the whole longitudinal cohort.

In summary, the current study demonstrates that variation in early childhood temperament is related to adult brain structure. These data reinforce the hypothesis that the dACC is a key brain structure in the physiology of BI, and provide a candidate biological basis for the associations between early childhood BI and adult functional outcomes. Future studies following cohorts incorporating longitudinal neuroimaging that begin earlier in childhood are needed to determine whether alterations are present early in childhood or emerge later in the course of development. In addition, future studies are required to determine the functional implications of the cortical thickness differences detected in this study. Regardless of the outcome of these additional studies, data from the current study highlight the importance of early intervention for children who are functionally impaired from high BI because of the potential for enduring effects on brain structure that occur into adulthood. &

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FIGURE S1 Whole-brain, vertexwise map demonstrating the relation between early childhood behavioral inhibition (BI) and cortical thickness in young adulthood. Note: Results are adjusted for anxiety during adolescence/young adulthood, the interaction between BI and anxiety, sex, and whole-hemisphere mean cortical thickness. Maps are thresholded at $p < .05$, uncorrected. Warmer colors indicate a positive relation between BI and cortical thickness, and cooler colors indicate a negative relation. Although no patches of cortex were significant after correcting for multiple comparisons across the prefrontal cortex, the highest peak in these maps is centered near the dorsal anterior cingulate cortex, consistent with the regional analyses.