

Structural Brain Correlates of Childhood Inhibited Temperament: An ENIGMA-Anxiety Mega-Analysis

Janna Marie Bas-Hoogendam^{a,b,c,d,*}, PhD , Rachel A. Bernstein^d, BA , Brenda E. Benson^d, PhD, Samuel E.C. Frank^d, BA , Kristin A. Buss^e, PhD , Kelley E. Gunther^{e,f}, PhD , Koraly Pérez-Edgar^e, PhD , Giovanni A. Salum^{g,h,i}, PhD , Andrea Jackowski^j, PhD , Rodrigo A. Bressan^l, PhD , André Zugman^d, PhD , Kathryn A. Degnan^k, PhD , Courtney A. Filippi^l, PhD , Nathan A. Fox^f, PhD, Heather A. Henderson^m, PhD , Alva Tangⁿ, PhD , Selin Zeytinoglu^{f,o}, PhD , Anita Harrewijn^p, PhD , Manon H.J. Hillegers^q, PhD, MD , Ryan L. Muetzel^q, PhD , Tonya White^{d,q}, MD, PhD , Marinus H. van IJzendoorn^{r,s}, PhD , Carl E. Schwartz^t, MD , Julia M. Felicione^{t,u}, PhD , Kathryn A. DeYoung^f, MA, MS , Alexander J. Shackman^f, PhD , Jason F. Smith^f, PhD, Rachael M. Tillman^f, PhD , Yvonne H.M. van den Berg^v, PhD , Antonius H.N. Cillessen^v, PhD, Karin Roelofs^v, PhD , Anna Tyborowska^v, PhD , Shirley Y. Hill^w, PhD , Marco Battaglia^{x,y}, MD, Marco Tettamanti^z, PhD , Lea R. Dougherty^f, PhD , Jingwen Jin^{aa}, PhD , Daniel N. Klein^{bb}, PhD , Hoi-Chung Leung^{bb}, PhD , Suzanne N. Avery^{cc}, PhD , Jennifer Urbano Blackford^{cc,dd}, PhD , Jacqueline A. Claus^{t,ee}, PhD, MD , James M. Bjork^{ff}, PhD , John M. Hettema^{gg}, MD, PhD , Ashlee A. Moore^{hh}, PhD , Roxann Roberson-Nayⁱⁱ, PhD , Chelsea Sawyers^{ff}, PhD , Elizabeth P. Hayden^{jj}, PhD , Pan Liu^{jj,kk}, PhD , Matthew R.J. Vandermeer^{jj}, PhD , H. Hill Goldsmith^{ll}, PhD , Elizabeth M. Planalp^{ll}, PhD , Thomas E. Nichols^{mm}, PhD , Paul M. Thompsonⁿⁿ, PhD , P. Michiel Westenberg^{a,c}, PhD , Nic J.A. van der Wee^{b,c}, PhD, MD , Nynke A. Groenewold^{oo}, PhD , Dan J. Stein^{oo}, PhD, MD , Anderson M. Winkler^{d,pp}, MD, DPhil , Daniel S. Pine^d, MD

Objective: Childhood inhibited temperament (cIT) is associated with an increased risk for developing internalizing psychopathology. Neurobiological characteristics identified by structural magnetic resonance imaging (MRI) may elucidate the neural substrates for cIT, but studies are scarce and often focus on particular regions of interest. Moreover, current findings lack replication. This preregistered analysis from the ENIGMA-Anxiety Working Group examined structural brain characteristics associated with cIT using a comprehensive whole-brain approach.

Method: Temperament assessments (behavioral observations, parent/teacher reports or self-reports on cIT before age 13 years) and MRI data (age at scan, 6–25 years) from international research sites (Europe, North America, South America) were pooled for mega-analysis. Following image processing and quality control, associations between cIT and brain structure were examined in 3,803 participants. Subcortical volumes, cortical thickness, and surface area (main analyses) and detailed subcortical characteristics (eg, subnuclei, subfields, partial volume effects; exploratory analyses) were considered.

Results: In the full sample, cIT showed no relation with brain structure, either as a main effect or in interactions with sex or age. Subgroup analyses (based on cIT assessment type) revealed cIT by sex interactions on mean cortical thickness ($p_{MC-FWER} = .037$) and thickness of the right superior parietal region ($p_{MC-FWER} = .029$) in youth with parent/teacher reports on cIT levels. Exploratory analyses revealed findings in the hippocampus, putamen, and caudate, but most did not survive statistical correction for multiple testing.

Conclusion: This mega-analysis found no consistent associations between cIT and regional brain structure, although the role of parietal regions warrants further investigation. Future studies should consider brain function in cIT, preferably using longitudinal designs.

Plain language summary: Inhibited temperament during childhood is a risk factor for the development of anxiety and depression later in life. A preregistered study from the international ENIGMA-Anxiety Working Group investigated whether characteristics of brain structure are associated with the level of childhood inhibited temperament, using brain scans and data on temperamental traits from participants aged 6 to 25 years, which have been previously acquired at research sites worldwide (total sample > 3,800 subjects). Analyses revealed no consistent correlations between brain structure and inhibited temperament.

Study Registration Information: Structural Brain Correlates of Childhood Inhibited Temperament: An ENIGMA-Anxiety Mega-analysis. [https://www.jaacap.org/article/S0890-8567\(22\)00299-4/fulltext](https://www.jaacap.org/article/S0890-8567(22)00299-4/fulltext)

Key words: temperament; magnetic resonance imaging (MRI); childhood; adolescence; anxiety disorders

J Am Acad Child Adolesc Psychiatry 2026;65(4):577-604. 

The term “temperament” refers to “a biological bias for particular feelings and actions that first appear during infancy or early childhood and that are sculpted by environments into a large, but still limited, number of personality traits.”¹ One of the most rigorously characterized temperament classifications distinguishes infants and young children based on their tendencies to approach or avoid unfamiliar people, objects, and unexpected events, especially in social contexts.^{2,3} Some infants explore new toys with enthusiastic curiosity, whereas others react in a more cautious or avoidant way; when meeting unfamiliar people, some toddlers approach them eagerly, whereas others cling to their parents. Individuals with the tendency to avoid the unfamiliar are labeled as manifesting “behavioral inhibition” or “inhibited temperament” (IT).^{4,5} IT is a moderately heritable trait that can be measured in multiple species, providing opportunities for translational research.⁶⁻⁸ In human beings, levels of IT can be quantified from the first year of life through direct behavioral observations or reports by caregivers or teachers. Similar approaches, as well as self-report questionnaires on current and/or retrospective levels of IT,² can be used later in life.

Variations in IT are present on a continuous scale within the population, and research suggests that around 20% of young children are characterized by high IT.⁹ Although temperament in unselected samples shows at least moderate continuity over time, these individuals with high levels of childhood IT (cIT) have much higher levels of stability.^{3,10-12} Considerable data suggest that temperament predicts personality traits later in life,^{13,14} and that high cIT has adverse long-term consequences^{15,16}: infants with cIT often become more reserved adults, and, on average, such infants exhibit poorer outcomes than noninhibited infants with respect to social relationships and internalizing psychopathology.¹³ Multiple studies have shown cIT (especially “social cIT,” when compared to “nonsocial cIT”¹⁷) to be associated with an elevated risk of developing social anxiety.¹⁸⁻²⁰ These findings have recently been strengthened by the results of a longitudinal twin sample (868 families) revealing that behavioral inhibition robustly predicts social anxiety.²¹ More specifically, almost half of all children with elevated and stable cIT will develop social anxiety disorder (SAD) later in life, compared with only 12% of noninhibited children.¹⁵ Furthermore, a twin study showed that cIT was associated with preadolescent social anxiety symptoms; social anxiety shared a substantial proportion of genetic and environmental variance with cIT, providing evidence for early cIT as a potential developmental endophenotype for later social anxiety.²² In addition, a recently

published study reporting on 110,367 children from a population-based pregnancy cohort study in Norway presented an association between early childhood temperament (shyness at age 5 years reported by the mother) and the presence of emotional disorders in adolescence.²³ Taken together, these findings indicate that cIT predicts risk for later psychopathology, especially SAD (large effect size; odds ratio = 5.84, 95% CI = 3.38-10.09, $p < .001$, as reported by Sandstrom *et al.*, 2020),^{16,24-28} although it should be noted that not all children with high levels of cIT early in life become anxious adults.²⁹⁻³²

Several neuroimaging studies have examined neurobiological correlates of cIT.³⁰ Such research is important, because brain characteristics—including brain structure, activity, and connectivity—may mediate the cIT-related risk for poor outcomes.³³ Some studies have used a cross-sectional approach, including children and early adolescents with high IT³⁴⁻³⁶ or investigating young adults who displayed inhibited behavior as a child (determined retrospectively) and at the time of MRI assessment.³⁷⁻⁴² Other studies had a longitudinal design, in which infant temperament was assessed early in life, whereas neuroimaging was performed during late childhood, adolescence, or young adulthood.⁴³⁻⁵⁶ These previous studies have connected cIT to structure and function of brain networks involved in emotion perception, experience, and regulation.² These brain networks involve the dorsal (caudal) and ventral (rostral) anterior cingulate cortex (ACC), insula, amygdala, dorsolateral and medial prefrontal cortex (PFC), orbitofrontal cortex (OFC) and striatum,^{2,33} all of which have also been implicated in familial risk for SAD.⁵⁷⁻⁶¹ In addition, translational work has indicated involvement of the hippocampus.^{8,62-64}

Despite this progress, the few available studies on the neuroanatomical correlates of cIT are often restricted to specific regions of interest, and cortical surface area and cortical thickness have been examined in only 1 study, with an exploratory approach.⁵⁵ Furthermore, most findings with respect to brain structure are unique to a specific sample³³ (Table 1^{34-37,39-42,44,45,51-55,65-79}), and cross-study comparisons are limited by relatively small sample sizes and failure to consider potential modifying variables such as age and biological sex.

In this ENIGMA-Anxiety project,⁸⁰ we aim to extend previous work by examining brain structure associated with cIT in a large dataset, assembling data acquired at research centers worldwide (18 samples, $n = 4,810$ before image processing and quality control). Compared to the individual studies (on relatively small [sample sizes ranging from 23 to 130] [Table 1] and homogeneous samples), this new study

TABLE 1 Previous Neuroimaging Findings Related to Childhood Inhibited Temperament, From Samples Included in the Present Mega-Analysis

Publications on cIT and brain structure					Publications on cIT and brain function/connectivity	
Sample	Sample size in present mega-analysis ^a	Sample size in previous work	Approach	Findings	Sample size in previous work	Findings
BRAINS study	n = 130	n = 130 ³⁵	ROI-based: bilateral amygdala, posterior insula, anterior insula, ACC, OFC, and vIPFC, as well as comparison occipital ROIs (inferior and middle occipital gyri).	Left posterior insula volume was positively correlated with total cIT score (no effect size reported).	n = 42 ³⁴ n = 67 ³⁶ n = 56 ⁶⁵	Increased connectivity between putative “salience processing” regions (amygdala and insula) and putative internal processing regions (vmPFC). Emotion-face masked dot-probe task: non-cIT children displayed greater activation vs cIT children in several regions in response to threat faces vs neutral faces, including striatum and prefrontal and temporal lobes. Dot-probe task (analysis in 3 prefrontal ROIs based on task activation): greater activation in the right dIPFC cluster in children with high cIT; no differences in amygdala, vIPFC, and mPFC ROIs.
Brazilian High Risk Cohort	n = 678	None	—	—	None	—
Cohort 3/4	n = 95	n = 53 ⁵²	ROI analysis of brain structure in adulthood. Cortical thickness: middle anterior part of cingulate gyrus and sulcus (dACC), short insular gyrus, subcallosal gyrus, and left orbitofrontal and right ventromedial ROIs. Bilateral volumes of the amygdala and hippocampus. Vertex-wise exploratory analyses in prefrontal cortex.	Early cIT predicted thinner cortex in the dACC (large effect size, partial $\eta^2 = 0.26$) and subcallosal gyrus (small effect size, partial $\eta^2 = 0.10$, uncorrected for multiple comparisons); other regions no relationship with cIT.	n = 32 ⁶⁶ n = 32 ⁴⁴ n = 39 ⁶⁷ n = 44 ⁶⁸ n = 32 ⁶⁹ n = 35 ⁷⁰ n = 35 ⁷¹ n = 27 ⁴⁵ n = 38 ⁷² n = 50 ⁷³ n = 83 ⁷⁴	Reward-contingency task: Adolescents characterized by an enduring pattern of cIT demonstrated enhanced sensitivity of the reward-related neural system. Monetary Incentive Delay task: greater striatal activation to incentives in adolescents with cIT; no significant interactions between early inhibited temperament and activity in the bilateral nucleus accumbens with changes in anxiety/anxiety levels at age 26 y. Social evaluation task: striatal sensitivity in adolescents varied as a function of temperament, the peer delivering the feedback, and feedback valence. Attention-bias task: young adults with cIT exhibited greater strength in threat-

(continued)

TABLE 1 Continued

Publications on cIT and brain structure					Publications on cIT and brain function/connectivity	
Sample	Sample size in present mega-analysis ^a	Sample size in previous work	Approach	Findings	Sample size in previous work	Findings
						related connectivity, differences manifested in connections between the amygdala and dlPFC and anterior insula. Emotional conflict task: adults with cIT exhibited greater dorsomedial prefrontal cortex activity during conflict detection and greater putamen activity during conflict adaptation. Implicit emotion-processing task: in the presence of fearful faces, adults with cIT exhibited greater activity in cingulate cortex, dlPFC, and striatum for high attention control trials compared with low attention control trials. The opposite pattern emerged in the presence of happy faces. Face processing task: adolescents with cIT showed exaggerated amygdala response during subjective fear ratings and deactivation during passive viewing, across all emotion faces. In addition, the cIT group showed an abnormally high amygdala response to a task condition marked by novelty and uncertainty. Connectivity: cIT was associated with differences in intrinsic functional connectivity in adulthood, between 3 amygdala subdivisions and prefrontal cortex, striatum, anterior insula, and cerebellum. Extinction recall task: cIT was associated with greater activation in subgenual ACC in response to cues signaling safety.

(continued)

TABLE 1 Continued

Publications on cIT and brain structure					Publications on cIT and brain function/connectivity	
Sample	Sample size in present mega-analysis ^a	Sample size in previous work	Approach	Findings	Sample size in previous work	Findings
Generation R-behavioral observations	n = 584	None	—	—	None	—
Generation R-questionnaire data	n = 1,982	None	—	—	None	—
Maryland-PAX	n = 220	None	—	—	None	—
Maryland-TAX	n = 53	None	—	—	None	—
Nijmegen Longitudinal Study	n = 71	None	—	—	None	—
Pittsburgh	n = 15	n = 23 ⁵¹	ROI-based approach with manually traced amygdala and OFC, followed by whole-brain VBM.	cIT related to greater right OFC volume and greater total amygdala volume in adolescence (no effect size reported).	None	—
San Raffaele	n = 20	None	—	—	None	—
SDAN	n = 55	None	—	—	None	—
Stony Brook Temperament Study	n = 74	None	—	—	None	—
TOTS	n = 96	n = 75 ⁵⁵	ROI-based: bilateral amygdala volume, with negative reactivity at 4 mo as predictor. Exploratory analysis: vertex-wise whole-brain cortical thickness and cortical surface area.	In children between 10 and 12 y of age, left amygdala volume increased more slowly in those with cIT (no effect size reported).	n = 43 ⁷⁵ n = 87 ⁵⁴ n = 53 ⁵³ n = 53 ⁷⁶ n = 55 ⁷⁷	Extinction recall task: cIT predicts a distinct pattern of hemodynamic—autonomic covariation when recalling extinguished threat and safety cues; interactions present in anterior insular cortex, anterior subdivision of the medial cingulate cortex, and dlPFC. Connectivity: in children with a history of high cIT, anxiety symptoms became more negatively correlated with dlPFC—amygdala connectivity when processing salient, proximal threats; the opposite developmental pattern was observed in low-cIT children. Virtual school paradigm: in adolescents with preadolescent

(continued)

TABLE 1 Continued

Publications on cIT and brain structure					Publications on cIT and brain function/connectivity	
Sample	Sample size in present mega-analysis ^a	Sample size in previous work	Approach	Findings	Sample size in previous work	Findings
Vanderbilt—children	n = 55	None	—	—	n = 37 ⁷⁸	social anxiety, greater cIT was associated with enhanced bilateral insula engagement while anticipating unpredictable-vs-nice social evaluation. High cIT predicted greater activity in dorsal ACC and bilateral insula. High cIT was associated with negative functional connectivity between insula and vmPFC, and negative evaluation was associated with increased amygdala activity (during feedback from unpredictable peers). Modified flanker task: significant cIT-by-anxiety-by-error condition interactions in cuneus, fusiform gyrus, lingual gyrus, orbitofrontal gyrus, and middle occipital gyrus. During anticipation and viewing of threat stimuli and social stimuli: high cIT is related to widespread alterations in prefrontal cortex function and connectivity
Vanderbilt—young adults	n = 150	n = 84 ⁴²	ROI-based approach focused on amygdala, with 3 complementary methods: manual segmentation, surface mapping, and VBM.	Inhibited adults had significantly larger volume in right amygdala, with a similar trend for left amygdala (manual segmentation), regions of increased convexity located primarily in basolateral and lateral subnuclei (surface mapping), and greater gray matter volume in	n = 20 ⁴¹ n = 33 ³⁹ n = 39 ⁴⁰ n = 34 ³⁷ n = 32 ⁷⁹	Faces task: cIT participants had faster amygdala responses to novel compared with familiar faces, and both longer and greater amygdala response to all faces; cIT young adults had increased BOLD response in amygdala when viewing both novel and recently familiarized faces (so sustained amygdala activation). In individuals with an inhibited temperament, the amygdala and hippocampus failed to habituate across repeated presentations of faces. Young adults with cIT: greater

(continued)

TABLE 1 Continued

Publications on cIT and brain structure

Publications on cIT and brain function/connectivity

Sample	Sample size in present mega-analysis ^a	Sample size in previous work	Approach	Findings	Sample size in previous work	Findings
Virginia Commonwealth University Juvenile Anxiety Study (VCU-JAS)	n = 129	None	—	both left and right amygdalae (VBM) (no effect size reported). Furthermore, inhibited adults had larger caudate volume (left; no effect size reported).	None	activation of a prefrontal network when anticipating viewing fear faces (but no functional differences in amygdala), and more negative connectivity between the rostral ACC and the bilateral amygdala. Higher social fearfulness was associated with slower habituation across regions of the social brain, including the hippocampus, amygdala, vmPFC, medial OFC, fusiform face area, primary visual cortex, and extrastriate visual cortex.
Western University	n = 87	None	—	—	None	—
Wisconsin Twin Project-RDoC twin study	n = 316	None	—	—	None	—

Note: ACC = anterior cingulate cortex; BOLD = blood oxygen level-dependent; cIT = childhood inhibited temperament; dACC = dorsal anterior cingulate cortex; dlPFC = dorsolateral prefrontal cortex; mPFC = medial prefrontal cortex; OFC = orbitofrontal cortex; ROI = region of interest; VBM = voxel-based morphometry; vlPFC = ventrolateral prefrontal cortex; vmPFC = ventromedial prefrontal cortex.

^aSample size before image processing and quality control.

is better powered because of the larger number of research participants available for analysis. Moreover, by combining data through a mega-analytic approach, the present study facilitates the differentiation of consistent, generalizable findings from false-positives that could emerge from smaller-sampled studies.⁸¹ Such work has the potential to establish reproducible anatomical correlates, and could inform the development of mechanistic studies and intervention research with clinical relevance.⁸²

We performed a mega-analysis of T₁-weighted anatomical MRI scans of the human brain with a whole-brain approach (regional and vertex-wise) on the total dataset, and considered the relationship between cIT and 3 distinct neuroanatomical metrics: volumes of subcortical structures, cortical thickness, and cortical surface area. As cortical thickness and cortical surface area are genetically and phenotypically independent, it is important to investigate them separately.⁸³ In addition to the main analyses, sensitivity analyses were performed in 3 subsets, based on the method and thus age at which cIT was determined: first, (early-life) behavioral observations; second, parental/teacher reports during childhood; and third, self-report measures acquired during late childhood and adolescence.² Importantly, the association between cIT and later (social) anxiety has been established for all 3 types of assessments (for some examples, see behavioral observations,^{18,19,84-86} parental reports,^{22,26,87,88} and self-report questionnaires^{89,90}; see also a recent meta-analysis reporting no significant effect of the method of measuring inhibited temperament on the cIT-anxiety association¹⁶). A fourth sensitivity analysis included only samples in which temperament was assessed during childhood (not retrospectively).

We expanded previous work by performing exploratory analyses on the relationship between cIT and amygdalar subnuclei, thalamic subnuclei, and hippocampal subfields,⁹¹⁻⁹³ the amount of gray matter inside each subcortical structure,⁹⁴ and the volumes of additional subcortical limbic structures that were more recently included in the FreeSurfer software package.⁹⁵

We expected to corroborate findings in brain circuits found previously (involved in processing fear, reward, and emotion regulation),^{2,33} with small-to-medium effect sizes.^{81,96-98} That is, based on earlier work on inhibited temperament (Table 1), we hypothesized that structural characteristics of the amygdala (larger volume^{42,51}), caudate (larger volume⁴²), caudal and rostral ACC (thinner cortex⁵²), insula (increased cortical thickness³⁵), and OFC (increased cortical thickness, especially in right OFC⁵¹) are neural substrates of cIT. Additional hypotheses, based on an endophenotype study in socially anxious families that revealed heritable brain alterations related to social

anxiety,⁵⁷ were the following: we expected that cIT is associated with increased volume of the putamen, decreased cortical thickness of the superior temporal gyrus, increased cortical thickness of the transverse temporal gyrus, and decreased surface area of the fusiform gyrus. In addition, we expected to find decreased volumes of the hippocampus.^{8,62,99-101} Furthermore, the whole-brain approach of the proposed study enabled us to explore and to potentially discover novel substrates for the risk-conferring cIT phenotype.

To the best of our understanding, this initiative is the first mega-analysis of brain structure associated with the temperamental risk for developing internalizing psychopathology. This provides the possibility of detecting novel cIT-related brain alterations and clarifying inconsistent findings in prior work.³³ We anticipated the large sample size to provide precise and relatively unbiased estimates of true effect sizes for multiple indices of cIT, providing a solid foundation to guide future research by individual investigators.

Furthermore, mega-analyses combine existing datasets to increase the overall sample size. This is particularly valuable for data acquired in vulnerable participants who are often difficult to recruit. Such studies exemplify next-generation science¹⁰²: previous studies within the ENIGMA-Consortium have resulted in important insights in the neurobiology of psychiatric conditions,¹⁰³⁻¹⁰⁷ and mega-analyses within the ENIGMA-Anxiety Working Group have revealed novel brain characteristics related to SAD,^{108,109} specific phobia,¹¹⁰ and anxiety in youth.¹¹¹ These insights reflect the advantages of large-scale data analyses for testing reproducibility and robustness of neuroimaging findings.¹⁰⁵ We expected the current project to provide similar insights concerning an important risk factor for social anxiety, increasing our understanding of the development of psychopathology in youth at risk. In addition, by preregistering the study in advance of performing the analyses, we aimed to contribute to a reduction of the potential publication bias in the field, and to advance a more complete and reliable scientific record on this topic.¹¹²

METHOD

Study Design and Setting

This preregistered study concerns a mega-analysis of T₁-weighted anatomical MRI scans of the human brain that have been previously acquired at research sites in Europe, North America, and South America (for scan characteristics, we refer to Table S2, available online). The project is part of the ENIGMA-Anxiety Working Group,⁸⁰ and analyses took place at the National Institute of Mental Health

(NIMH; Bethesda, MD). See Table S1, available online, for Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Participants

Individual participant data from studies where participants underwent MRI scanning between 6 and 25 years of age (inclusion criterion 1) and possessed at least 1 measurement of childhood inhibited temperament (cIT; inclusion criterion 2) were considered for inclusion. These inclusion criteria are based on the course of normative brain development into young adulthood¹¹³⁻¹¹⁵ and the emergence of internalizing psychopathology during adolescence.^{116,117} Regardless of age at scan, all participants were required to have data on cIT (childhood defined as age \leq 12 years). These temperament assessments should include measures of the tendency to withdraw from novel stimuli or to avoid unknown people, indices of fear toward novelty, and/or scores of social reticence. To make optimal use of the available data, various methods to acquire this information were allowed: we included behavioral observations in childhood, parent or teacher reports, self-report questionnaires on current temperament (children or young adolescents), and self-report questionnaires on retrospective temperament (young adults). Several studies acquired information using multiple methods.

Studies varied in their designs, with temperament assessments performed at or around the time of scan (“cross-sectional”) or preceding the MRI scan (“longitudinal”). For studies in which MRI scans were acquired at multiple time points, we selected the scan closest to the time point of the temperament assessment. (Internalizing) psychopathology was not an exclusion criterion, to allow for investigation of the full spectrum of cIT, and was included as a descriptive variable when available. An overview of the temperament measures acquired in each sample, as well as a description of the design of the datasets included in this analysis, are provided in Table 2¹¹⁸⁻¹⁷⁴ and in Supplement 1, available online.

Results on structural brain characteristics of cIT have been reported previously for several samples included in the present mega-analysis^{35,42,51,52,55}; however, only 1 study investigated the neural substrates of cIT using a whole-brain vertex-wise approach.⁵⁵

Ethics

The individual research protocols were approved by local institutional review boards and ethics committees. All adult participants and parents of participants younger than 18 years of age provided written informed consent at their local research site. Principal investigators from the

individual research sites signed a memorandum of understanding, which included regulations about data use, participant deidentification, data transfer methods, data ownership, and confidentiality and security practices.^{80,175} Each site also obtained approval from their local officials to share data.

Variables

Independent Variable: Data on Childhood Inhibited Temperament (cIT). As summarized in Table 2, studies varied in the way in which cIT was assessed. To optimally use all of the available information, we used a continuous approach to investigate the relationship between measures of cIT (predictor) and structural brain characteristics, based on the sample-specific temperament measures as provided by the participating sites (see Statistical Analyses).

Dependent Variable: Subcortical Volumes, Cortical Thickness, and Cortical Surface Area Derived From Structural MRI Data

Ten ENIGMA-Anxiety sites sent individual participant structural MRI data to the corresponding author and the research group at NIMH between January 2021 and December 2021. In addition, structural MRI data from the Wisconsin Twin project^{171,172} were downloaded from the NIMH data archive (September 2021). In September 2022, data from a new ENIGMA-Anxiety site (Virginia Commonwealth University) were added to the dataset and shared with the corresponding author and the research group at NIMH. Because of data-sharing restrictions, data from the Generation R study could not be shared internationally; therefore, analyses of these data took place locally in Rotterdam, the Netherlands, and group-level outcomes were merged with the results obtained at NIMH.

Additional Descriptive Data

Research sites were asked to provide information with respect to variables of interest: namely, demographic information (age, sex, IQ, socioeconomic status [SES], ancestry), information from clinical interviews concerning anxiety (generalized anxiety disorder [GAD], panic disorder [PD], social anxiety disorder [SAD], specific phobia [SP], other anxiety disorders) and other psychiatric disorders (major depressive disorder [MDD], obsessive compulsive disorder [OCD], posttraumatic stress disorder [PTSD], substance use dependence [SUD], other psychiatric disorders), psychotropic medication use at the time of scan, and several questionnaires on psychopathology (see Supplement 2, available online). Availability of these variables varied per sample (Table S3, available online).

TABLE 2 Characteristics of Samples After Image Processing and Quality Control

Sample (location)	Type of sample	n (n female) with MRI and cIT data	Design ^a	Age MRI scan (range; mean \pm SD)	Age cIT (range; mean \pm SD)	Measure of cIT (range; mean \pm SD)	Sub-group ^b	SES	Ancestry ^c	IQ MRI scan (range; mean \pm SD)	Notes
BRAINS study (Pennsylvania State University, University Park, Pennsylvania) 34,36,65,118-124	Oversampled for high/low cIT.	129 (72)	C	9.2-13.2 y (10.8 \pm 1.0)	9.2-13.2 y (10.8 \pm 1.0)	BIQ, parent rated; 34-165 (95.3 \pm 32.6); cross-sectional measure.	2	NA	African American (2.8%), Asian/Pacific Islander (2.8%), Hispanic (2.8%), Mixed Race (3.7%), White (Non-Hispanic; 88.1%).	71-149 (112.2 \pm 14.4)	IQ data available for 124 participants; data on ancestry available for 109 participants.
Brazilian High Risk Cohort (National Institute of Developmental Psychiatry (INPD), São Paulo, Brazil) ¹²⁵	Community sample and a high-risk sample of children with increased familial risk for mental disorders.	502 (233)	C	5.8-13.0 y (9.8 \pm 1.7)	5.8-13.0 y (9.8 \pm 1.7)	EATQ-R-shyness scale; 1.0-5.0 (2.7 \pm 1.1); cross-sectional measure.	3	Low (10.3%), middle (76.0%) and high (13.7%).	Asian (0.4%), Between White and Black (Brown; 32.0%), Black (12.4%), Indigenous (0.3%), White (54.9%).	57-152 (102.8 \pm 16.4)	IQ data available for 502 participants.
Cohort 3/4 (University of Maryland, College Park Maryland, ^{44,45,48,52,66-71,126}	Community sample: prospective longitudinal study on infants thought likely to display behavioral inhibition later in infancy and early childhood.	88 (50)	L	13.3-21.1 y (18.0 \pm 1.9)	Around 24 mo (no data at individual level).	Standard laboratory observations at age 2: composite score of stranger, robot, tunnel episodes; -1.3 -1.2 (-0.04 \pm 0.6); significant correlation with scores obtained at age 14 mo ($r = 0.3$).	1	Parents of the infants were in the middle to upper-middle class; 61.5% of mothers held college degrees.	Predominately Caucasian (98% White).	83-137 (114.3 \pm 10.4)	IQ data available for 84 participants.
Generation R —behavioral observations (Erasmus University Medical Center, Rotterdam, the Netherlands) ¹²⁷⁻¹³⁰	Community sample.	498 (248)	L	8.7-12.0 y (10.2 \pm 0.6)	34.7-44.2 mo (37.4 \pm 1.4)	Standard laboratory observations: stranger approach and jumping spider episode from the Lab-TAB; -1.16 to 1.36 (-0.01 \pm 0.37); cross-sectional measure.	1	Educational level mother at 5 y: 2.8% primary, 31.2% secondary, 52.3% higher; (missing: 13.8%).	55.8% Dutch, 8.1% Non-Dutch Western, 33.9% Non-Dutch non-Western, 2.2% missing.	No IQ scores at (around) time of scan.	
Generation R- questionnaire data (Erasmus University Medical Center, Rotterdam, the Netherlands) ¹²⁷⁻¹²⁹	Community sample.	1,604 (833)	L	8.6-12.0 y (10.0 \pm 0.5)	4.5-11.8 mo (6.7 \pm 1.1)	Infant Behavior Questionnaire —Revised (IBQ-r) —fear subscale; maternal report; 0.0-1.8 (0.4 \pm 0.3); cross-sectional measure.	2	Educational level mother at 5 y: 2.8% primary, 31.2% secondary, 52.3% higher; (missing: 13.8%).	55.8% Dutch, 8.1% Non-Dutch Western, 33.9% Non-Dutch non-Western, 2.2% missing.	No IQ scores at (around) time of scan.	Age of IBQ-R assessment was missing for 271 children.

(continued)

TABLE 2 Continued

Sample (location)	Type of sample	n (n female) with MRI and cIT data	Design ^a	Age MRI scan (range; mean ± SD)	Age cIT (range; mean ± SD)	Measure of cIT (range; mean ± SD)	Sub-group ^b	SES	Ancestry ^c	IQ MRI scan (range; mean ± SD)	Notes
Maryland-PAX (University of Maryland, College Park, Maryland) ¹³¹⁻¹³⁴	30-m Longitudinal study on a sample of first-year university students enriched for internalizing risk.	139 (81)	C	18-19 y (18.2 ± 0.4)	Retrospective: remembered inhibited behaviors in childhood	RMBI; 2-31 (15.0 ± 6.7); measures repeated at 3 follow-ups, with very good within-subject stability (from baseline to 6 mo, from baseline to 24 mo, and from baseline to 30 mo follow-up: all $r > 0.76$, $p < .001$).	3	NA	African American (8.6%), Asian (17.3%), Hispanic/Latino (4.1%), Multiracial/other (9.0%) White (61%).	NA	
Maryland-TAX (University of Maryland, College Park, Maryland) ¹³⁵	Cross-sectional community sample enriched for elevated social anxiety symptoms.	53 (28)	C	13-17 y (15.0 ± 1.2)	Retrospective: remembered inhibited behaviors in childhood.	RSRI-adolescent rated 1. 0-3.6 (2.3 ± 0.5); cross-sectional measure.	3	NA	African American (27.8%), Asian (5.6%), Hispanic (9.2%), Multiracial/other (7.4%), White (50%).	NA	
Nijmegen Longitudinal Study (Radboud University, Nijmegen, the Netherlands) ¹³⁶⁻¹³⁸	Longitudinal community sample.	68 (31)	L	17 y	1.20-1.28 y (1.24 ± 0.02)	Behavioral observations at 15 mo of age: 6-17 (9.5 ± 2.6); cross-sectional measure.	1	NA	NA	NA	
Pittsburgh (University of Pittsburgh School of Medicine, Pittsburgh, USA) ^{51,139-141}	High and low-risk (control) children/adolescents from ongoing family studies.	15 (3)	L	19.2-24. 8 y (21.5 ± 1.7)	4.1-6.4 y (5.1 ± 0.7)	Laboratory observations during peer play; sum score of amount of time staring at the other child, amount of time spent proximal to the parent, and latency to speak; 14.0-951.3 (213.5 ± 260.5); average over 3 sessions that were separated by no less than 1 wk and no more than 2 mo. Lack of session effect indicates stability of behavioral measures; cf. Hill <i>et al.</i> ¹³⁹	1	Majority of children from parents with professional, semiprofessional, and skilled occupation.	"All children were from white families."	88-124 (105.6 ± 12.5)	IQ data available for 11 participants.

(continued)

TABLE 2 Continued

Sample (location)	Type of sample	n (n female) with MRI and cIT data	Design ^a	Age MRI scan (range; mean \pm SD)	Age cIT (range; mean \pm SD)	Measure of cIT (range; mean \pm SD)	Sub-group ^b	SES	Ancestry ^c	IQ MRI scan (range; mean \pm SD)	Notes
San Raffaele (Vita-Salute San Raffaele University and San Raffaele Scientific Institute, Milan, Italy) ¹⁴²⁻¹⁴⁵	Community sample.	20 (8)	L	13-16 y (14.8 \pm 1.1)	Around age 7 y (no data at individual level) ¹⁴³	Empirical composite index of multiple scales with moderate to high cross-correlations ¹⁴³ ; 0-23 (8.8 \pm 6.9); cross-sectional measure.	2	Majority high SES (low/middle/high: 11%/33%/55%).	"All children were White and of Italian ancestry."	NA	
SDAN (National Institute of Mental Health, Bethesda, Maryland) ¹⁴⁶⁻¹⁴⁸	Treatment-seeking children and control group of healthy volunteers.	41 (20)	C	7.3-14.6 y (10.4 \pm 1.8)	8.1-12.8 y (10.5 \pm 1.6)	BIQ-child rated; 47-171 (114.5 \pm 28.6); cross-sectional measure.	3	Range 20-82, mean 30.9 (data for n = 36).	5.5% Asian, 10.9% Black or African American, 16.4% multiple, 5.5% unknown, 61.8% White.	78-145 (113.5 \pm 14.9).	IQ data available for 37 participants.
Stony Brook Temperament Study (Stony Brook University, Stony Brook, New York) ¹⁴⁹⁻¹⁵⁷	Community sample; MRI subsample oversampled for youth with temperamental high negative emotionality, low positive emotionality, and high behavioral inhibition at age 3 y.	74 (31)	L	9-12 y (10.2 \pm 0.9)	2.9-4.0 y (3.4 \pm 0.3)	Lab-TAB: 3 Kagan-like tasks around age 3 (log-transformed sum-score); 0.2-1.4 (0.7 \pm 0.2); Lab-TAB was repeated at age 6 (2 tasks), small but significant within-subject correlations. ¹⁵⁰	1	Majority of sample middle-class based on the Hollingshead Four Factor Index of Social Status. Majority of parents were married (94.6%) and well educated (67.6% of families had at least 1 parent who graduated from college).	"Majority of the MRI subsample was White and Non-Hispanic (77.0%)."	NA	
TOTS (University of Maryland, College Park, Maryland) ^{52-55,77,158-160}	Longitudinally followed sample of children selected at 4 mo of age based on their behavior in the laboratory.	27 (15)	L	9.1-19.5 y (12.4 \pm 3.3)	1.9-2.7 y (2.2 \pm 0.2)	Standard laboratory observations at age 2 y (composite score of stranger, robot, tunnel episodes); -1.0 to 1.2 (0.0 \pm 0.5); tasks were repeated at age 3 y, significant within-subject correlations (r = 0.3).	1	For the majority of the children, the mother graduated from graduate school (34.4%) or college (44.8%).	14.6% African American, 65.6% Caucasian, 5.2% Hispanic, 14.6% other/mixed.	78-134 (111.0 \pm 15.8)	IQ scores available for 24 participants.
Vanderbilt-children (Vanderbilt University Medical Center, Nashville, Tennessee) ⁷⁸	Study with extreme discordant phenotypes approach: inhibited and uninhibited children at the extreme ends.	55 (33)	C	8-12 y (9.3 \pm 1.1)	8-12 y (9.3 \pm 1.1)	BIQ-child rated; 33-181 (111.4 \pm 31.7); cross-sectional measure.	3	NA	NA	93-136 (115.7 \pm 10.3)	IQ scores available for 47 participants.

(continued)

TABLE 2 Continued

Sample (location)	Type of sample	n (n female) with MRI and cIT data	Design ^a	Age MRI scan (range; mean ± SD)	Age cIT (range; mean ± SD)	Measure of cIT (range; mean ± SD)	Sub-group ^b	SES	Ancestry ^c	IQ MRI scan (range; mean ± SD)	Notes
Vanderbilt—young adults (Vanderbilt University Medical Center, Nashville, Tennessee) 39,41,42,79,161	Study with extreme discordant phenotypes approach: inhibited and uninhibited young adults at the extreme ends.	145 (79)	C	18-25 y (21.8 ± 2.0)	Retrospective: remembered inhibited behaviors in childhood.	RSRI; 1.1-4. 4 (2.3 ± 0.9); cross-sectional measure.	3	NA	NA	93-141 (112.2 ± 13.8)	IQ scores available for 14 participants.
Virginia Commonwealth University Juvenile Anxiety Study (VCU-JAS) (VCU, Richmond, Virginia) 22,162-164	Twin study of pre-adolescents, using an epidemiological sampling design unselected for any particular outcome phenotypes.	126 (75)	C	9.2-14.3 y (11.3 ± 1.4)	Retrospective: remembered inhibited behaviors in early childhood (2-6 y).	Retrospective BIQ —parent rated, range 30-186 (88.9 ± 30.0).	2	NA	Caucasian	76-139 (111.9 ± 12.9)	IQ scores available for 108 participants.
Western University (The Brain and Mind Institute, Western University, London, Ontario, Canada) 165-170	Children selected based on presence/absence maternal depression.	82 (36)	L	9.2-12.4 y (11.1 ± 0.7)	3.0-4.0 y (3.4 ± 0.3)	Lab-TAB around age 3 y: risk room, stranger approach and jumping spider; log-transformed composite scores; -0.7-1.1 (0.0 ± 0.4); stable over time as indicated by Lab-TAB at age 5 y. ¹⁶⁶	1	Majority (50.6%) of the families were middle class with an annual family income of \$40,000-\$100,000 CAD.	"Majority of the sample was White (97%)."	82-147 (113.0 ± 14.0)	IQ based on scores on the Peabody Picture Vocabulary Test (data for 81 participants).
Wisconsin Twin Project-RdoC twin study (University of Wisconsin—Madison, Madison, Wisconsin) 21,171-174	Longitudinally followed samples of twins, recruited from statewide birth records for birth cohorts 1989–2004.	152 (93)	L	15.1-23.9 y (17.9 ± 1.8)	6.5-9.1 y (7.5 ± 0.5)	Ratings on Approach and Shyness from a home visit, and scores from videotaped reactions to the "Conversation with a Stranger" episode of Lab-TAB; 0.7-5.4 (2.9 ± 1.2) cross-sectional measure.	1	Median family income above \$50,000; majority of parents completed college. ¹⁷⁴	"Majority of the sample was White/Caucasian." ¹⁷⁴	NA	
Total		3,818 (1,969)									

Note: BI = behavioral inhibition; BIQ = Behavioral Inhibition Questionnaire; CBQ = Child Behavior Questionnaire; cIT = childhood inhibited temperament; EATQ-R = Early Adolescent Temperament Questionnaire; Lab-TAB = Laboratory Temperament Assessment Battery; MRI = magnetic resonance imaging; NA = not available; RMBI = Retrospective Measure of Behavioral Inhibition; RSRI = Retrospective Self-report of Inhibition; SES = socioeconomic status.

^aWith respect to timepoint temperament assessment and MRI scan, for data used in this study: cross-sectional (C) or longitudinal (L).

^bSubgroups for sensitivity analysis: 1: (early-life) behavioral observations; 2: parental/teacher reports during childhood; 3: self-report measures acquired during late childhood and adolescence.

^cInformation on ancestry was derived from the original papers and is thus reflective of the concept of "ancestry" as it was defined by the individual research sites. No attempt was made to acquire new information for the current work.

Study Size and Bias

We aimed to assemble the largest dataset possible, consisting of previously acquired samples with structural MRI data of the human brain from participants 6 to 25 years of age (inclusion criterion 1) and a characterization of cIT for each participant (inclusion criterion 2). Therefore, we reached out to all members within the ENIGMA-Anxiety Working Group, based on information about their samples that they had previously provided to the Working Group (see the description of this procedure in Bas-Hoogendam *et al.*⁸⁰). In addition, we contacted research groups based on literature searches and personal contacts of the coordinators (JMBH and DSP). This secured unpublished data, which minimizes the risk of publication biases. The initial sample consisted of 5,098 MRI scans (Table 3), whereas temperament data were available for 4,810 participants, leading to a pooled dataset of 4,810 MRI scans for further processing (Table 3).

Image Processing

Image processing took place following the procedure described previously.⁹⁷ To start, structural MRI scans were organized according to the Brain Imaging Data Structure (BIDS)¹⁷⁶ specification, and MRI Quality Control (MRIQC)¹⁷⁷ was used for initial quality checking (QC). Next, images were processed with FreeSurfer software (version 7.0.0)¹⁷⁸ to obtain volumes of subcortical structures and regional measures of cortical surface area (CSA) and cortical thickness (CT). In addition, advanced methods were used to obtain volumes of amygdalar subnuclei, thalamic subnuclei, and hippocampal subfields,⁹¹⁻⁹³ to compute the amount of gray matter inside each subcortical structure (partial volume effect [PVE]; see the description in the Supplemental section of Abend *et al.*⁹⁴), and to acquire volumes of subcortical limbic structures that were recently included in the FreeSurfer software package (hypothalamus, mammillary bodies (part of hypothalamus), basal forebrain, septal nuclei, nucleus accumbens, and fornix).⁹⁵ These outcome measures were investigated in preregistered exploratory analyses (reported in Supplement 2, available online), and the results of these analyses can advance the field, as they have the potential to enable the generation of new hypotheses.

Data were visually checked for gross over- or underestimation of the white/pial surfaces by 2 independent raters, and additional semi-automated QC was performed by using the ratio between the Euler characteristic and the number of vertices in the surfaces before topology correction, defining site-specific thresholds using a receiver operating characteristic curve constructed using the results of the visual inspection.¹⁷⁵ After registration to a common space ("FsAverage"), measurements of CT and CSA were

resampled to an icosahedron recursively subdivided 4 times ("fsaverage4"), which was used as a common grid for interpolation.¹⁷⁹ Data availability after processing and quality control is shown in Table 3.

Statistical Analyses

cIT as Continuous Predictor. Studies included in this analysis varied in the way in which cIT was assessed (Table 2), and data required for between-sample harmonization of cIT measures (ie, data on multiple measures acquired within the same participants) are lacking. Here, we used a dimensional approach and used continuous scores of cIT as predictor in the analyses. To allow for joint inference across all samples while accounting for the variability in cIT measures between samples, a variation of the method of nonparametric combination (NPC)^{180,181} was used. Specifically, we allowed the relationship between cIT and brain structures to be modeled as variable across sites, and then nonparametrically combined the resulting statistics across sites using the Stouffer combining function. The modification over the original NPC is that we allowed each subject to make 1 contribution to the joint result across samples, as opposed to each subject contributing with multiple metrics within a given sample. Models included cIT (continuous variable), sex, age, age squared, and their interactions (all allowed to vary among sites), as well as scanner (Table 4). It should be noted that in each sample, higher scores reflect a higher level of cIT. We tested 6 contrasts: main effect of cIT (positive and negative), 2-way interaction between cIT and sex (positive and negative), 2-way interaction between cIT and age, and 3-way interaction between cIT, age and sex (Table 4).

Linear and quadratic effects of age were combined using an *F* test. As the design is fully separable, effects across sites could be combined nonparametrically, thus allowing a joint test that benefits from the various cIT measures in their native scales, eschews the need for explicit data harmonization across the multiple samples, and, together with inference through permutation testing, allows multiple-testing correction with minimal assumptions.¹⁸² Moreover, the use of NPC with a separable design allowed data analysis to occur locally for sites that were unable to share data (as was the case with the Generation R study); all that was needed was the group-level test statistics, as well as the same statistics after permutation of the data. It should be noted, however, that these advantages come with a few drawbacks: effect sizes are not computed, as NPC uses a combination of statistics based on different measures of cIT, each with a different scale; furthermore, interaction effects are hard to explore, given the restrictions on data sharing from some sites.

TABLE 3 Data Inclusion for Each Sample; Sample Size per (Sub)-Analysis

Total remaining (n) following pre-processing and quality control

Sample	Initial no. MRI scans (n)^a	Initial no. with cIT data (n)^a	Main analysis (n)	Subgroup analysis 1 (n)^b	Subgroup analysis 2 (n)^b	Subgroup analysis 3 (n)^b	Subgroup analysis 4 (n)^b
BRAINS study	131	130	129		129		129
Brazilian High Risk Cohort	688	678	502			502	502
Cohort 3/4	121	95	88	88			88
Generation R-sample with behavioral observation	584 ^a	584 ^a	498 ^a	498 ^a			498 ^a
Generation R-sample with questionnaire data	1,982 ^a	1,982 ^a	1,604 ^a		1,604 ^a		1,604 ^a
Maryland-PAX	220	220	139			139	
Maryland-TAX	54	53	53			53	
Nijmegen Longitudinal Study	71	71	68	68			68
Pittsburgh	64	15	0 ^c				
San Raffaele	20	20	20		20		20
SDAN	55	55	41			41	41
Stony Brook Temperament Study	74	74	74	74			74
TOTS	129	96	27	27			27
Vanderbilt—children	55	55	55			55	55
Vanderbilt—young adults	150	150	145			145	
VCU-JAS	133	129	126		126		
Western University	87	87	82	82			82
Wisconsin Twin Project	480	316	152	152			152
Total	5,098	4,810	3,803	989	1,879	935	3,340

^aData with superscript letter “a” were processed locally in Rotterdam, the Netherlands. All other numbers available at Section on Development and Affective Neuroscience (SDAN), National Institute of Mental Health, Bethesda, Maryland.

^bSubgroup sensitivity analyses: 1: (early life) behavioral observations; 2: parent/teacher reports during childhood; 3: self-report measures acquired during late childhood /adolescence; 4: cIT measured during childhood.

^cData from Pittsburgh needed to be dropped from the final analyses, because the number of participants made the design rank deficient (statistical issue unrelated to data quality).

TABLE 4 Variables and Contrasts for the Analyses

Global analysis		Regional analysis		Regional analysis with global brain measures		Vertex-wise analysis		Vertex-wise analysis with global brain measures	
Dependent variables	Independent variables	Dependent variables	Independent variables	Dependent variables	Independent variables	Dependent variables	Independent variables	Dependent variables	Independent variables
Total ICV	Of interest	SV (16 regions)	Of interest	SV (16 regions)	Of interest	Vertex-wise CSA (2,562 vertices)	Of interest	Vertex-wise CSA (2,562 vertices)	Of interest
Total CSA	cIT	CSA (68 regions)	cIT	CSA (68 regions)	cIT	Vertex-wise CT (2,562 vertices)	cIT	Vertex-wise CT (2,562 vertices)	cIT
Mean CT	Age ^a	CT (68 regions)	Age	CT (68 regions)	Age		Age		Age
	Sex Age ²		Sex Age ²		Sex Age ²		Sex Age ²		Sex Age ²
	Sex by age Sex by age ² cIT by age cIT by sex cIT by age ² cIT by sex by age cIT by sex by age ²		Sex by age Sex by age ² cIT by age cIT by sex cIT by age ² cIT by sex by age cIT by sex by age ²		Sex by age Sex by age ² cIT by age cIT by sex cIT by age ² cIT by sex by age cIT by sex by age ²		Sex by age Sex by age ² cIT by age cIT by sex cIT by age ² cIT by sex by age cIT by sex by age ²		Sex by age Sex by age ² cIT by age cIT by sex cIT by age ² cIT by sex by age cIT by sex by age ²
	Nuisance: Scanner		Nuisance: Scanner		Nuisance: Scanner, total CSA, mean CT, total ICV		Nuisance: Scanner		Nuisance: Scanner, total CSA, mean CT, total ICV
Contrasts of interest		Contrasts of interest		Contrasts of interest		Contrasts of interest		Contrasts of interest	
1	Positive effect cIT	1	Positive effect cIT	1	Positive effect cIT	1	Positive effect cIT	1	Positive effect cIT
2	Negative effect cIT	2	Negative effect cIT	2	Negative effect cIT	2	Negative effect cIT	2	Negative effect cIT
3	cIT by sex (positive)	3	cIT by sex (positive)	3	cIT by sex (positive)	3	cIT by sex (positive)	3	cIT by sex (positive)
4	cIT by sex (negative)	4	cIT by sex (negative)	4	cIT by sex (negative)	4	cIT by sex (negative)	4	cIT by sex (negative)

(continued)

TABLE 4 Continued

Global analysis		Regional analysis		Regional analysis with global brain measures		Vertex-wise analysis		Vertex-wise analysis with global brain measures	
Dependent variables	Independent variables	Dependent variables	Independent variables	Dependent variables	Independent variables	Dependent variables	Independent variables	Dependent variables	Independent variables
5	cIT by age ^b	5	cIT by age ^b	5	cIT by age ^b	5	cIT by age ^b	5	cIT by age ^b
6	cIT by sex by age	6	cIT by sex by age	6	cIT by sex by age	6	cIT by sex by age	6	cIT by sex by age

Note: cIT = childhood inhibited temperament; CSA = cortical surface area; CT = cortical thickness; ICV = intracranial volume; SV = subcortical volumes.
^aAge represents age at timepoint of magnetic resonance imaging scan.
^bLinear and quadratic effects of age are combined using an F test.

Analyses were performed using the Permutation Analysis of Linear Models software (PALM; <https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/PALM>) with 2,000 permutations, followed by the fitting of a generalized Pareto distribution to the tail of the permutation distribution,¹⁸¹ thus dispensing with the need to perform a computationally prohibitive larger number of permutations.

Global, Regional, and Local (Vertex-wise) Analyses. We investigated the association between cIT and 3 global brain measures: total intracranial volume (ICV), total cortical surface area (CSA), and mean cortical thickness (CT).

We also investigated the relationship between cIT and subcortical volumes (SV; 8 regions in each hemisphere) and regional estimates of CSA and CT (34 bilateral regions according to the Desikan–Killiany parcellation¹⁸³), in 2 models, one with and one without global brain measures (ie, total CSA, mean CT, and total intracranial volume) as additional nuisance variables (Table 4). Symptom scores and diagnostic information were not included in the models, as availability of clinical information for specific diagnoses varied widely across samples (Table S3, available online). Vertex-wise CSA and CT were investigated (2,562 vertices per hemisphere) in models similar to those used in the regional analyses (Table 4).

Sensitivity Analyses per Subgroup Based on cIT Assessment Type. To examine the neurobiological substrates of cIT in more homogenous but smaller samples, we repeated the analyses described above in subgroups of the dataset based on the type of cIT assessment (which closely varied with age at assessment). We defined the following subgroups: first, (early-life) behavioral observations; second, parent/teacher reports during childhood; and third, self-report measures acquired during late childhood and adolescence. Allocation of the samples to the subgroups is provided in Table 2, and sample sizes for each analysis are described in Table 3.

Sensitivity Analysis Excluding Retrospective Measures of cIT. In a fourth sensitivity analysis, we selected only those samples in which temperament was assessed during childhood, meaning that samples with retrospective measures on temperament (Maryland-TAX, Maryland-PAX, Vanderbilt-adults, and Virginia Commonwealth University Juvenile Anxiety Study) (Table 2) were excluded.

Correction for Multiple Testing. Multiple testing correction used the distribution of the maximum statistic,^{182,184} thus allowing control over the family-wise error rate (FWER). Correction considered all tests within each metric

(ie, 68 cortical regions each for CSA and CT, and 16 SV), all 3 sets of metrics, and all contrasts (ie, MC-FWER).¹⁸⁴ Results at more liberal levels of correction for multiple testing (eg, only within a metric [M-FWER] or only across contrasts [C-FWER]) are reported in Supplement 3, available online.

Timeline for Completion of the Study

Data collection (ie, retrieval of MRI data, cIT information, and further descriptive data from participating sites) was locked on December 31, 2022. Image processing and organization of data took place from January 2022 to December 2023; analyses as described above took place from January to November 2024.

Data Access Certification

The first and last authors of this paper (JMBH, RB, BB, AMW, DSP) declared that they did not perform any cIT analyses on the MRI data for the purpose of the mega-analysis described in this preregistration before submitting the registered report of this study, although AMW and DSP were involved in a previous study on the relationship between cIT and brain structure (subset of the TOTS sample).⁵⁵

RESULTS

Analyses in Full Mega-Analytic Sample

Following image processing and quality control, the analysis included cIT and MRI data from 3,803 participants (1,966 female), originating from 17 independent samples (Table 3).

Analyses revealed no significant effects of cIT, nor interactions between levels of cIT, sex, or age, on any of the brain measures when applying FWER correction considering all imaging metrics and contrasts (MC-FWER) (Figure 1). There were no significant effects either when more liberal levels of correction were used (C-FWER correction, considering only the 6 contrasts of interest; M-FWER correction, considering only the number of tests within each metric). Results of exploratory analyses are reported in Supplement 3, available online.

Sensitivity Analyses in Subgroups

As preregistered, we repeated analyses in subsamples based on cIT assessment type (Table 3). The first sensitivity analysis included data from 989 participants for whom cIT was determined based on behavioral observations, mostly early in life. Regional analyses in this subgroup did not yield any significant results when MC-FWER correction was applied; vertex-wise analyses on CT revealed 1 isolated

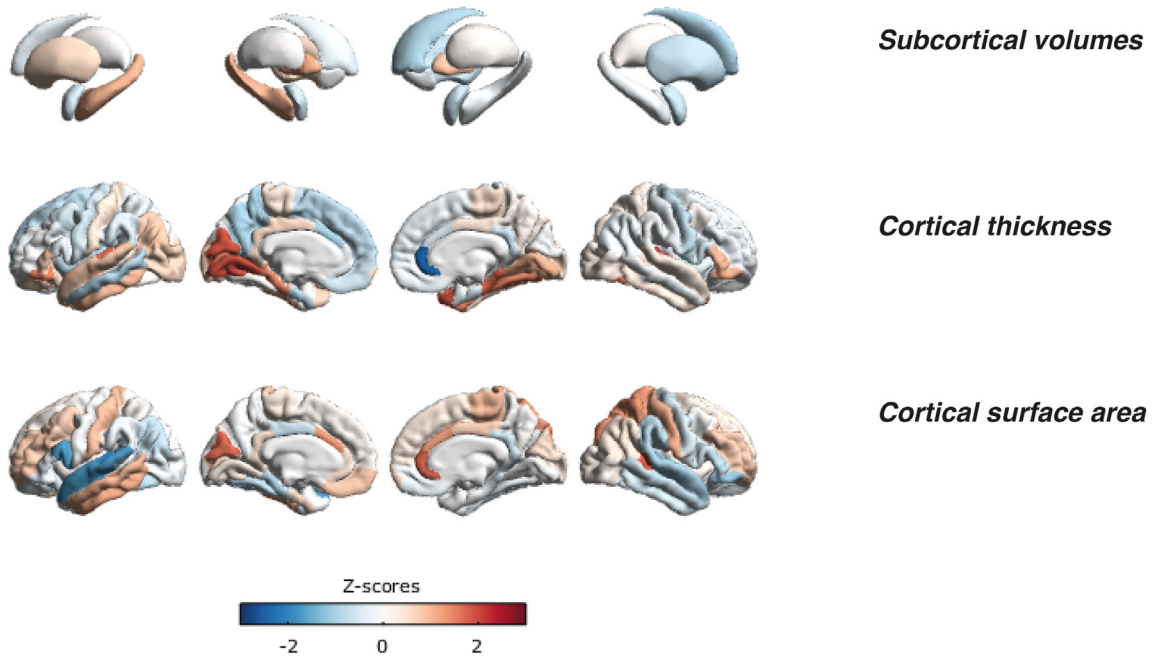
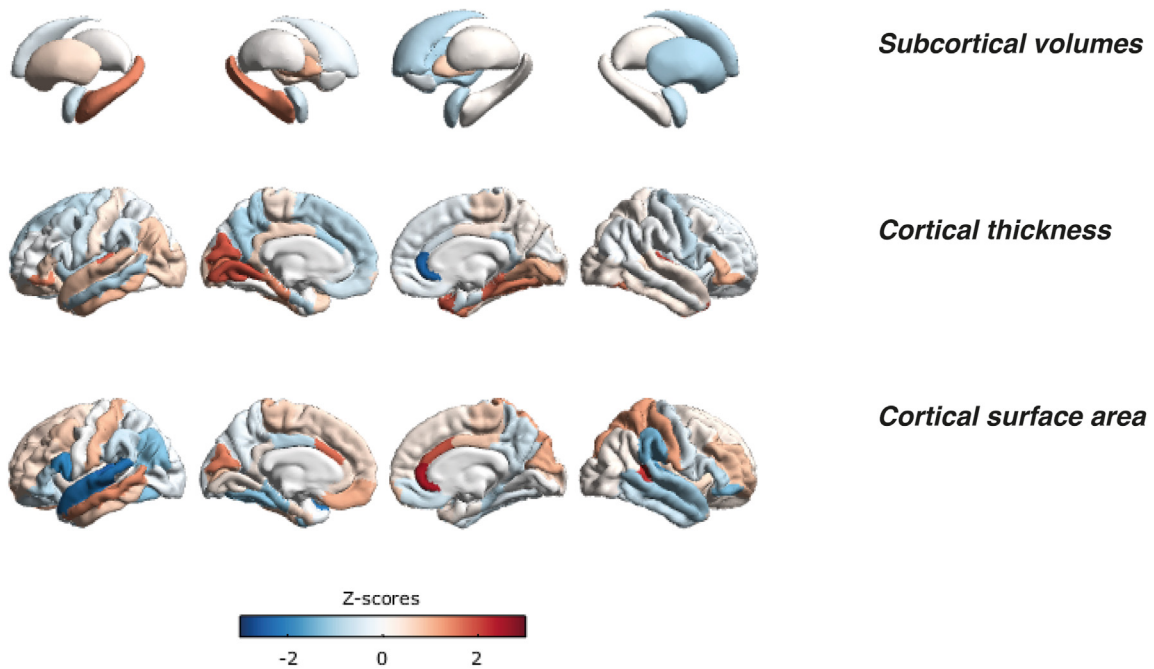
vertex (located at the border of the right superior parietal region and supramarginal gyrus) with a significant cIT by sex by age interaction at the MC-FWER-corrected significance level ($p_{MC-FWER} = .020$). Findings at M-FWER corrected significance level (for both main and exploratory analyses) are reported in Supplement 3, available online.

The sensitivity analyses in the second subgroup (1,879 participants for whom the level of cIT was established based on parent or teacher reports during childhood) revealed significant negative cIT by sex interactions with respect to global mean CT ($p_{MC-FWER} = .037$) and CT of the right superior parietal region ($p_{MC-FWER} = .029$), at the most stringent level of thresholding. Vertex-wise analyses revealed negative cIT by sex interactions as well, in 2 independent vertices in the left inferior parietal area ($p_{MC-FWER} = .006$ and $p_{MC-FWER} = .003$, respectively). Because most data within this subgroup originated from Generation R ($n = 1,604$), we repeated the analyses without Generation R data (remaining $n = 275$). In these analyses, the negative cIT by sex interactions with respect to mean CT and CT in the right superior parietal region ($p_{MC-FWER} = .106$ and $p_{MC-FWER} = .136$, respectively) did not survive, nor did we replicate the interaction effects from the vertex-wise analysis. As we considered unraveling site-specific effects out of the scope of the present preregistered work, we did not explore this interaction further. Additional findings within subgroup 2 (at M-FWER corrected level) are listed in Supplement 3, available online.

Analyses in the third subgroup (935 participants who completed self-report measures on the level of cIT) and in the fourth subgroup (3,340 participants in whom cIT was actually assessed during childhood, excluding those with retrospective measures of cIT) did not reveal significant findings when MC-FWER correction was applied. Findings from exploratory analyses are included in Supplement 3, available online.

DISCUSSION

This preregistered mega-analytic study from the ENIGMA-Anxiety Working Group explored structural brain characteristics related to childhood inhibited temperament (cIT: $n = 3,803$ from 17 independent samples). We used a comprehensive approach considering subcortical volumes, cortical thickness, and cortical surface area as well as multiple other subcortical and cortical indices in exploratory analyses. Contrary to expectations, cIT showed no association with brain structure, nor interactions with either sex or age in the full sample, using the preregistered statistical threshold.

FIGURE 1 Z Statistics for the Regional Analyses Exploring the Positive Effect of Childhood Inhibited Temperament on Brain Structure**Regional analyses without global brain measures****Regional analyses with global brain measures**

Note: Contrast of interest 1; none of these z statistics were statistically significant following family-wise error rate correction for multiple testing across all metrics and all contrasts (MC-FWER). Figure created with ENIGMA-Toolbox.¹⁸⁵

When considering subgroups (defined based on cIT assessment type), a significant interaction arose in the sensitivity analyses in subgroup 2. In this group, cIT level was based on assessments by parents or teachers, using questionnaires such as the Infant Behavior Questionnaire (IBQ-r; Generation R), the Behavioral Inhibition Questionnaire (BIQ; BRAINS study and VCU-JAS study), or a composite measure of cIT reported by teachers (San Raffaele sample). Individuals in this subgroup were between 8 and 16 years of age at the time of the MRI scan, and analyses revealed significant negative cIT by sex interactions with respect to global mean cortical thickness (CT) as well as when considering CT of the right superior parietal region, at the most stringent level of thresholding. These results lost significance after excluding data from Generation R, suggesting either power issues or site-specific differences rather than universal cIT relations with brain structure. However, several isolated findings from vertex-wise analyses also implicated parietal regions, namely the right superior parietal region (sensitivity analysis subgroup 1) and the left inferior parietal area (sensitivity analysis subgroup 2).

Findings in the parietal region relate to other reports in current literature. For example, a recently published Mendelian randomization study related increased volume of the left superior parietal area to the onset of anxiety disorders.¹⁸⁶ A study in SAD patients reported cortical thickness alterations in the right superior parietal lobule, angular gyrus, right precuneus, and inferior parietal lobule.¹⁸⁷ Thickening of the right parietal cortex was also found in a study comparing medication-naïve patients with SAD and major depressive disorder with healthy participants,¹⁸⁸ suggesting that structural brain characteristics of the superior parietal region could be transdiagnostically implicated in internalizing disorders. As summarized by Sylvester *et al.*,¹⁸⁹ such findings could relate to the role of the superior parietal lobule in functions performed by the dorsal attention network, and multiple studies reported associations between this network and both anxiety and temperament.¹⁹⁰⁻¹⁹⁵ A multimodal meta-analysis examining neural correlates of personality dimensions among 3,053 healthy participants further implicated these brain areas in harm avoidance, at both the structural and functional level.¹⁹⁶ Thus, further research in parietal structure and function as it relates to inhibited behavior appears to be warranted.

Exploratory analyses using detailed analytical techniques (analyses on amygdalar and thalamic subnuclei, hippocampal subfields, partial volume effects, volumes of additional subcortical limbic structures) are reported in Supplement 3, available online, and revealed some findings in hypothesized regions of interest. However, only 1 finding

for the left hippocampus (PVE analysis; $p_{MC-FWER} = .036$, subgroup 4) survived MC-FWER correction for multiple testing. Other results at lower thresholds (Supplement 3, available online) might also be informative, as they were present in regions that we *a priori* hypothesized to be related to cIT, namely the caudate and putamen. Interestingly, volumetric differences in putamen volume were also recently reported in ENIGMA-Anxiety mega-analyses on adult patients with SAD¹⁰⁹ and individuals with specific phobia,¹¹⁰ using a dichotomous approach comparing patients and controls. Analyses of amygdalar subnuclei and hippocampal subfields did not yield results at the MC-FWER, M-FWER, or C-FWER corrected significance level.

Previous studies in smaller samples (published before 2020; most of them included in the present mega-analysis) did report associations between the level of cIT and brain structure (Table 1). These explorations were often limited to specific regions of interest, and findings were inconsistent and lacked replication. None of these findings were replicated in this large sample in which multiple measures of cIT were combined. These null findings illustrate the value of big datasets and preregistered analyses. As outlined in a recent review paper on the advantages and challenges of big data in psychiatric neuroimaging,¹⁹⁷ underpowered studies are often associated with sampling variability. This could, in combination with publication bias, create the illusion of strong effect sizes, whereas real brain-behavior associations are probably smaller¹⁹⁷ (see also the report by Scheel *et al.* highlighting the excess of positive results in standard psychology research compared with registered reports,¹⁹⁸ and an editorial highlighting the value of null findings¹⁹⁹).

The present findings suggest that although inhibited temperament is a partly heritable and early observable trait with a persistent character during the lifespan which is associated with the development of psychopathology,²⁰⁰ this trait shows no relation to brain structure as measured using current 3T MRI scans. These findings are consistent with recent research on the structural correlates of other relevant constructs. For example, Xu *et al.* (2024) explored neuroanatomical correlates of child psychiatric problems in 11,271 children 9 to 10 years of age.²⁰¹ Using multivariate machine learning techniques, the authors reported a highly reliable and generalizable brain-behavior association between child attention problems and brain morphometry, but a robust multivariate pattern of neuroanatomy related to internalizing disorders was not demonstrated.²⁰¹ Similar results also arose from other recent studies exploring neuroanatomical data from the ABCD Study²⁰² and the BIS-subscale of the Carver-White BIS/BAS questionnaire.^{203,204} Functional indices may be more sensitive than

structural indices to internalizing problems and their risk factors, such as cIT, at this point in the lifespan,⁸ a hypothesis that seems to be supported by the evidence summarized in Table 1. Alternatively, cross-sample variation in cIT assessments (hence, phenotypic variability) could have reduced sensitivity in our analyses on associations between cIT and brain structure. This hypothesis received some support from the sensitivity analyses (although even within these subgroups, substantial variation was present in the method of cIT assessment and thus potentially in levels of cIT; see Fleece and Teglas [2024] outlining how informant discrepancies, for example between parents' and teachers' ratings of cIT in children, emphasize the transactional nature of inhibited behavior²⁰⁵).

The strengths of the current study are the unique sample size, including data from ENIGMA-Anxiety Working Group research sites from multiple countries and continents,⁸⁰ as well as the preregistered and comprehensive standardized method to assess brain structure in relation to cIT, combined with stringent statistical correction for multiple testing. Given that there is no established way to harmonize cIT measures, when designing the study and preregistering it, we opted for an approach that would not require data to be harmonized across samples. For scanner effects within sample, we note that whereas some samples used multiple scanners, others did not; because harmonization alters the data in ways that cannot be accounted for by the NPC approach, we opted to treat the sites identically, following the classical strategy of including 1 regressor per scanner, an approach that works symmetrically for sites that use one or multiple of them.

Limitations inherent to the composed nature of the dataset deserve to be mentioned as well. For example, sample heterogeneity between datasets could not be avoided, leading to the above-mentioned cross-sample variation in cIT assessment methods, but also a large age range of included participants (cIT defined as "temperament during childhood, up to and including 12 years of age"; MRI scan acquired between 6 and 25 years of age). Combined with the cross-sectional nature of the current analysis, these limitations precluded exploring developmental patterns in more specific age groups, for example as reported by Filippi *et al.* (2020).⁵⁵ Major developmental changes occur in brain structure during childhood and adolescence, processes that are characterized by substantial interindividual variability,²⁰⁶ and region-specific developmental changes in our hypothesized regions of interest in particular (see, for example, work of the ENIGMA Lifespan Working Group¹¹⁴). Hence, future work might consider hypotheses on developmental interactions

between the level of cIT and structural brain characteristics. Such work could define *a priori* hypotheses on these patterns and test them in groups of participants in which MRI data were acquired within smaller age ranges. In particular, longitudinal studies with consistent assessments of cIT are recommended to disentangle such effects on brain structure at the individual level.

Next, as clinical data were not consistently available across samples, it was not possible to take levels of psychopathology (in the children and/or their parents) into account. Another methodological limitation is inherent to the use of NPC: although this method allowed a joint test that combined the various cIT measures in their native scales and yielded interpretable test statistics (z values) (Figure 1),¹⁸⁴ it does not produce standardized effect sizes, and unpacking interaction effects across different sites is complicated. Furthermore, information on environmental factors that might play a role in the development of internalizing psychopathology in children with high levels of cIT was lacking. For example, a recent study using longitudinal growth modeling exploring the development of social anxiety in youth 9 to 15 years of age demonstrated an interaction between infant temperament and parenting style at 36 months.²⁰⁷ Another parental factor that has been identified to influence the relation between temperament of a child and the development of psychopathology is the interparental relationship quality²⁰⁸; however, a 20-year longitudinal, multi-informant, and multi-methods study on 128 children that reported an indirect association between high cIT and high loneliness during adolescence did not provide evidence for a moderating effect of infant parenting on this relationship.²⁰⁹ Future longitudinal large-scale studies are needed to explore these complex relationships and interactions between innate, biological, and environmental factors that play a role in the development of anxiety in youth.

To conclude, this preregistered mega-analysis did not find consistent structural brain characteristics related to cIT in a large dataset consisting of 3,803 structural MRI scans collected from 17 independent research samples around the world that were analyzed using a single analytic pipeline and quality control. Findings from subgroup analyses and exploratory analyses point at changes in parietal regions as well as subcortical regions such as the hippocampus, putamen, and caudate in relation to cIT, but these findings did not survive statistical correction for multiple testing. Future large-scale studies, preferably using longitudinal designs and looking at brain function, are recommended to further unravel this neurobiological risk signature for internalizing psychopathology.

CRedit authorship contribution statement

Janna Marie Bas-Hoogendam: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Rachel A. Bernstein:** Writing – review & editing, Project administration, Data curation. **Brenda E. Benson:** Project administration, Data curation, Writing – review & editing. **Samuel E.C. Frank:** Writing – review & editing, Data curation. **Kristin A. Buss:** Writing – review & editing, Data curation. **Kelley E. Gunther:** Data curation, Writing – review & editing. **Koraly Pérez-Edgar:** Writing – review & editing, Data curation. **Giovanni A. Salum:** Writing – review & editing, Data curation. **Andrea Jackowski:** Data curation, Writing – review & editing. **Rodrigo A. Bressan:** Writing – review & editing, Data curation. **André Zugman:** Writing – review & editing, Data curation. **Kathryn A. Degnan:** Writing – review & editing, Data curation. **Courtney A. Filippi:** Writing – review & editing, Data curation. **Nathan A. Fox:** Writing – review & editing, Data curation, Conceptualization. **Heather A. Henderson:** Writing – review & editing, Data curation. **Alva Tang:** Writing – review & editing, Data curation. **Selin Zeytinoglu:** Data curation, Writing – review & editing. **Anita Harrewijn:** Writing – review & editing, Data curation. **Manon H.J. Hillegers:** Writing – review & editing, Data curation. **Ryan L. Muetzel:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Tonya White:** Writing – review & editing, Data curation. **Marinus H. van IJzendoorn:** Writing – review & editing, Data curation. **Carl E. Schwartz:** Writing – review & editing. **Julia Felicione:** Writing – review & editing. **Kathryn A. DeYoung:** Writing – review & editing, Data curation. **Alexander J. Shackman:** Writing – review & editing, Data curation. **Jason F. Smith:** Writing – review & editing, Data curation. **Rachael M. Tillman:** Writing – review & editing, Data curation. **Yvonne H.M. van den Berg:** Writing – review & editing, Data curation. **Antonius H.N. Cillessen:** Writing – review & editing, Data curation. **Karin Roelofs:** Writing – review & editing, Data curation. **Anna Tyborowska:** Writing – review & editing, Data curation. **Shirley Y. Hill:** Writing – review & editing, Data curation. **Marco Battaglia:** Data curation, Writing – review & editing. **Marco Tettamanti:** Writing – review & editing, Data curation. **Lea R. Dougherty:** Writing – review & editing, Data curation. **Jingwen Jin:** Writing – review & editing, Data curation. **Daniel N. Klein:** Writing – review & editing, Data curation. **Hoi-Chung Leung:** Writing – review & editing, Data curation. **Suzanne N. Avery:** Writing – review & editing, Data curation. **Jennifer Urbano Blackford:** Writing –

review & editing, Conceptualization, Data curation. **Jacqueline A. Clauss:** Writing – review & editing, Data curation. **James M. Bjork:** Writing – review & editing, Data curation. **John M. Hettema:** Writing – review & editing, Data curation. **Ashlee A. Moore:** Writing – review & editing, Data curation. **Roxann Roberson-Nay:** Writing – review & editing, Data curation. **Chelsea Sawyers:** Writing – review & editing, Data curation. **Elizabeth P. Hayden:** Writing – review & editing, Data curation. **Pan Liu:** Writing – review & editing, Data curation. **Matthew R.J. Vandermeer:** Writing – review & editing, Data curation. **H. Hill Goldsmith:** Writing – review & editing, Data curation. **Elizabeth M. Planalp:** Writing – review & editing, Data curation. **Thomas E. Nichols:** Writing – review & editing, Methodology, Conceptualization. **Paul M. Thompson:** Writing – review & editing, Methodology, Conceptualization. **P. Michiel Westenberg:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Nic J.A. van der Wee:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Nynke A. Groenewold:** Methodology, Conceptualization, Writing – review & editing. **Dan J. Stein:** Writing – review & editing, Methodology, Conceptualization. **Anderson M. Winkler:** Writing – review & editing, Visualization, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Daniel S. Pine:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization.

Accepted June 27, 2025.

^aLeiden University, Leiden, the Netherlands; ^bLeiden University Medical Center, Leiden, the Netherlands; ^cLeiden Institute for Brain and Cognition, Leiden, the Netherlands; ^dNational Institute of Mental Health, Bethesda, Maryland; ^eThe Pennsylvania State University, University Park, Pennsylvania; ^fUniversity of Maryland, College Park, Maryland; ^gHospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil; ^hNational Institute of Developmental Psychiatry, São Paulo, Brazil; ⁱChild Mind Institute, New York, New York; ^jFederal University of São Paulo, São Paulo, Brazil; ^kThe Catholic University of America, Washington, DC; ^lNew York University Grossman School of Medicine, New York, New York; ^mUniversity of Waterloo, Waterloo, Ontario, Canada; ⁿUniversity of Texas at Dallas, Richardson, Texas; ^oTulane University, New Orleans, Louisiana; ^pErasmus University Rotterdam, Rotterdam, the Netherlands; ^qErasmus University Medical Center, Rotterdam, the Netherlands; ^rUCL, London, United Kingdom; ^sUniversidad San Sebastián, Santiago de Chile, Chile; ^tMassachusetts General Hospital, Harvard Medical School, Boston, Massachusetts; ^uTufts University, Medford, Massachusetts; ^vRadboud University, Behavioural Science Institute, Nijmegen, the Netherlands; ^wUniversity of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; ^xUniversity of Toronto, Toronto, Ontario, Canada; ^yCentre for Addiction and Mental Health, Toronto, Ontario, Canada; ^zUniversity of Milan-Bicocca, Milan, Italy; ^{aa}The University of Hong Kong, Hong Kong; ^{bb}Stony Brook University, Stony Brook, New York; ^{cc}Vanderbilt University Medical Center, Nashville, Tennessee; ^{dd}Munroe-Meyer Institute, University of Nebraska Medical Center, Omaha, Nebraska; ^{ee}University of Maryland Medical School, Baltimore, Maryland; ^{ff}Virginia Commonwealth University, Richmond, Virginia; ^{gg}Texas A&M University, Bryan, Texas; ^{hh}State University of New York, Oswego, New York; ⁱⁱUniversity of Virginia, Charlottesville, Virginia; ^{jj}Western University, London, Ontario, Canada; ^{kk}University of Alberta, Edmonton, Alberta, Canada; ^{ll}University of Wisconsin-Madison, Madison, Wisconsin;

^{mm}University of Oxford, Oxford, United Kingdom; ⁿⁿUniversity of Southern California, Marina del Rey, California; ^{oo}University of Cape Town, Cape Town, South Africa; ^{pp}The University of Texas Rio Grande Valley, Brownsville, Texas

This project is funded by a Rubicon grant from the Dutch Research Council NWO (019.201SG.022), awarded to Janna Marie Bas-Hoogendam. Rachel Bernstein, Brenda E. Benson, Samuel E. C. Frank, André Zugman, Anderson M. Winkler and Daniel S. Pine were supported by the National Institute of Mental Health Intramural Research Program Project ZIAMH002782. Kristin A. Buss received funding from the National Institute of Mental Health (R01MH094633S1). Koralý Pérez-Edgar received funding from the National Institute of Mental Health (R01MH094633 and R01MH094633S1). Kathryn A. Degnan, Nathan A. Fox and Heather A. Henderson received funding from the National Institute of Mental Health (U01MH093349). Selin Zeytinoglu received funding from the National Institute of Mental Health (F32MH127869). Anita Harrewijn received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement (No 101026595). Manon H. J. Hillegers, Ryan L. Muetzel, Tonya White reported that the Generation R Study is supported by Erasmus MC, Erasmus University Rotterdam, the Rotterdam Homecare Foundation, the Municipal Health Service Rotterdam area, the Stichting Trombosedienst & Arsenlaboratorium Rijnmond, the Netherlands Organization for Health Research and Development (ZonMw), and the Ministry of Health, Welfare and Sport. Tonya White was supported by the Intramural Research Program of the National Institute of Mental Health (ZIA MH002986). Alexander J. Shackman, Kathryn A. De Young, and Jason F. Smith were supported by multiple grants from the National Institute of Mental Health (R01-AA031261, R01-AA030042, R01-MH131264, R01-MH107444 and R01-MH121409). Karin Roelofs received funding from the European Research Council (ERC_CoG-2017_772337). Shirley Y. Hill received grant support from the National Institute on Alcohol Abuse and Alcoholism (AA05909, AA08082 and AA018289). Daniel N. Klein received funding from the National Institute of Mental Health (R01-MH069942). Jennifer Urbano Blackford received funding from the National Institute of Mental Health (K01-MH083052). Jacqueline A. Clauss received funding from the National Institute of Mental Health (F30-MH097344). James M. Bjork received funding from the National Institutes of Health (U01-DA051037). John M. Hettema received funding from the National Institutes of Health (R01MH098055) and the Brain and Behavior Research Foundation (PT110243/21984). Ashlee A. Moore was supported by the National Institute of Mental Health (F31MH11229). Roxann Roberson-Nay received funding from the National Institute of Mental Health (R01MH098055). Chelsea Sawyers reported support from the National Institutes of Health (T32MH020030). Elizabeth P. Hayden and Pan Liu declared that the BrainsCAN initiative at Western University has been funded by the Canada First Research Excellence Fund (CFREF). Matthew R. J. Vandermeer received the Canadian Institutes for Health Research (CIHR) Frederick Banting & Charles Best Canada Graduate Scholarships Doctoral Award. H. Hill Goldsmith reported support from the National Institute of Mental Health (R01MH101504 and R01MH059785), as well as infrastructure support by the Waisman Center via core support from the National Institute of Child Health and Human Development (P30 HD003352 and U54 HD090256). Elizabeth M. Planalp was supported by the National Institute of Mental Health (K01MH113710). Thomas E. Nichols was supported by the National Institutes of Health (R01MH096906). Paul M. Thompson reported grants from the National Institute of Mental Health (R01MH116147, R01MH121246, R01MH123163), support from the Milken Foundation and the Baszucki Brain Research Fund. P. Michiel Westenberg was supported by the Leiden University Research Profile Health, Prevention, and the Human Life Cycle. Nynke A. Groenewold declared that this work was made possible in part by a grant from Carnegie Corporation of New York. The statements made and views expressed are solely the responsibility of the author. Dan J. Stein was funded by the South African Medical Research Council.

The preregistration of this study was presented as an abstract at the 34th ECNP Congress, October 2-5 2021, hybrid attendance; the 8th biennial VNOP Conference, May 24-25, 2022, Utrecht, the Netherlands; and the Reading Emotions Conference, June 8-9, 2022, online.

Data Sharing: This project used MRI and temperament data collected at various institutions. They were brought together for mega-analysis at NIMH. With respect to data sharing, there will probably be data sharing restrictions imposed by 1) ethical review boards of the participating sites and consent documents and 2) national and trans-national data sharing laws, such as the GDPR, and 3) institutional processes, some of which require a signed data

transfer agreement for limited and predefined data use. Despite these limitations, data sharing might still be possible, by means of submitting a detailed analysis plan to the ENIGMA-Anxiety Working Group. If approved, access to relevant data can be provided, dependent on data availability, local PI approval, and compliance with all supervening regulations.

Anderson M. Winkler and Thomas E. Nichols served as the statistical experts for this research.

Disclosure: Janna Marie Bas-Hoogendam received support from a Talent Acceleration grant from Medical Delta (NL) and a grant from NeurolabNL (Small projects for NWA routes 21/22; NWA.1418.22.025). Kelley E. Gunther received a National Science Foundation Graduate Research Fellowship (DGE1255832). Koralý Pérez-Edgar has reported financial support from NIDA U01DA055361-01, the National Institute of Mental Health (R56MH126349, R01MH109692, R01MH130007) and received royalties from Springer Press, honoraria for lectures to professional audiences, and honoraria for editing the journal *Developmental Psychology*. Giovanni A. Salum declared that the BHRCS was supported with grants from the National Institute of Development Psychiatric for Children and Adolescent (INPD) (grants CNPq 465550/2014-2 and FAPESP 2014/50917-0). Andrea P. Jackowski has reported grant support from CNPq 427215/2018-8 and FAPESP (13/08531-5). Kathryn A. Degnan has reported grant support from a subaward from the National Institute of Mental Health (U01MH093349) via University of Maryland. Courtney A. Filippi has received support intramural Research Program of the National Institute of Mental Health (NIMH; ZIA-MH-002782), NIMH extramural funding (R00MH125878), and the NARSAD Young Investigator Grant from the Brain and Behavior Foundation (#28024). Nathan A. Fox reported grant support from the National Institute of Mental Health (U01MH093349), the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), the National Science Foundation (NSF), the National Institutes of Health Environmental influences on Child Health Outcomes (NIH ECHO) consortium, the Russell Sage Foundation, and the Lumos Foundation, royalties from Springer Press, Guilford Press and Harvard University Press and honoraria for lectures to professional audiences. Heather A. Henderson has reported grant support from the Social Sciences and Humanities Research Council of Canada, Canada Foundation for Innovation. Carl E. Schwartz reported grants from the National Institute of Mental Health (Career Development Award, NIH/ NIMH R01MH07484 and NIH/NIMH R01MH071467). Karin Roelofs and Anna Tyborowska stated that past MR scanning of the Nijmegen Longitudinal Study (NLS) was funded by European Research Council starting grant (ERC_StG2012_313749 awarded to KR) and a FP7-HEALTH-2013-INNOVATION grant (602805-2). Shirley Y. Hill reported grant support from the National Institute on Alcohol Abuse and Alcoholism (AA021746 and AA021746-05S1-Supplement). Lea R. Dougherty has reported grants from the National Institute of Mental Health (R01MH122487 and R01MH121385). Daniel N. Klein reported multiple grants from the National Institute of Health (R01 MH133652; NIEHS R01 ES0 13744; NIEHS R01 ES033436). Roxann Roberson-Nay reported grants from the National Institute of Mental Health (R01MH101518; 5R21MH106924) and the Brain and Behavior Research Foundation (Independent Investigator, 21976). Paul M. Thompson has received partial research support from Biogen, Inc., unrelated to this work, as well as a NIH Big Data to Knowledge (BD2K) award (U54 EB020403). Nic J. A. van der Wee reported support from the EU Innovative Medicines Initiative 'Psychiatric Ratings using Intermediate Stratified Markers' 2 (IMI PRISM 2) with grant agreement nr 101034377. Dan J. Stein received research grants and/or consultancy honoraria from Discovery Vitality, Johnson & Johnson, Kanna, Lundbeck, Orion, Sanofi, Servier and Takeda. Rodrigo A. Bressan, Alva Tang, Marinus H. van IJzendoorn, Julia M. Felicione, Kathryn A. DeYoung, Alexander J. Shackman, Jason F. Smith, Rachael M. Tillman, Yvonne H. M. van den Berg, Antonius H. N. Cillessen, Marco Battaglia, Marco Tettamanti, Jingwen Jin, Hoi-Chung Leung, and Suzanne N. Avery have reported no biomedical financial interests or potential conflicts of interest.

*Correspondence to Janna Marie Bas-Hoogendam, PhD, Institute of Psychology, Leiden University, Pieter de la Court Building, Room 3.B47, Wassenaarseweg 52, 2333 AK Leiden, the Netherlands; e-mail: j.m.hoogendam@fsw.leidenuniv.nl

0890-8567/\$36.00/©2025 American Academy of Child and Adolescent Psychiatry. Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

<https://doi.org/10.1016/j.jaac.2025.06.026>

REFERENCES

- Kagan J, Snidman NC. *The Long Shadow of Temperament*. Harvard University Press; 2009.
- Clauss JA, Avery SN, Blackford JU. The nature of individual differences in inhibited temperament and risk for psychiatric disease: a review and meta-analysis. *Prog Neurobiol*. 2015;127-128:23-45. <https://doi.org/10.1016/j.pneurobio.2015.03.001>
- Pérez-Edgar K, Fox NA. Temperament and anxiety disorders. *Child Adolesc Psychiatr Clin N Am*. 2005;14(4):681-706. <https://doi.org/10.1016/j.jc.2005.05.008>
- Pérez-Edgar KE, Guyer AE. Behavioral Inhibition: temperament or prodrome? *Curr Behav Neurosci Rep*. Published online June 24, 2014. <https://doi.org/10.1007/s40473-014-0019-9>
- Kagan J, Reznick JS, Snidman N. The physiology and psychology of behavioral inhibition in children. *Child Dev*. 1987;58(6):1459-1473.
- Fox AS, Harris RA, Del Rosso L, *et al*. Infant inhibited temperament in primates predicts adult behavior, is heritable, and is associated with anxiety-relevant genetic variation. *Mol Psychiatry*. Published online 2021. <https://doi.org/10.1038/s41380-021-01156-4>
- Shackman AJ, Fox AS, Oler JA, *et al*. Heightened extended amygdala metabolism following threat characterizes the early phenotypic risk to develop anxiety-related psychopathology. *Mol Psychiatry*. 2017;22(5):724-732. <https://doi.org/10.1038/mp.2016.132>
- Fox AS, Kalin NH. A translational neuroscience approach to understanding the development of social anxiety disorder and its pathophysiology. *Am J Psychiatry*. 2014;171(11):1162-1173. <https://doi.org/10.1176/appi.ajp.2014.14040449>
- Sylvester CM, Pine DS. The biological bridge between behavioral inhibition and psychopathology BT—behavioral inhibition: integrating theory, research, and clinical perspectives. In: Pérez-Edgar K, Fox NA, eds. *Behavioral Inhibition*. Springer; 2018:309-335. https://doi.org/10.1007/978-3-319-98077-5_14
- Mumper EE, Klein DN. Behavioral inhibition and psychopathology in childhood. *The Handbook of Solitude: Psychological Perspectives on Social Isolation, Social Withdrawal, and Being Alone*. Wiley Online Library; 2021:89-104.
- Fox NA, Henderson HA, Marshall PJ, Nichols KE, Ghera MM. Behavioral inhibition: linking biology and behavior within a developmental framework. *Annu Rev Psychol*. 2004;56(1):235-262. <https://doi.org/10.1146/annurev.psych.55.090902.141532>
- Bornstein MH, Putnick DL, Gartstein MA, Hahn C, Auestad N, O'Connor DL. Infant temperament: stability by age, gender, birth order, term status, and socioeconomic status. *Child Dev*. 2015;86(3):844-863. <https://doi.org/10.1111/cdev.12367>
- Tang A, Crawford H, Morales S, Degnan KA, Pine DS, Fox NA. Infant behavioral inhibition predicts personality and social outcomes three decades later. *Proc Natl Acad Sci*. Published online April 17, 2020:201917376. <https://doi.org/10.1073/pnas.1917376117>
- Caspi A, Roberts BW, Shiner RL. Personality development: stability and change. *Annu Rev Psychol*. 2004;56(1):453-484. <https://doi.org/10.1146/annurev.psych.55.090902.141913>
- Clauss JA, Blackford JU. Behavioral inhibition and risk for developing social anxiety disorder: a meta-analytic study. *J Am Acad Child Adolesc Psychiatry*. 2012;51(10):1066-1075. <https://doi.org/10.1016/j.jaac.2012.08.002>
- Sandstrom A, Uher R, Pavlova B. Prospective association between childhood behavioral inhibition and anxiety: a meta-analysis. *Res Child Adolesc Psychopathol*. 2020;48(1):57-66. <https://doi.org/10.1007/s10802-019-00588-5>
- Tan E, Zeytinoglu S, Morales S, *et al*. Social vs non-social behavioral inhibition: differential prediction from early childhood of long-term psychosocial outcomes. *Dev Sci*. 2024;27(1). <https://doi.org/10.1111/desc.13427>
- Schwartz CE, Snidman N, Kagan J. Adolescent social anxiety as an outcome of inhibited temperament in childhood. *J Am Acad Child Adolesc Psychiatry*. 1999;38(8):1008-1015. <https://doi.org/10.1097/00004583-199908000-00017>
- Biederman J, Hirshfeld-Becker DR, Rosenbaum JF, *et al*. Further evidence of association between behavioral inhibition and social anxiety in children. *Am J Psychiatry*. 2001;158(10):1673-1679. <https://doi.org/10.1176/appi.ajp.158.10.1673>
- Fox NA, Buzzell GA, Morales S, Valadez EA, Wilson M, Henderson HA. Understanding the emergence of social anxiety in children with behavioral inhibition. *Biol Psychiatry*. 2021;89(7):681-689. <https://doi.org/10.1016/j.biopsych.2020.10.004>
- Goldsmith HH, Hilton EC, Phan JM, *et al*. Childhood inhibition predicts adolescent social anxiety: findings from a longitudinal twin study. *Dev Psychopathol*. Published online 2022:1-20. <https://doi.org/10.1017/S0954579422000864>
- Bourdon JL, Savage JE, Verhulst B, *et al*. The genetic and environmental relationship between childhood behavioral inhibition and preadolescent anxiety. *Twin Res Hum Genet*. 2019;22(1):48-55. <https://doi.org/10.1017/thg.2018.73>
- Bakken NR, Hannigan LJ, Shadrin A, *et al*. Childhood temperamental, emotional, and behavioral characteristics associated with mood and anxiety disorders in adolescence: a prospective study. *Acta Psychiatr Scand*. 2023;147(2):217-228. <https://doi.org/10.1111/acps.13522>
- Bas-Hoogendam JM, Roelofs EF, Westenberg PM, van der Wee NJA. Pathogenesis of SAD. In: Simon NM, Hollander E, Rothbaum BO, Stein DJ, eds. *Textbook of Anxiety, Trauma and OCD-Related Disorders*. 3rd ed. American Psychiatric Association Publishing; 2020:429-444.
- Bas-Hoogendam JM, Blackford JU, Brühl AB, Blair KS, van der Wee NJA, Westenberg PM. Neurobiological candidate endophenotypes of social anxiety disorder. *Neurosci Biobehav Rev*. 2016;71:362-378. <https://doi.org/10.1016/j.neubiorev.2016.08.040>
- Chronis-Tuscano A, Degnan KA, Pine DS, *et al*. Stable early maternal report of behavioral inhibition predicts lifetime social anxiety disorder in adolescence. *J Am Acad Child Adolesc Psychiatry*. 2009;48(9):928-935. <https://doi.org/10.1097/CHI.0b013e3181ae09df>
- Essex MJ, Klein MH, Slattery MJ, Goldsmith HH, Kalin NH. Early risk factors and developmental pathways to chronic high inhibition and social anxiety disorder in adolescence. *Am J Psychiatry*. 2010;167(1):40-46. <https://doi.org/10.1176/appi.ajp.2009.07010051>
- Morales S, Tang A, Bowers ME, *et al*. Infant temperament prospectively predicts general psychopathology in childhood. *Dev Psychopathol*. 2022;34(3):774-783. <https://doi.org/10.1017/S0954579420001996>
- Poole KL, Tang A, Schmidt LA. The temperamentally shy child as the social adult: an exemplar of multifinality BT—behavioral inhibition: integrating theory, research, and clinical perspectives. In: Pérez-Edgar K, Fox NA, eds. *Behavioral Inhibition*. Springer; 2018:185-212. https://doi.org/10.1007/978-3-319-98077-5_9
- Liu P, Pérez-Edgar KE. Developmental pathways from early behavioral inhibition to later anxiety: an integrative review of developmental psychopathology research and translational implications. *Adolesc Res Rev*. 2019;4(1):45-58. <https://doi.org/10.1007/s40894-018-0092-5>
- Klein DN, Mumper EE. Behavioral inhibition as a precursor to psychopathology BT—behavioral inhibition: integrating theory, research, and clinical perspectives. In: Pérez-Edgar K, Fox NA, eds. *Behavioral Inhibition*. Springer; 2018:283-307. https://doi.org/10.1007/978-3-319-98077-5_13
- Sylvester CM. Editorial: Several roads lead to anxiety. *J Am Acad Child Adolesc Psychiatry*. 2020;59(6):703-704. <https://doi.org/10.1016/j.jaac.2019.09.030>
- Blackford JU, Clauss JA, Benningfield MM. The Neurobiology of behavioral inhibition as a developmental mechanism. In: Pérez-Edgar K, Fox NA, eds. *Behavioral Inhibition*. Springer; 2018:113-134. https://doi.org/10.1007/978-3-319-98077-5_6
- Taber-Thomas BC, Morales S, Hillary FG, Pérez-Edgar KE. Altered topography of intrinsic functional connectivity in childhood risk for social anxiety. *Depress Anxiety*. 2016;33:995-1004. <https://doi.org/10.1002/da.22508>
- Auday ES, Pérez-Edgar KE. Limbic and prefrontal neural volume modulate social anxiety in children at temperamental risk. *Depress Anxiety*. 2019;36(8):690-700. <https://doi.org/10.1002/da.22941>
- Auday ES, Taber-Thomas BC, Pérez-Edgar KE. Neural correlates of attention bias to masked facial threat cues: examining children at-risk for social anxiety disorder. *Neuroimage Clin*. 2018;19:202-212. <https://doi.org/10.1016/j.nicl.2018.04.003>
- Clauss JA, Avery SN, Vanderklok RM, *et al*. Neurocircuitry underlying risk and resilience to social anxiety disorder. *Depress Anxiety*. 2014;31(10):822-833. <https://doi.org/10.1002/da.22265>
- Blackford JU, Clauss JA, Avery SN, Cowan RL, Benningfield MM, Vanderklok RM. Amygdala-cingulate intrinsic connectivity is associated with degree of social inhibition. *Biol Psychol*. 2014;99:15-25. <https://doi.org/10.1016/j.biopsycho.2014.02.003>
- Blackford JU, Avery SN, Cowan RL, Shelton RC, Zald DH. Sustained amygdala response to both novel and newly familiar faces characterizes inhibited temperament. *Soc Cogn Affect Neurosci*. 2011;6(5):621-629. <https://doi.org/10.1093/scan/nsq073>
- Blackford JU, Allen AH, Cowan RL, Avery SN. Amygdala and hippocampus fail to habituate to faces in individuals with an inhibited temperament. *Soc Cogn Affect Neurosci*. 2013;8(2):143-150. <https://doi.org/10.1093/scan/nsr078>
- Blackford JU, Avery SN, Shelton RC, Zald DH. Amygdala temporal dynamics: temperamental differences in the timing of amygdala response to familiar and novel faces. *BMC Neurosci*. 2009;10:145. <https://doi.org/10.1186/1471-2202-10-145>
- Clauss JA, Scay AL, Vanderklok RM, *et al*. Structural and functional bases of inhibited temperament. *Soc Cogn Affect Neurosci*. 2014;9(12):2049-2058. <https://doi.org/10.1093/scan/nsu019>
- Schwartz CE, Wright CI, Shin LM, Kagan J, Rauch SL. Inhibited and uninhibited infants “grown up”: adult amygdala response to novelty. *Science* (1979). 2003;300(5627):1952-1953. <https://doi.org/10.1126/science.1083703>
- Guyer AE, Nelson EE, Perez-Edgar K, *et al*. Striatal functional alteration in adolescents characterized by early childhood behavioral inhibition. *J Neurosci*. 2006;26(24):639LP-6405. <https://doi.org/10.1523/JNEUROSCI.0666-06.2006>
- Pérez-Edgar K, Roberson-Nay R, Hardin MG, *et al*. Attention alters neural responses to evocative faces in behaviorally inhibited adolescents. *Neuroimage*. 2007;35(4):1538-1546. <https://doi.org/10.1016/j.neuroimage.2007.02.006>

46. Schwartz CE, Kunwar PS, Greve DN, *et al.* Structural differences in adult orbital and ventromedial prefrontal cortex predicted by infant temperament at 4 months of age. *Arch Gen Psychiatry*. 2010;67(1):78-84. <https://doi.org/10.1001/archgenpsychiatry.2009.171>
47. Schwartz CE, Kunwar PS, Greve DN, Kagan J, Snidman NC, Bloch RB. A phenotype of early infancy predicts reactivity of the amygdala in male adults. *Mol Psychiatry*. 2012; 17(10):1042-1050. <https://doi.org/10.1038/mp.2011.96>
48. Pérez-Edgar K, Hardee JE, Guyer AE, *et al.* DRD4 and striatal modulation of the link between childhood behavioral inhibition and adolescent anxiety. *Soc Cogn Affect Neurosci*. 2014;9(4):445-453. <https://doi.org/10.1093/scan/nst001>
49. Troller-Renfree SV, Buzzell GA, Bowers ME, *et al.* Development of inhibitory control during childhood and its relations to early temperament and later social anxiety: unique insights provided by latent growth modeling and signal detection theory. *J Child Psychol Psychiatry*. 2019;60(6):622-629. <https://doi.org/10.1111/jcpp.13025>
50. Lahat A, Benson BE, Pine DS, Fox NA, Ernst M. Neural responses to reward in childhood: relations to early behavioral inhibition and social anxiety. *Soc Cogn Affect Neurosci*. Published online August 16, 2018;281-289. <https://doi.org/10.1093/scan/nsw122>
51. Hill SY, Tessner K, Wang S, Carter H, McDermott M. Temperament at 5 years of age predicts amygdala and orbitofrontal volume in the right hemisphere in adolescence. *Psychiatry Res Neuroimaging*. 2010;182(1):14-21. <https://doi.org/10.1016/j.psychres.2009.11.006>
52. Sylvester CM, Barch DM, Harms MP, *et al.* Early childhood behavioral inhibition predicts cortical thickness in adulthood. *J Am Acad Child Adolesc Psychiatry*. 2016; 55(2):122-129. <https://doi.org/10.1016/j.jaac.2015.11.007>
53. Clarkson T, Eaton NR, Nelson EE, *et al.* Early childhood social reticence and neural response to peers in preadolescence predict social anxiety symptoms in midadolescence. *Depress Anxiety*. 2019;36(8):676-689. <https://doi.org/10.1002/da.22910>
54. Abend R, Swetlitz C, White LK, *et al.* Levels of early-childhood behavioral inhibition predict distinct neurodevelopmental pathways to pediatric anxiety. *Psychol Med*. 2020; 50(1):96-106. <https://doi.org/10.1017/S0033291718003999>
55. Filippi CA, Sachs JF, Phillips D, *et al.* Infant behavioral reactivity predicts change in amygdala volume 12 years later. *Dev Cogn Neurosci*. 2020;42. <https://doi.org/10.1016/j.dcn.2020.100776>
56. Troller-Renfree SV, Buzzell GA, Pine DS, Henderson HA, Fox NA. Consequences of not planning ahead: reduced proactive control moderates longitudinal relations between behavioral inhibition and anxiety. *J Am Acad Child Adolesc Psychiatry*. 2019;58(8): 768-775. <https://doi.org/10.1016/j.jaac.2018.06.040>
57. Bas-Hoogendam JM, van Steenberg H, Tissier RLM, Houwing-Duistermaat JJ, Westenberg PM, van der Wee NJA. Subcortical brain volumes, cortical thickness and cortical surface area in families genetically enriched for social anxiety disorder-A multiplex multigenerational neuroimaging study. *EBioMedicine*. Published online September 26, 2018:410-428. <https://doi.org/10.1016/j.ebiom.2018.08.048>
58. Bas-Hoogendam JM, van Steenberg H, van der Wee NJA, Westenberg PM. Amygdala hyperreactivity to faces conditioned with a social-evaluative meaning—a multiplex, multigenerational fMRI study on social anxiety endophenotypes. *Neuroimage Clin*. Published online 2020:102247. <https://doi.org/10.1016/j.nicl.2020.102247>
59. Bas-Hoogendam JM, Harrewijn A, Tissier RLM, *et al.* The Leiden Family Lab Study on Social Anxiety Disorder: a multiplex, multigenerational family study on neurocognitive endophenotypes. *Int J Methods Psychiatr Res*. 2018;27(2):e1616. <https://doi.org/10.1002/mpr.1616>
60. Bas-Hoogendam JM, van Steenberg H, Blackford JU, Tissier RLM, van der Wee NJA, Westenberg PM. Impaired neural habituation to neutral faces in families genetically enriched for social anxiety disorder. *Depress Anxiety*. 2019;36(12):1143-1153. <https://doi.org/10.1002/da.22962>
61. Bas-Hoogendam JM, van Steenberg H, Tissier RLM, van der Wee NJA, Westenberg PM. Altered neurobiological processing of unintentional social norm violations: a multiplex, multigenerational fMRI study on social anxiety endophenotypes. *Biol Psychiatry Cogn Neurosci Neuroimaging*. 2020;5(10):981-990. <https://doi.org/10.1016/j.bpsc.2019.03.003>
62. Oler JA, Fox AS, Shelton SE, *et al.* Amygdalar and hippocampal substrates of anxious temperament differ in their heritability. *Nature*. 2010;466(7308):864-868. <https://doi.org/10.1038/nature09282>
63. Clinton SM, Shupe EA, Glover ME, *et al.* Modeling heritability of temperamental differences, stress reactivity, and risk for anxiety and depression: relevance to Research Domain Criteria (RDoC). *Eur J Neurosci*. 2022;55(9):2076-2107. <https://doi.org/10.1111/ejn.15158>
64. McNaughton N, Gray JA. Anxiolytic action on the behavioural inhibition system implies multiple types of arousal contribute to anxiety. *J Affect Disord*. 2000;61(3): 161-176. [https://doi.org/10.1016/s0165-0327\(00\)00344-x](https://doi.org/10.1016/s0165-0327(00)00344-x)
65. Fu X, Taber-Thomas BC, Pérez-Edgar K. Frontolimbic functioning during threat-related attention: relations to early behavioral inhibition and anxiety in children. *Biol Psychol*. 2017;122:98-109. <https://doi.org/10.1016/j.biopsycho.2015.08.010>
66. Bar-Haim Y, Fox NA, Benson B, *et al.* Neural correlates of reward processing in adolescents with a history of inhibited temperament. *Psychol Sci*. 2009;20(8):1009-1018. <https://doi.org/10.1111/j.1467-9280.2009.02401.x>
67. Guyer AE, Benson B, Choate VR, *et al.* Lasting associations between early-childhood temperament and late-adolescent reward-circuitry response to peer feedback. *Dev Psychopathol*. 2014;26(1):229-243. <https://doi.org/10.1017/S0954579413000941>
68. Hardee JE, Benson BE, Bar-Haim Y, *et al.* Patterns of neural connectivity during an attention bias task moderate associations between early childhood temperament and internalizing symptoms in young adulthood. *Biol Psychiatry*. 2013;74(4):273-279. <https://doi.org/10.1016/j.biopsych.2013.01.036>
69. Helfinstein SM, Benson B, Perez-Edgar K, *et al.* Striatal responses to negative monetary outcomes differ between temperamentally inhibited and non-inhibited adolescents. *Neuropsychologia*. 2011;49(3):479-485. <https://doi.org/10.1016/j.neuropsychologia.2010.12.015>
70. Jarcho JM, Fox NA, Pine DS, *et al.* The neural correlates of emotion-based cognitive control in adults with early childhood behavioral inhibition. *Biol Psychol*. 2013;92(2): 306-314. <https://doi.org/10.1016/j.biopsycho.2012.09.008>
71. Jarcho JM, Fox NA, Pine DS, *et al.* Enduring influence of early temperament on neural mechanisms mediating attention-emotion conflict in adults. *Depress Anxiety*. 2014; 31(1):53-62. <https://doi.org/10.1002/da.22140>
72. Roy AK, Benson BE, Degnan KA, *et al.* Alterations in amygdala functional connectivity reflect early temperament. *Biol Psychol*. 2014;103:248-254. <https://doi.org/10.1016/j.biopsycho.2014.09.007>
73. Shechner T, Fox NA, Mash JA, *et al.* Differences in neural response to extinction recall in young adults with or without history of behavioral inhibition. *Dev Psychopathol*. 2018;30(1):179-189. <https://doi.org/10.1017/S0954579417000554>
74. Tang A, Harrewijn A, Benson B, *et al.* Striatal activity to reward anticipation as a moderator of the association between early behavioral inhibition and changes in anxiety and depressive symptoms from adolescence to adulthood. *JAMA Psychiatry*. 2022; 79(12):1199-1208. <https://doi.org/10.1001/jamapsychiatry.2022.3483>
75. Michalska KJ, Feldman JS, Ivie EJ, *et al.* Early-childhood social reticence predicts SCR-BOLD coupling during fear extinction recall in preadolescent youth. *Dev Cogn Neurosci*. 2019;36:100605. <https://doi.org/10.1016/j.dcn.2018.12.003>
76. Jarcho JM, Davis MM, Shechner T, *et al.* Early-childhood social reticence predicts brain function in preadolescent youths during distinct forms of peer evaluation. *Psychol Sci*. 2016;27(6):821-835. <https://doi.org/10.1177/0956797616638319>
77. Smith AR, White LK, Leibenluft E, *et al.* The heterogeneity of anxious phenotypes: neural responses to errors in treatment-seeking anxious and behaviorally-inhibited youth. *J Am Acad Child Adolesc Psychiatry*. 2020;59(6):759-769. <https://doi.org/10.1016/j.jaac.2019.05.014>
78. Clauss JA, Benningfield MM, Rao U, Blackford JU. Altered prefrontal cortex function marks heightened anxiety risk in children. *J Am Acad Child Adolesc Psychiatry*. 2016; 55(9):809-816. <https://doi.org/10.1016/j.jaac.2016.05.024>
79. Avery SN, Blackford JU. Slow to warm up: the role of habituation in social fear. *Soc Cogn Affect Neurosci*. 2016;11(11):1832-1840. <https://doi.org/10.1093/scan/nsw095>
80. Bas-Hoogendam JM, Groenewold NA, Aghajani M, *et al.* ENIGMA-Anxiety Working Group: rationale for and organization of large-scale neuroimaging studies of anxiety disorders. *Hum Brain Mapp*. 2022;43(1):83-112. <https://doi.org/10.1002/hbm.25100>
81. Boedhoe PSW, Heymans MW, Schmaal L, *et al.* An empirical comparison of meta- and mega-analysis with data from the ENIGMA Obsessive-Compulsive Disorder Working Group. *Front Neuroinform*. 2019;12. <https://doi.org/10.3389/fninf.2018.00102>
82. Zacharek SJ, Kribakaran S, Kitt ER, Gee DG. Leveraging big data to map neurodevelopmental trajectories in pediatric anxiety. *Dev Cogn Neurosci*. 2021;50:100974. <https://doi.org/10.1016/j.dcn.2021.100974>
83. Winkler AM, Kochunov P, Blangero J, *et al.* Cortical thickness or grey matter volume? The importance of selecting the phenotype for imaging genetics studies. *Neuroimage*. 2010;53(3):1135-1146. <https://doi.org/10.1016/j.neuroimage.2009.12.028>
84. Hirshfeld-Becker DR, Biederman J, Henin A, *et al.* Behavioral inhibition in preschool children at risk is a specific predictor of middle childhood social anxiety: a five-year follow-up. *J Dev Behav Pediatr*. 2007;28(3):225-233. <https://doi.org/10.1097/01.DBP.0000268559.34463.d0>
85. Buzzell GA, Troller-Renfree SV, Barker TV, *et al.* A neurobehavioral mechanism linking behaviorally inhibited temperament and later adolescent social anxiety. *J Am Acad Child Adolesc Psychiatry*. 2017;56(12):1097-1105. <https://doi.org/10.1016/j.jaac.2017.10.007>
86. Buzzell GA, Morales S, Bowers ME, *et al.* Inhibitory control and set shifting describe different pathways from behavioral inhibition to socially anxious behavior. *Dev Sci*. 2021;24(1):e13040. <https://doi.org/10.1111/desc.13040>
87. Vreeke LJ, Muris P, Mayer B, *et al.* The assessment of an inhibited, anxiety-prone temperament in a Dutch multi-ethnic population of preschool children. *Eur Child Adolesc Psychiatry*. 2012;21(11):623-633. <https://doi.org/10.1007/s00787-012-0299-0>

88. Marysko M, Finke P, Wiebel A, Resch F, Moehler E. Can mothers predict childhood behavioural inhibition in early infancy? *Child Adolesc Ment Health*. 2010;15(2):91-96. <https://doi.org/10.1111/j.1475-3588.2009.00539.x>
89. Muris P, Meesters C. Reactive and regulative temperament in youths: psychometric evaluation of the Early Adolescent Temperament Questionnaire—Revised. *J Psychopathol Behav Assess*. 2009;31(1):7-19. <https://doi.org/10.1007/s10862-008-9089-x>
90. Lawson KM, Atherton OE, Robins RW. The structure of adolescent temperament and associations with psychological functioning: a replication and extension of Snyder *et al.* (2015). *J Pers Soc Psychol*. Published online February 2021. <https://doi.org/10.1037/pspp0000380>
91. Saygin ZM, Kliemann D, Iglesias JE, *et al.* High-resolution magnetic resonance imaging reveals nuclei of the human amygdala: manual segmentation to automatic atlas. *Neuroimage*. 2017;155:370-382. <https://doi.org/10.1016/j.neuroimage.2017.04.046>
92. Iglesias JE, Augustinack JC, Nguyen K, *et al.* A computational atlas of the hippocampal formation using ex vivo, ultra-high resolution MRI: application to adaptive segmentation of in vivo MRI. *Neuroimage*. 2015;115:117-137. <https://doi.org/10.1016/j.neuroimage.2015.04.042>
93. Iglesias JE, Insausti R, Lerma-Usabiaga G, *et al.* A probabilistic atlas of the human thalamic nuclei combining ex vivo MRI and histology. *Neuroimage*. 2018;183:314-326. <https://doi.org/10.1016/j.neuroimage.2018.08.012>
94. Abend R, Gold AL, Britton JC, *et al.* Anticipatory threat responding: associations with anxiety, development, and brain structure. *Biol Psychiatry*. 2020;87(10):916-925. <https://doi.org/10.1016/j.biopsych.2019.11.006>
95. Greve DN, Billot B, Cordero D, *et al.* A deep learning toolbox for automatic segmentation of subcortical limbic structures from MRI images. *Neuroimage*. 2021;244:118610. <https://doi.org/10.1016/j.neuroimage.2021.118610>
96. Groenewold N, Bas-Hoogendam JM, Amod AR, *et al.* F27. subcortical volumes in social anxiety disorder: preliminary results from Enigma-Anxiety. *Biol Psychiatry*. 2018;83(9, Suppl):S247-S248. <https://doi.org/10.1016/j.biopsych.2018.02.640>
97. Harrewijn A, Cardinale EM, Groenewold NA, *et al.* Cortical and subcortical brain structure in generalized anxiety disorder: findings from 28 research sites in the ENIGMA-Anxiety Working Group. *Transl Psychiatry*. 2021;11(1):502. <https://doi.org/10.1038/s41398-021-01622-1>
98. Groenewold NA, Bas-Hoogendam JM, Aghajani M, *et al.* Brain characteristics associated with anxiety disorders: an update from the ENIGMA-Anxiety Working Group. *J Neural Transm*. 2021;128:1767-1812. <https://doi.org/10.1007/s00702-021-02422-z>
99. Cha J, Greenberg T, Song I, Blair Simpson H, Posner J, Mujica-Parodi LR. Abnormal hippocampal structure and function in clinical anxiety and comorbid depression. *Hippocampus*. 2016;26(5):545-553. <https://doi.org/10.1002/hipo.22566>
100. Montag C, Reuter M, Jurkiewicz M, Markert S, Panksepp J. Imaging the structure of the human anxious brain: a review of findings from neuroscientific personality psychology. *Rev Neurosci*. 2013;24(2):167-190. <https://doi.org/10.1515/revneuro-2012-0085>
101. Moon CM, Jeong GW. Abnormalities in gray and white matter volumes associated with explicit memory dysfunction in patients with generalized anxiety disorder. *Acta Radiol*. 2017;58(3):353-361. <https://doi.org/10.1177/0284185116649796>
102. Blackford JU. Leveraging statistical methods to improve validity and reproducibility of research findings. *JAMA Psychiatry*. 2017;74(2):119-120. <https://doi.org/10.1001/jamapsychiatry.2016.3730>
103. Thompson P, Jahanshad N, Ching CRK, *et al.* ENIGMA and global neuroscience: a decade of large-scale studies of the brain in health and disease across more than 40 countries. *Transl Psychiatry*. 2020;10(100). <https://doi.org/10.1038/s41398-020-0705-1>
104. Bearden CE, Thompson PM. Emerging global initiatives in neurogenetics: the Enhancing Neuroimaging Genetics through Meta-analysis (ENIGMA) Consortium. *Neuron*. 2017;94(2):232-236. <https://doi.org/10.1016/j.neuron.2017.03.033>
105. Ching CRK, Hibar DP, Gurholt TP, *et al.* What we learn about bipolar disorder from large-scale neuroimaging: findings and future directions from the ENIGMA Bipolar Disorder Working Group. *Hum Brain Mapp*. 2022;43:56-82. <https://doi.org/10.1002/hbm.25098>
106. Hoogman M, van Rooij D, Klein M, *et al.* Consortium neuroscience of attention deficit/hyperactivity disorder and autism spectrum disorder: the ENIGMA adventure. *Hum Brain Mapp*. 2022;43(1):37-55. <https://doi.org/10.1002/hbm.25029>
107. van den Heuvel OA, Boedhoe PSW, Bertolin S, *et al.* An overview of the first 5 years of the ENIGMA Obsessive-Compulsive Disorder Working Group: the power of worldwide collaboration. *Hum Brain Mapp*. 2022;n/a(43):23-36. <https://doi.org/10.1002/hbm.24972>
108. Bas-Hoogendam JM, Groenewold NA, Amod AR, *et al.* Subcortical brain volumes in social anxiety disorder: an Enigma-Anxiety international mega-analysis of 37 samples. *Biol Psychiatry*. 2023;93(9):S85-S86. <https://doi.org/10.1016/j.biopsych.2023.02.222>
109. Groenewold NA, Bas-Hoogendam JM, Amod AR, *et al.* Volume of subcortical brain regions in social anxiety disorder: mega-analytic results from 37 samples in the ENIGMA-Anxiety Working Group. *Mol Psychiatry*. Published online 2023. <https://doi.org/10.1038/s41380-022-01933-9>
110. Hilbert K, Boeken OJ, Langhammer T, *et al.* Cortical and subcortical brain alterations in specific phobia and its animal and blood-injection-injury subtypes: a mega-analysis from the ENIGMA Anxiety Working Group. *Am J Psychiatry*. Published online June 11, 2024;appiajp20230032. <https://doi.org/10.1176/appi.ajp.20230032>
111. Bruin WB, Zhutovsky P, van Wingen GA, *et al.* Brain-based classification of youth with anxiety disorders: transdiagnostic examinations within the ENIGMA-Anxiety database using machine learning. *Nat Ment Health*. 2024;2(1):104-118. <https://doi.org/10.1038/s44220-023-00173-2>
112. Novins DK, Althoff RR, Billingsley MK, *et al.* Study registration: encouraging the practice of hypothetical-deductive research in the journal. *J Am Acad Child Adolesc Psychiatry*. 2018;57(12):901-902. <https://doi.org/10.1016/j.jaac.2018.10.004>
113. Crone EA, Dahl RE. Understanding adolescence as a period of social-affective engagement and goal flexibility. *Nat Rev Neurosci*. 2012;13(9):636-650. <https://doi.org/10.1038/nrn3313>
114. Dima D, Modabbernia A, Papachristou E, *et al.* Subcortical volumes across the lifespan: data from 18,605 healthy individuals aged 3–90 years. *Hum Brain Mapp*. 2022;43(1):452-469. <https://doi.org/10.1002/hbm.25320>
115. Frangou S, Modabbernia A, Williams SCR, *et al.* Cortical thickness across the lifespan: data from 17,075 healthy individuals aged 3–90 years. *Hum Brain Mapp*. 2022;43:431-451. <https://doi.org/10.1002/hbm.25364>
116. Paus T, Keshavan M, Giedd JN. Why do many psychiatric disorders emerge during adolescence? *Nat Rev Neurosci*. 2008;9(12):947-957. <https://doi.org/10.1038/nrn2513>
117. Beesdo-Baum K, Knappe S, Asselmann E, *et al.* The “Early Developmental Stages of Psychopathology (EDSP) study”: a 20-year review of methods and findings. *Soc Psychiatry Psychiatr Epidemiol*. 2015;50(6):851-866. <https://doi.org/10.1007/s00127-015-1062-x>
118. Morales S, Taber-Thomas BC, Pérez-Edgar KE. Patterns of attention to threat across tasks in behaviorally inhibited children at risk for anxiety. *Dev Sci*. Published online January 19, 2017. <https://doi.org/10.1111/desc.12391>
119. Wise S, Huang-Pollock C, Pérez-Edgar K. Implementation of the diffusion model on dot-probe task performance in children with behavioral inhibition. *Psychol Res*. Published online 2021. <https://doi.org/10.1007/s00426-021-01532-3>
120. Anaya B, Vallorani AM, Pérez-Edgar K. Individual dynamics of delta–beta coupling: using a multilevel framework to examine inter- and intraindividual differences in relation to social anxiety and behavioral inhibition. *J Child Psychol Psychiatry*. 2021;62(6):771-779. <https://doi.org/10.1111/jcpp.13319>
121. Anaya B, Vallorani A, Pérez-Edgar K. Dyadic behavioral synchrony between behaviorally inhibited and non-inhibited peers is associated with concordance in EEG frontal alpha asymmetry and delta-beta coupling. *Biol Psychol*. 2021;159:108018. <https://doi.org/10.1016/j.biopsycho.2021.108018>
122. Liu P, Taber-Thomas BC, Fu X, Pérez-Edgar KE. Biobehavioral markers of attention bias modification in temperamental risk for anxiety: a randomized control trial. *J Am Acad Child Adolesc Psychiatry*. 2018;57(2):103-110. <https://doi.org/10.1016/j.jaac.2017.11.016>
123. Suarez GL, Morales S, Metcalf K, Pérez-Edgar KE. Perinatal complications are associated with social anxiety: indirect effects through temperament. *Infant Child Dev*. 2019;28(3):e2130. <https://doi.org/10.1002/icd.2130>
124. Thai N, Taber-Thomas BC, Pérez-Edgar KE. Neural correlates of attention biases, behavioral inhibition, and social anxiety in children: an ERP study. *Dev Cogn Neurosci*. 2016;19:200-210. <https://doi.org/10.1016/j.dcn.2016.03.008>
125. Salum GA, Gadelha A, Pan PM, *et al.* High risk cohort study for psychiatric disorders in childhood: rationale, design, methods and preliminary results. *Int J Methods Psychiatr Res*. 2015;24(1):58-73. <https://doi.org/10.1002/mpr.1459>
126. Fox NA, Henderson HA, Rubin KH, Calkins SD, Schmidt LA. Continuity and discontinuity of behavioral inhibition and exuberance: psychophysiological and behavioral influences across the first four years of life. *Child Dev*. 2001;72(1):1-21. <https://doi.org/10.1111/1467-8624.00262>
127. White T, Muetzel RL, El Marroun H, *et al.* Paediatric population neuroimaging and the Generation R Study: the second wave. *Eur J Epidemiol*. 2018;33(1):99-125. <https://doi.org/10.1007/s10654-017-0319-y>
128. Jansen PW, Raat H, Mackenbach JP, *et al.* Socioeconomic inequalities in infant temperament. *Soc Psychiatry Psychiatr Epidemiol*. 2009;44(2):87-95. <https://doi.org/10.1007/s00127-008-0416-z>
129. Jaddoe VVW, van Duijn CM, van der Heijden AJ, *et al.* The Generation R Study: design and cohort update until the age of 4 years. *Eur J Epidemiol*. 2008;23(12):801. <https://doi.org/10.1007/s10654-008-9309-4>
130. Pappa I, Mileva-Seitz VR, Szekely E, *et al.* DRD4 VNTRs, observed stranger fear in preschoolers and later ADHD symptoms. *Psychiatry Res*. 2014;220(3):982-986. <https://doi.org/10.1016/j.psychres.2014.09.004>

131. Hur J, Smith JF, DeYoung KA, *et al.* Anxiety and the neurobiology of temporally uncertain threat anticipation. *J Neurosci.* 2020;40(41):7949LP-7964LP. <https://doi.org/10.1523/JNEUROSCI.0704-20.2020>
132. Hur J, DeYoung KA, Islam S, Anderson AS, Barstead MG, Shackman AJ. Social context and the real-world consequences of social anxiety. *Psychol Med.* 2020;50(12):1989-2000. <https://doi.org/10.1017/S0033291719002022>
133. Grogans SE, Hur J, Barstead MG, *et al.* Neuroticism/negative emotionality is associated with increased reactivity to uncertain threat in the bed nucleus of the stria terminalis, not the amygdala. *J Neurosci.* Published online July 15, 2024:e1868232024. <https://doi.org/10.1523/jneurosci.1868-23.2024>
134. Hur J, Kuhn M, Grogans SE, *et al.* Anxiety-related frontocortical activity is associated with dampened stressor reactivity in the real world. *Psychol Sci.* Published online June 3, 2022:09567976211056635. <https://doi.org/10.1177/09567976211056635>
135. Hur J, Tillman RM, Kim HC, *et al.* Adolescent social anxiety is associated with diminished discrimination of anticipated threat and safety in the bed nucleus of the stria terminalis. *J Psychopathol Clin Sci.* 2025;134(1):41-56. <https://doi.org/10.1037/abn0000940>
136. Tyborowska A, Volman I, Niermann HCM, *et al.* Early-life and pubertal stress differentially modulate grey matter development in human adolescents. *Sci Rep.* 2018; 8(1):9201. <https://doi.org/10.1038/s41598-018-27439-5>
137. Van Bakel HJA, Riksen-Walraven JM. Stress reactivity in 15-month-old infants: links with infant temperament, cognitive competence, and attachment security. *Dev Psychobiol.* 2004;44(3):157-167. <https://doi.org/10.1002/dev.20001>
138. Van Bakel HJA, Riksen-Walraven JM. Parenting and development of one-year-olds: links with parental, contextual, and child characteristics. *Child Dev.* 2002;73(1):256-273. <https://doi.org/10.1111/1467-8624.00404>
139. Hill SY, Lowers L, Locke J, Snidman N, Kagan J. Behavioral inhibition in children from families at high risk for developing alcoholism. *J Am Acad Child Adolesc Psychiatry.* 1999;38(4):410-417. <https://doi.org/10.1097/00004583-199904000-00013>
140. Hill SY, Shen S, Lowers L, Locke-Wellman J, Matthews AG, McDermott M. Psychopathology in offspring from multiplex alcohol dependence families with and without parental alcohol dependence: a prospective study during childhood and adolescence. *Psychiatry Res.* 2008;160(2):155-166. <https://doi.org/10.1016/j.jpsychires.2010.08.005>
141. Hill SY, Tessner KD, McDermott MD. Psychopathology in offspring from families of alcohol dependent female probands: a prospective study. *J Psychiatr Res.* 2011;45(3):285-294. <https://doi.org/10.1016/j.jpsychires.2010.08.005>
142. Battaglia M, Zanoni A, Taddei M, *et al.* Cerebral responses to emotional expressions and the development of social anxiety disorder: a preliminary longitudinal study. *Depress Anxiety.* 2012;29(1):54-61. <https://doi.org/10.1002/da.20896>
143. Battaglia M, Ogliaeri A, Zanoni A, *et al.* Influence of the serotonin transporter promoter gene and shyness on children's cerebral responses to facial expressions. *Arch Gen Psychiatry.* 2005;62(1):85-94. <https://doi.org/10.1001/archpsyc.62.1.85>
144. Battaglia M, Ogliaeri A, Zanoni A, *et al.* Children's discrimination of expressions of emotions: relationship with indices of social anxiety and shyness. *J Am Acad Child Adolesc Psychiatry.* 2004;43(3):358-365. <https://doi.org/10.1097/01.chi.0000103177.91435.1b>
145. Taddei M, Tettamanti M, Zanoni A, Cappa S, Battaglia M. Brain white matter organisation in adolescence is related to childhood cerebral responses to facial expressions and harm avoidance. *Neuroimage.* 2012;61(4):1394-1401. <https://doi.org/10.1016/j.neuroimage.2012.03.062>
146. Gold AL, Steuber ER, White LK, *et al.* Cortical thickness and subcortical gray matter volume in pediatric anxiety disorders. *Neuropsychopharmacology.* 2017;42:2423-2433. <https://doi.org/10.1038/npp.2017.83>
147. Gold AL, Abend R, Britton JC, *et al.* Age differences in the neural correlates of anxiety disorders: an fMRI study of response to learned threat. *Am J Psychiatry.* 2020;177(5):454-463. <https://doi.org/10.1176/appi.ajp.2019.19060650>
148. Linke JO, Abend R, Kircanski K, *et al.* Shared and anxiety-specific pediatric psychopathology dimensions manifest distributed neural correlates. *Biol Psychiatry.* 2021; 89(6):579-587. <https://doi.org/10.1016/j.biopsych.2020.10.018>
149. Dyson MW, Klein DN, Olinio TM, Dougherty LR, Durbin CE. Social and non-social behavioral inhibition in preschool-age children: differential associations with parent reports of temperament and anxiety. *Child Psychiatry Hum Dev.* 2011;42(4):390-405. <https://doi.org/10.1007/s10578-011-0225-6>
150. Dyson MW, Olinio TM, Durbin CE, *et al.* The structural and rank-order stability of temperament in young children based on a laboratory-observational measure. *Psychol Assess.* 2015;27(4):1388-1401. <https://doi.org/10.1037/pas0000104>
151. Hubachek S, Bortdorf M, Riggins T, Leong HC, Klein DN, Dougherty LR. Hippocampal subregion volume in high-risk offspring is associated with increases in depressive symptoms across the transition to adolescence. *J Affect Disord.* 2021;281:358-366. <https://doi.org/10.1016/j.jad.2020.12.017>
152. Klein DN, Finsaas MC. The Stony Brook Temperament Study: early antecedents and pathways to emotional disorders. *Child Dev Perspect.* 2017;11(4):257-263. <https://doi.org/10.1111/cdep.12242>
153. Kopala-Sibley DC, Cyr M, Finsaas MC, *et al.* Early childhood parenting predicts late childhood brain functional connectivity during emotion perception and reward processing. *Child Dev.* 2020;91(1):110-128. <https://doi.org/10.1111/cdev.13126>
154. Laptook RS, Klein DN, Olinio TM, Dyson MW, Carlson G. Low positive affectivity and behavioral inhibition in preschool-age children: a replication and extension of previous findings. *Pers Individ Diff.* 2010;48(5):547-551. <https://doi.org/10.1016/j.paid.2009.12.003>
155. Mumfer EE, Dyson MW, Finsaas MC, Olinio TM, Klein DN. Life stress moderates the effects of preschool behavioral inhibition on anxiety in early adolescence. *J Child Psychol Psychiatry.* 2020;61(2):167-174. <https://doi.org/10.1111/jcpp.13121>
156. Olinio TM, Klein DN, Dyson MW, Rose SA, Durbin CE. Temperamental emotionality in preschool-aged children and depressive disorders in parents: associations in a large community sample. *J Abnorm Psychol.* 2010;119(3):468. <https://doi.org/10.1037/a0020112>
157. Stumper A, Danzig AP, Dyson MW, Olinio TM, Carlson GA, Klein DN. Parents' behavioral inhibition moderates association of preschoolers' BI with risk for age 9 anxiety disorders. *J Affect Disord.* 2017;210:35-42. <https://doi.org/10.1016/j.jad.2016.12.008>
158. White LK, Degnan KA, Henderson HA, *et al.* Developmental relations among behavioral inhibition, anxiety, and attention biases to threat and positive information. *Child Dev.* 2017;88(1):141-155. <https://doi.org/10.1111/cdev.12696>
159. Hane AA, Fox NA, Henderson HA, Marshall PJ. Behavioral reactivity and approach-withdrawal bias in infancy. *Dev Psychol.* 2008;44(5):1491. <https://doi.org/10.1037/a0012855>
160. Harrewijn A, Abend R, Linke J, *et al.* Combining fMRI during resting state and an attention bias task in children. *Neuroimage.* 2020;205:116301. <https://doi.org/10.1016/j.neuroimage.2019.116301>
161. Thorntona-Wells TA, Avery SN, Blackford JU. Using novel control groups to dissect the amygdala's role in Williams syndrome. *Dev Cogn Neurosci.* 2011;1(3):295-304. <https://doi.org/10.1016/j.dcn.2011.03.003>
162. Carney DM, Moroney E, Machlin L, *et al.* The Twin Study of Negative Valence Emotional Constructs. *Twin Res Hum Genet.* 2016;19(5):456-464. <https://doi.org/10.1017/thg.2016.59>
163. Hettema JM, Bourdon JL, Sawyers C, *et al.* Genetic and environmental risk structure of internalizing psychopathology in youth. *Depress Anxiety.* 2020;37(6):540-548. <https://doi.org/10.1002/da.23024>
164. Lee M, Aggen SH, Carney DM, *et al.* Latent structure of negative valence measures in childhood. *Depress Anxiety.* 2017;34(8):742-751. <https://doi.org/10.1002/da.22656>
165. Liu P, Vandermeer MRJ, Mohamed Ali O, *et al.* Maternal depression, child temperament, and early-life stress predict never-depressed preadolescents' functional connectivity during a negative-mood induction. *Clin Psychol Sci.* Published online June 3, 2021:21677026211016420. <https://doi.org/10.1177/21677026211016419>
166. Liu P, Kryski KR, Smith HJ, Joannise MF, Hayden EP. Transactional relations between early child temperament, structured parenting, and child outcomes: a three-wave longitudinal study. *Dev Psychopathol.* 2020;32(3):923-933. <https://doi.org/10.1017/S0954579419000841>
167. Liu P, Vandermeer MRJ, Joannise MF, Barch DM, Dozois DJA, Hayden EP. Neural activity during self-referential processing in children at risk for depression. *Biol Psychiatry Cogn Neurosci Neuroimaging.* 2020;5(4):429-437. <https://doi.org/10.1016/j.bpsc.2019.12.012>
168. Liu P, Vandemeer MRJ, Joannise MF, Barch DM, Dozois DJA, Hayden EP. Depressive self-schemas are associated with smaller regional grey matter volume in never-depressed preadolescents. *Neuroimage Clin.* 2020;28:102422. <https://doi.org/10.1016/j.nicl.2020.102422>
169. Vandermeer MRJ, Liu P, Mohamed Ali O, *et al.* Orbitofrontal cortex grey matter volume is related to children's depressive symptoms. *Neuroimage Clin.* 2020;28:102395. <https://doi.org/10.1016/j.nicl.2020.102395>
170. Vandermeer MRJ, Sheikh HI, Singh SS, *et al.* The BDNF gene val66met polymorphism and behavioral inhibition in early childhood. *Soc Dev.* 2018;27(3):543-554. <https://doi.org/10.1111/sode.12292>
171. Schmidt NL, Lemery-Chalfant K, Goldsmith HH. Wisconsin Twin Project Overview: temperament and affective neuroscience. *Twin Res Hum Genet.* 2019;22(6):794-799. <https://doi.org/10.1017/thg.2019.108>
172. Schmidt NL, Brooker RJ, Carroll IC, *et al.* Longitudinal research at the interface of affective neuroscience, developmental psychopathology, health and behavioral genetics: findings from the Wisconsin Twin Project. *Twin Res Hum Genet.* 2019;22(4):233-239. <https://doi.org/10.1017/thg.2019.55>
173. Goldsmith HH, Lemery-Chalfant K, Schmidt NL, Arneson CL, Schmidt CK. Longitudinal analyses of affect, temperament, and childhood psychopathology. *Twin Res Hum Genet.* 2007;10(1):118-126. <https://doi.org/10.1375/twin.10.1.118>

174. Planalp EM, Goldsmith HH. Observed profiles of infant temperament: stability, heritability, and associations with parenting. *Child Dev.* 2020;91(3):e563-e580. <https://doi.org/10.1111/cdev.13277>
175. Zugman A, Harrewijn A, Cardinale EM, *et al.* Mega-analysis methods in ENIGMA: the experience of the Generalized Anxiety Disorder Working Group. *Hum Brain Mapp.* 2022;43(1):255-277. <https://doi.org/10.1002/hbm.25096>
176. Gorgolewski KJ, Auer T, Calhoun VD, *et al.* The brain imaging data structure, a format for organizing and describing outputs of neuroimaging experiments. *Sci Data.* 2016;3. <https://doi.org/10.1038/sdata.2016.44>
177. Esteban O, Birman D, Schaer M, Koyejo OO, Poldrack RA, Gorgolewski KJ. MRIQC: advancing the automatic prediction of image quality in MRI from unseen sites. *PLoS One.* 2017;12(9):e0184661. <https://doi.org/10.1371/journal.pone.0184661>
178. Fischl B, Salat DH, Busa E, *et al.* Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron.* 2002;33(3):341-355. [https://doi.org/10.1016/s0896-6273\(02\)00569-x](https://doi.org/10.1016/s0896-6273(02)00569-x)
179. Winkler AM, Sabuncu MR, Yeo BTT, *et al.* Measuring and comparing brain cortical surface area and other areal quantities. *Neuroimage.* 2012;61(4):1428-1443. <https://doi.org/10.1016/j.neuroimage.2012.03.026>
180. Pesarin F, Salmaso L. *Permutation Tests for Complex Data: Theory, Applications and Software.* Wiley; 2010.
181. Winkler AM, Ridgway GR, Douaud G, Nichols TE, Smith SM. Faster permutation inference in brain imaging. *Neuroimage.* 2016;141:502-516. <https://doi.org/10.1016/j.neuroimage.2016.05.068>
182. Westfall PH, Young SS. *Resampling-Based Multiple Testing: Examples and Methods for p-Value Adjustment.* Wiley; 1993.
183. Desikan RS, Ségonne F, Fischl B, *et al.* An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage.* 2006;31(3):968-980. <https://doi.org/10.1016/j.neuroimage.2006.01.021>
184. Winkler AM, Webster MA, Brooks JC, Tracey I, Smith SM, Nichols TE. Non-parametric combination and related permutation tests for neuroimaging. *Hum Brain Mapp.* 2016;37(4):1486-1511. <https://doi.org/10.1002/hbm.23115>
185. Larivière S, Paquola C, Park B yong, *et al.* The ENIGMA Toolbox: multiscale neural contextualization of multisite neuroimaging datasets. *Nat Methods.* 2021;18(7):698-700. <https://doi.org/10.1038/s41592-021-01186-4>
186. Teng F, Wang M, Lu Z, *et al.* Causal relationship between cortical structural changes and onset of anxiety disorder: evidence from Mendelian randomization. *Cereb Cortex.* 2024;34(11). <https://doi.org/10.1093/cercor/bhae440>
187. Brühl AB, Hänggi J, Baur V, *et al.* Increased cortical thickness in a frontoparietal network in social anxiety disorder. *Hum Brain Mapp.* 2014;35(7):2966-2977. <https://doi.org/10.1002/hbm.22378>
188. Zhao K, Liu H, Yan R, *et al.* Cortical thickness and subcortical structure volume abnormalities in patients with major depression with and without anxious symptoms. *Brain Behav.* 2017;7(8):e00754. <https://doi.org/10.1002/brb3.754>
189. Sylvester CM, Corbetta M, Raichle ME, *et al.* Functional network dysfunction in anxiety and anxiety disorders. *Trends Neurosci.* 2012;35(9):527-535. <https://doi.org/10.1016/j.tins.2012.04.012>
190. Bas-Hoogendam JM, van Steenbergen H, Cohen Kadosh K, Westenberg PM, van der Wee NJA. Intrinsic functional connectivity in families genetically enriched for social anxiety disorder—an endophenotype study. *EBioMedicine.* 2021;69. <https://doi.org/10.1016/j.ebiom.2021.103445>
191. He Y, Xu T, Zhang W, Zuo XN. Lifespan anxiety is reflected in human amygdala cortical connectivity. *Hum Brain Mapp.* 2016;37(3):1178-1193. <https://doi.org/10.1002/hbm.23094>
192. Liao W, Chen H, Feng Y, *et al.* Selective aberrant functional connectivity of resting state networks in social anxiety disorder. *Neuroimage.* 2010;52(4):1549-1558. <https://doi.org/10.1016/j.neuroimage.2010.05.010>
193. De la Peña-Arteaga V, Chavarría-Elizondo P, Juaneda-Seguí A, *et al.* Trait anxiety is associated with attentional brain networks. *Eur Neuropsychopharmacol.* 2024;83:19-26. <https://doi.org/10.1016/j.euroneuro.2024.02.013>
194. Sylvester CM, Smyser CD, Smyser T, *et al.* Cortical functional connectivity evident after birth and behavioral inhibition at age 2. *Am J Psychiatry.* 2018;175(2):180-187. <https://doi.org/10.1176/appi.ajp.2017.17010018>
195. Filippi CA, Winkler AM, Kanel D, *et al.* Neural correlates of novelty-evoked distress in 4-month-old infants: a synthetic cohort study. *Biol Psychiatry Cogn Neurosci Neuroimaging.* Published online April 18, 2024. <https://doi.org/10.1016/j.bpsc.2024.03.008>
196. Zhong S, Lin J, Zhang L, *et al.* Neural correlates of harm avoidance: a multimodal meta-analysis of brain structural and resting-state functional neuroimaging studies. *Transl Psychiatry.* 2024;14(1). <https://doi.org/10.1038/s41398-024-03091-8>
197. Jahanshad N, Lenzini P, Bijsterbosch J. Current best practices and future opportunities for reproducible findings using large-scale neuroimaging in psychiatry. *Neuropsychopharmacology.* Published online 2024. <https://doi.org/10.1038/s41386-024-01938-8>
198. Scheel AM, Schijen MRMJ, Lakens D. An excess of positive results: comparing the standard psychology literature with registered reports. *Adv Methods Pract Psychol Sci.* 2021;4(2). <https://doi.org/10.1177/25152459211007467>
199. Stein DJ, van Honk J, Thompson PM. The importance and limitations of null findings. *Am J Psychiatry.* 2024;181(12):1033-1035. <https://doi.org/10.1176/appi.ajp.20240957>
200. Bas-Hoogendam JM. Genetic vulnerability to social anxiety disorder. In: Blackford JU, Milad MR, eds. *Current Topics in Behavioral Neurosciences.* Springer; 2024. https://doi.org/10.1007/7854_2024_544
201. Xu B, Wang H, Dall'Aglio L, *et al.* Beyond out-of-sample: robust and generalizable multivariate neuroanatomical patterns of psychiatric problems in youth. *Mol Psychiatry.* Published online 2024. <https://doi.org/10.1038/s41380-024-02855-4>
202. Casey BJ, Cannonier T, Conley MI, *et al.* The Adolescent Brain Cognitive Development (ABCD) Study: imaging acquisition across 21 sites. *Dev Cogn Neurosci.* 2018;32:43-54. <https://doi.org/10.1016/j.dcn.2018.03.001>
203. Ide JS, Li HT, Chen Y, *et al.* Gray matter volumetric correlates of behavioral activation and inhibition system traits in children: an exploratory voxel-based morphometry study of the ABCD project data. *Neuroimage.* 2020;220. <https://doi.org/10.1016/j.neuroimage.2020.117085>
204. Hammoud RA, Ammar LA, McCall SJ, Shamseddeen W, Elbejjani M. Brain volumes, behavioral inhibition, and anxiety disorders in children: results from the Adolescent Brain Cognitive Development Study. *BMC Psychiatry.* 2024;24(1):257. <https://doi.org/10.1186/s12888-024-05725-z>
205. Fleece H, Teghli H. Behavioral inhibition and social competence through the eyes of parent and teacher informants. *Behav Sci.* 2024;14(11). <https://doi.org/10.3390/bs14111080>
206. Mills KL, Siegmund KD, Tamnes CK, *et al.* Inter-individual variability in structural brain development from late childhood to young adulthood. *Neuroimage.* 2021;242:118450. <https://doi.org/10.1016/j.neuroimage.2021.118450>
207. Lorenzo NE, Novick DR, Seddio K, *et al.* Bidirectional and interactive effects of child temperament and parenting in early childhood on the trajectory of social anxiety in adolescence. *Depress Anxiety.* 2022;39(3):192-200. <https://doi.org/10.1002/da.23224>
208. Phillips EM, Brock RL. Interplay between interparental relationship quality and infant temperament predicts toddler transdiagnostic psychopathology. *J Fam Psychol.* Published online August 5, 2024. <https://doi.org/10.1037/fam0001263>
209. Verhagen M, Derks M, Roelofs K, Maciejewski D. Behavioral inhibition, negative parenting, and social withdrawal: longitudinal associations with loneliness during early, middle, and late adolescence. *Child Dev.* 2023;94(2):512-528. <https://doi.org/10.1111/cdev.13874>