Structural brain correlates of childhood inhibited temperament: an ENIGMA-anxiety mega-analysis

Bas-Hoogendam, J.M.; Bernstein, R.A.; Benson, B.E.; Frank, S.E.C.; Buss, K.A.; Gunther, K.E.; ... ; Pine, D.S.

META-ANALYSIS

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[j], 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 Tang[n], 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. Clauss[t,ee], PhD, MD, James M. Bjork[ff], PhD, John M. Hettema[gg], MD, PhD, Ashlee A. Moore[hh], PhD, Roxann Roberson-Nay[ii], 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. Thompson[nn], 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 ($p_{MC\text{-}FWER} = .037$) and thickness of the right superior parietal region ($p_{MC\text{-}FWER} = .029$) 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.

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

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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 IT[34-36] 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 sample[33] (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 vlPFC, 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 dlPFC cluster in children with high cIT; no differences in amygdala, vlPFC, 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 η^2 = 0.26) and subcallosal gyrus (small effect size, partial η^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 activitiy 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**
						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.
Vanderbilt—children	n = 55	None	—	—	n = 37[78]	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**
				both left and right amygdalae (VBM) (no effect size reported). Furthermore, inhibited adults had larger caudate volume (left; no effect size reported).		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.
Virginia Commonwealth University Juvenile Anxiety Study (VCU-JAS)	n = 129	None	—	—	None	—
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.*

[a]*Sample 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 T_1-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 association[16]). 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 volume[42]), caudal and rostral ACC (thinner cortex[52]), insula (increased cortical thickness[35]), and OFC (increased cortical thickness, especially in right OFC[51]) 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 science[102]: 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.[103] 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 T_1-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 adulthood[113-115] 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 2[118-174] 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 ± 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
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 ± 1.0)	9.2-13.2 y (10.8 ± 1.0)	BIQ, parent rated; 34-165 (95.3 ± 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 ± 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 ± 1.7)	5.8-13.0 y (9.8 ± 1.7)	EATQ-R-shyness scale; 1.0-5.0 (2.7 ± 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 ± 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 ± 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 ± 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 ± 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 ± 0.6)	34.7-44.2 mo (37.4 ± 1.4)	Standard laboratory observations: stranger approach and jumping spider episode from the Lab-TAB; −1.16 to 1.36 (−0.01 ± 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 ± 0.5)	4.5-11.8 mo (6.7 ± 1.1)	Infant Behavior Questionnaire —Revised (IBQ-r) —fear subscale; maternal report; 0.0-1.8 (0.4 ± 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 *et al.*[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 ± 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
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 ± 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 ± 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 ± 1.8)	8.1-12.8 y (10.5 ± 1.6)	BIQ-child rated; 47-171 (114.5 ± 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 ± 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 ± 0.9)	2.9-4.0 y (3.4 ± 0.3)	Lab-TAB: 3 Kagan-like tasks around age 3 (log-transformed sum-score); 0.2-1.4 (0.7 ± 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 ± 3.3)	1.9-2.7 y (2.2 ± 0.2)	Standard laboratory observations at age 2 y (composite score of stranger, robot, tunnel episodes); −1.0 to 1.2 (0.0 ± 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 ± 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 ± 1.1)	8-12 y (9.3 ± 1.1)	BIQ-child rated; 33-181 (111.4 ± 31.7); cross-sectional measure.	3	NA	NA	93-136 (115.7 ± 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.

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

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

[c]Information 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

[a]*Data 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.*

[b]*Subgroup 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.*

[c]*Data 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		Sex		Sex		Sex		Sex
	Age^2		Age^2		Age^2		Age^2		Age^2
	Sex by age		Sex by age		Sex by age		Sex by age		Sex by age
	Sex by age^2		Sex by age^2		Sex by age^2		Sex by age^2		Sex by age^2
	cIT by age		cIT by age		cIT by age		cIT by age		cIT by age
	cIT by sex		cIT by sex		cIT by sex		cIT by sex		cIT by sex
	cIT by age^2		cIT by age^2		cIT by age^2		cIT by age^2		cIT by age^2
	cIT by sex by age		cIT by sex by age		cIT by sex by age		cIT by sex by age		cIT by sex by age
	cIT by sex by age^2		cIT by sex by age^2		cIT by sex by age^2		cIT by sex by age^2		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.*

[a]*Age representes age at timepoint of magnetic resonance imaging scan.*

[b]*Linear 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 parcellation[183]), 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 ($p_{\text{MC-FWER}} = .020$). Findings at M-FWER corrected significance level (for both main and exploratory analyses) are reported in Supplement 3, available online.

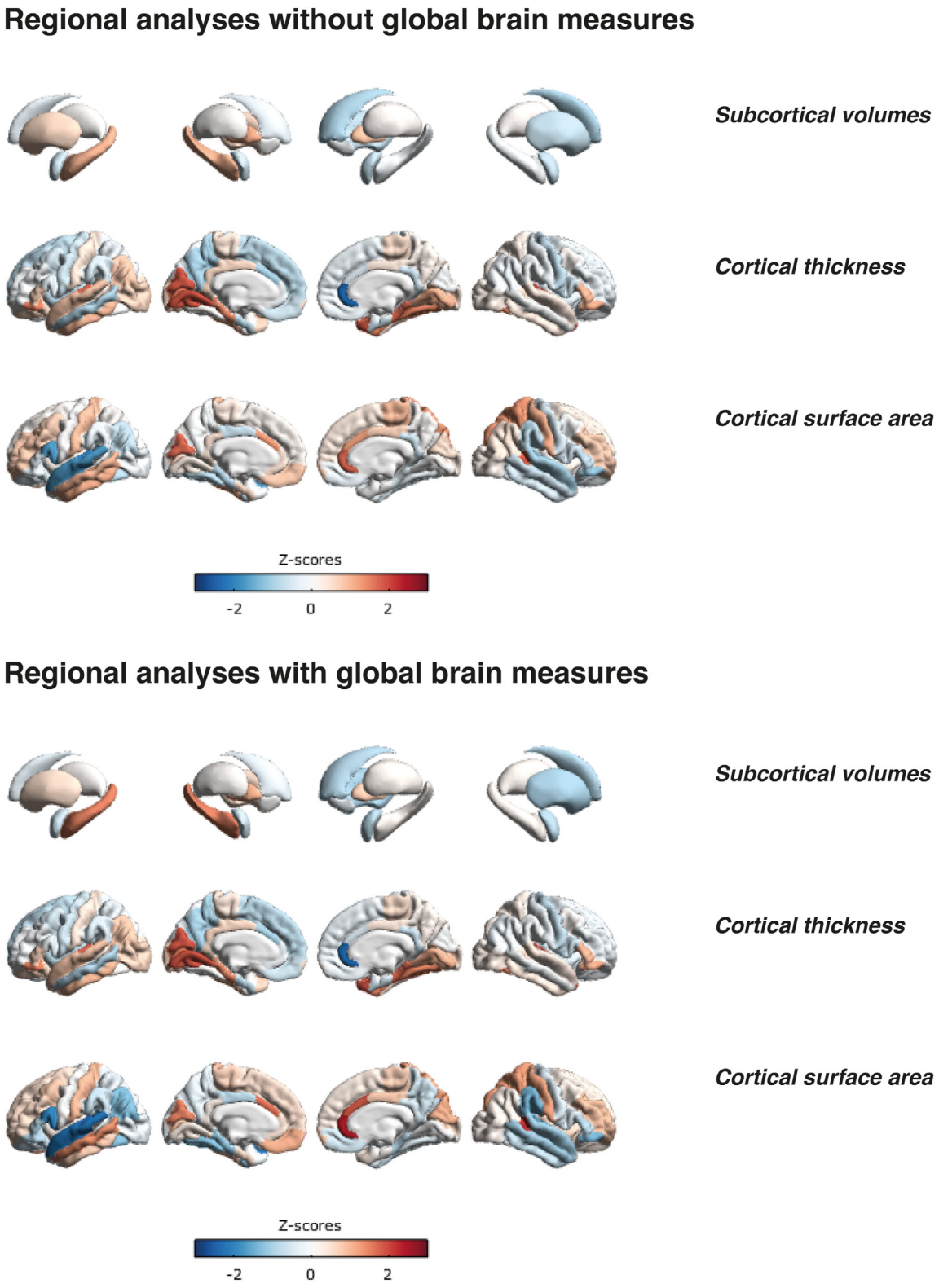
The sensitivity analyses in the second subgroup (1,879 participants for whom the level of cIT was established based on parent or teacher reports during childhood) revealed significant negative cIT by sex interactions with respect to global mean CT ($p_{\text{MC-FWER}} = .037$) and CT of the right superior parietal region ($p_{\text{MC-FWER}} = .029$), 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 ($p_{\text{MC-FWER}} = .006$ and $p_{\text{MC-FWER}} = .003$, 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 ($p_{\text{MC\_FWER}} = .106$ and $p_{\text{MC\_FWER}} = .136$, 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



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-naive 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; $p_{\text{MC-FWER}} = .036$, 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 SAD[109] 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 smaller[197] (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 findings[199]).

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 Study[202] 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 Teglasi [2024] outlining how informant discrepancies, for example between parents' and teachers' ratings of cIT in children, emphasize the transactional nature of inhibited behavior[205]).

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 Group[114]). 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 quality[208]; 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.

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[a]Leiden University, Leiden, the Netherlands; [b]Leiden University Medical Center, Leiden, the Netherlands; [c]Leiden Institute for Brain and Cognition, Leiden, the Netherlands; [d]National Institute of Mental Health, Bethesda, Maryland; [e]The Pennsylvania State University, University Park, Pennsylvania; [f]University of Maryland, College Park, Maryland; [g]Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil; [h]National Institute of Developmental Psychiatry, São Paulo, Brazil; [i]Child Mind Institute, New York, New York; [j]Federal University of São Paulo, São Paulo, Brazil; [k]The Catholic University of America, Washington, DC; [l]New York University Grossman School of Medicine, New York, New York; [m]University of Waterloo, Waterloo, Ontario, Canada; [n]University of Texas at Dellas, Richardson, Texas; [o]Tulane University, New Orleans, Louisiana; [p]Erasmus University Rotterdam, Rotterdam, the Netherlands; [q]Erasmus University Medical Center, Rotterdam, the Netherlands; [r]UCL, London, United Kingdom; [s]Universidad San Sebastián, Santiago de Chile, Chile; [t]Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts; [u]Tufts University, Medford, Massachusetts; [v]Radboud University, Behavioural Science Institute, Nijmegen, the Netherlands; [w]University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; [x]University of Toronto, Toronto, Ontario, Canada; [y]Centre for Addiction and Mental Health, Toronto, Ontario, Canada; [z]University 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; [ii]University 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; [nn]University 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

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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.

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*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

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