Association between Stress and Frontotemporal Regions in 9-12-Year-Old Children: Evidence from Multimodal Neuroimaging

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Abstract

This study is the first to employ an approach integrating multimodal data with machine learning methods to investigate the neural correlates of stress in 78 school-age children (39 females, mean age 10.18 years). Results demonstrated that children's stress levels were significantly positively correlated with gray matter volume in the medial orbitofrontal cortex, insula, superior temporal gyrus, and supplementary motor area, while being significantly negatively correlated with functional connectivity strength between the insula and inferior parietal lobule. This suggests that prefrontal-limbic-temporal brain regions involved in emotional processing may play a key role in individual differences in children' s stress, while increased functional synchronization between the insula and inferior parietal lobule—responsible for integrating internal and external information (e.g., positive self-evaluation and external negative stimuli)—is closely associated with reduced stress in children. Prediction analysis based on structural networks revealed that sensorimotor, frontoparietal, salience, visual, and cerebellar networks exhibited good predictive power for children' s stress levels. The study not only enriches empirical evidence regarding the neural basis of childhood stress but also has important implications for early prevention strategies and intervention approaches for childhood stress.

Full Text

The Association Between Stress and Frontotemporal Regions in Children Aged 9-12: Evidence from Multimodal Brain Imaging

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Abstract

This study examined, for the first time, the neural correlates of stress in 78 school-age children (39 females, mean age = 10.18 years) using a multimodal approach combined with machine learning. Results revealed that children' s stress levels were significantly positively correlated with gray matter volume in the medial orbitofrontal cortex, insula, superior temporal gyrus, and supplementary motor area, while being significantly negatively correlated with functional connectivity strength between the insula and inferior parietal lobule. These findings suggest that prefrontal-limbic-temporal brain regions involved in emotional processing may play a key role in individual differences in childhood stress, whereas increased functional synchrony between the insula (which integrates internal and external information such as positive self-evaluation and external negative stimuli) and the inferior parietal lobule is closely associated with reduced stress in children. Structural network-based predictive analysis demonstrated that sensorimotor, frontoparietal, salience, visual, and cerebellar networks exhibited good predictive power for children' s stress levels. This study not only enriches the empirical evidence on the neural basis of childhood stress but also provides insights for early prevention strategies and intervention approaches for childhood stress.

Keywords: stress, children, gray matter volume, resting-state functional connectivity, machine learning, structural network

Introduction

Stress refers to a state of tension that occurs when an individual perceives an imbalance between demands and their ability to meet those demands, typically prompting psychological and physiological responses to adapt to this state [**?**]. While stress is commonly believed to primarily affect adult populations [**?**], school-age children also encounter stressful events, which mainly stem from family life, academic pressures, peer relationships, and life adaptation [**?**, **?**]. As children begin participating in school social activities during the school-age period, their primary contacts shift from parents to teachers and peers, and this more complex external environment increases the incidence of negative events [**?**]. Simultaneously, children' s brain development and psychological functions remain immature, making them less capable of responding appropriately to negative events and more vulnerable to their effects, ultimately leading to stress symptoms [**?**]. More importantly, early-life stress impacts children' s cognitive and emotional development and serves as a robust predictor of lifelong psychiatric illness [**?**, **?**]. As revealed by the stress sensitization model, childhood adversity increases individuals' susceptibility to stressful life events later in life, thereby elevating the risk for mental illness [**?**, **?**]. Although numerous studies have explored the neural mechanisms of stress in adults from a neuroscience perspective, research on the neural basis of childhood stress remains relatively limited. Therefore, identifying the neurobiological markers of childhood stress is a critical issue for understanding its detrimental effects on individuals and provides robust evidence for developing early prevention and intervention strategies for childhood stress [?, ?].

In recent years, an increasing number of voxel-based morphometry (VBM) and resting-state functional MRI (rs-fMRI) studies have revealed relationships between childhood stress and brain structural and functional differences from a neurobiological perspective. VBM technology examines structural changes in brain regions based on morphological metrics and is used to investigate relationships between psychological phenomena and local brain tissue changes, with gray matter volume being one of its commonly used indicators [?, ?]. Existing VBM evidence has primarily focused on individuals with severe traumatic experiences (such as violence and abuse) or clinical patients with stress-related disorders (such as post-traumatic stress disorder [PTSD], anxiety disorders, and depression). Specifically, Lim et al.' s voxel-based meta-analysis demonstrated that in individuals who experienced childhood maltreatment, gray matter abnormalities appeared in the relatively late-developing prefrontal-limbic-temporal regions involved in emotional and cognitive control functions [?]. This finding has been corroborated in patients with PTSD, anxiety disorders, and major depression (see review [?]). Given that extreme stress events occur relatively infrequently in daily life [?], researchers have begun to investigate more common stressful life events to explore the neural basis of general stress levels. For instance, Ringwald et al. used a stressful life events scale to assess individuals' stress levels (e.g., job changes, financial crises) and found that higher stress levels in adults were associated with smaller gray matter volume in the medial orbitofrontal cortex (mOFC) [?]. A comparative study examining age-related differences in gray matter volume associated with stress further revealed that stress levels were negatively correlated with gray matter volume in the OFC, insula, and amygdala in adults, but positively correlated in adolescents [?], providing partial empirical support for investigating the relationship between general life stress and brain structure in children.

Rs-fMRI can measure spontaneous neural activity changes in the brain at rest [?] and, compared to task-based fMRI, its results are independent of experimental tasks. Functional connectivity (FC) is a commonly used effective indicator in rs-fMRI research, reflecting the coordination between brain regions and its relationship with specific psychological and behavioral functions [?]. This technique has yielded certain achievements in stress-related research, with numerous studies indicating that childhood traumatic stress is associated with altered connectivity strength between brain regions, including the OFC, hippocampus, amygdala, and insula [?, ?, ?]. Thomason et al. demonstrated that in high-trauma subjects aged 9-15, FC strength in the insula-amygdala and OFC-insula was significantly higher than in control groups [?]. For adolescents aged 15-17, higher exposure to childhood violence was associated with weaker connectivity between the insula and inferior parietal lobule (IPL) [?]. Meanwhile, task-based fMRI studies have shown that childhood stress is associated with abnormal activation in the aforementioned brain regions. Compared to control groups, children with PTSD exhibited abnormal activation in the prefrontal cortex, insula, and superior temporal gyrus (STG) during emotional processing tasks (e.g., emotion recognition and implicit emotion tasks), along with faster recognition of fearful expressions and lower accuracy in color judgment of negative words [?, ?].

In summary, although numerous studies have explored the relationship between stress and brain structure and function, only a few have focused on the neural correlates of childhood stress, and even fewer have directly examined the relationship between daily life stressors in children and neural structure and function. Most studies have focused on clinical children meeting PTSD diagnostic criteria [?] or children who have experienced severe traumatic events (e.g., sexual abuse, physical abuse, and caregiver abandonment) [?, ?]. Such severe stress events occur infrequently in daily life and do not encompass stressors from family life, academic life, peer relationships, and life adaptation, making it difficult to generalize findings to the general child population. Therefore, this study employed the Scale of Stressful Life Events for Primary School Students, which comprehensively covers daily stressors in children, to measure childhood stress levels [?]. Second, current neuroimaging evidence on childhood stress is largely based on single-modality analytical approaches, with few studies using multimodal data to investigate the neural basis of childhood stress. Researchers have noted that brain structural and functional networks develop synchronously, with structural networks providing an internal physiological framework for the development and integration of functional networks; therefore, both structural and functional modalities should be considered when examining the potential harm of adverse psychological phenomena on individual development [?]. For example, Späti et al. (2015) demonstrated through analyses of cortical thickness and FC in patients with major depressive disorder that prefrontal cortical thinning during depressive episodes may impair anterior cingulate cortex neural activity [?]. Based on this, this study combined structural MRI (sMRI) and rs-fMRI to explore potential neural correlates of childhood stress. First, gray matter volume was used as a VBM indicator to analyze brain regions significantly correlated with childhood stress levels. Subsequently, these brain regions were used as regions of interest (ROIs) to estimate FC significantly associated with childhood stress. Given that the hippocampus and amygdala are important brain regions for childhood stress [?, ?], FC between these regions and the whole brain was also examined. Based on previous research, we hypothesized that childhood stress levels would be primarily associated with gray matter volume and functional connectivity in prefrontal-limbic-temporal brain regions such as the OFC, insula, hippocampus, and amygdala. In addition to the aforementioned node-level analyses, this study also employed machine learning methods to predict individual differences in childhood stress at the network level, thereby providing robust support for the brain-stress relationship. In summary, this study examined the neural basis of childhood stress from a multimodal perspective and provided more targeted evidence for intervention strategies for daily stress in children and treatment protocols for traumatic stress [**?**, **?**].

2.1 Participants

Participants in this study were recruited from two public elementary schools in southwestern China, with a total of 139 elementary school students initially recruited. All participants were right-handed and, according to self-reports and school records, were in good mental health with no history of psychiatric or neurological disorders or use of psychotropic medications. Examination of school records was conducted after obtaining consent from participants and their parents. A total of 139 valid questionnaires were collected, of which 130 participants underwent rs-MRI scanning between April 2018 and October 2018. After excluding participants with poor data quality or excessive head motion (see Section 2.3.3), 78 participants (39 females, mean age = 10.18 ± 1.02 years) were included in the final analysis. All participants and their parents signed informed consent forms prior to the experiment. Each participant received stationery as a reward after completing the experiment, and parents were provided with a project indicator test report. All experimental procedures were approved by the Southwest University Academic Ethics Committee.

2.2 Measures

2.2.1 Scale of Stressful Life Events for Primary School Students We used the Scale of Stressful Life Events for Primary School Students (SSLEPSS) developed by Liu Shudan et al. (2016) for Chinese elementary school students. The scale comprises five dimensions with 30 items total. The family life stress dimension includes 9 items (e.g., "parents arguing or fighting"), teacher-student relationship stress includes 5 items (e.g., "teacher severely criticized me"), academic adaptation stress includes 5 items (e.g., "cannot do homework"), peer relationship stress includes 7 items (e.g., "had a disagreement with a good friend"), and life adaptation stress includes 4 items (e.g., "cannot fall asleep at noon or night"). Participants first reported whether each stressful event occurred in the past six months ("yes" or "no"), then rated its impact on a 5-point scale, where 1 indicated "no impact" and 5 indicated "extremely upset." Higher total scores represent higher stress levels. The scale includes both traumatic stress events with substantial impact on children (e.g., "parental separation or divorce") and daily stressors with potential impact (e.g., "forced to sleep"), enabling more comprehensive measurement of childhood stress. In this study, the scale' s Cronbach' s α coefficient was 0.92.

2.2.2 Positive and Negative Affect Schedule We used the Positive and Negative Affect Schedule (PANAS) developed by Watson et al. (1988). The scale includes positive and negative affect subscales rated on a 5-point Likert scale from 1 ("not at all") to 5 ("extremely"), with higher scores indicating stronger experience of positive or negative emotions. In this study, Cronbach' s

α coefficients were 0.79 for the positive affect subscale and 0.75 for the negative affect subscale. Only the negative affect subscale scores were used in this study.

2.3 Imaging Data Acquisition and Preprocessing

2.3.1 Data Acquisition All participants completed a 5-minute structural scan and an 8-minute resting-state functional scan. All imaging data were acquired using a 3.0 T Siemens Trio MRI scanner at the Southwest University Brain Imaging Center. Before formal scanning, each participant practiced with a mock scan to adapt to the scanning environment and reduce head motion. Prior to formal scanning, participants' heads were adjusted to a comfortable position and secured with foam padding. During scanning, participants were instructed to keep their eyes open, lie flat and rest, and avoid thinking or falling asleep. T1-weighted structural images were acquired using magnetization-prepared rapid acquisition gradient echo (MPRAGE) sequences with the following parameters: echo time (TE) = 3.48 ms, repetition time (TR) = 2530 ms, inversion time (TI) = 1900 ms, flip angle (FA) = 7°, field of view (FOV) = 256 × 256 mm^2, matrix size = 256 × 256, slice distance = 1.0 mm, voxel size = 1 × 1 × 1 mm^3. Resting-state functional images were acquired using gradient echo-echo planar imaging (GRE-EPI) sequences with parameters: TE = 30 ms, TR = 2000 ms, FA = 90°, FOV = 224 × 224 mm^2, matrix size = 64 × 64, number of slices = 33, thickness = 3.5 mm, distance between slices = 1 mm, voxel size = 3.5 × 3.5 × 3.5 mm^3. A total of 180 continuous images were obtained.

2.3.2 Preprocessing of Structural and Resting-State Data Structural MRI data were preprocessed using the CAT12 toolbox (http://www.neuro.uni-jena.de/cat12-html/cat.html) running on MATLAB R2014a with SPM 12 (http://www.fil.ion.ucl.ac.uk/spm/). Preprocessing steps included: (1) exclusion of brain images with poor quality or anatomical abnormalities; (2) tissue segmentation, dividing T1-weighted images into gray matter, white matter, and cerebrospinal fluid; (3) registration to MNI (Montreal Neurological Institute) space using DARTEL (diffeomorphic anatomical registration through exponentiated Lie algebra) with nonlinear modulation using Jacobian determinants, resulting in normalized voxel size of 1.5 × 1.5 × 1.5 mm^3; (4) smoothing with an 8 mm kernel.

Resting-state MRI data were preprocessed using DPARSF 5.2 software (http://www.restfmri.net/forum/DPARSF) running on MATLAB R2014a [?]. Preprocessing steps included: (1) data quality inspection; (2) removal of the first 10 time points followed by slice timing correction for remaining slices; (3) head motion realignment; (4) normalization to MNI standard space template using DARTEL, resulting in normalized voxel size of 3 × 3 × 3 mm^3; (5) smoothing with a 6 mm kernel; (6) regression of head motion signals using the Friston 24-parameter model and white matter/cerebrospinal fluid signals, with all participants showing mean framewise displacement (mean FD) less than 0.5 mm; (7) extraction of low-frequency signals in the 0.01–0.1 Hz band.

2.3.3 Data Quality Control and Head Motion Before preprocessing, two doctoral students in psychology conducted initial visual screening of structural data for all 139 participants (rating data quality subjectively four times total), excluding 49 participants with structural abnormalities or substantial artifacts (15 females). Next, based on widely used resting-state head motion criteria, head motion control was applied to the remaining 81 participants, excluding those with mean FD_{Jenkinson} values $\geq$ 0.5 mm [**?**]. This study further excluded three participants with stress scores of 0, resulting in 78 participants (39 females) included in the final statistical analysis. Given that head motion during scanning may potentially affect the neural correlates of variables of interest [**?**], we ensured that variables of interest were not significantly correlated with head motion indices. Current data showed no significant correlation between head motion (mean FD) and stress scores (correlation between head motion and raw stress scores: $r = 0.04$, $p = 0.71$; correlation between head motion and square-root transformed stress scores [stress_{sqrt}; see Section 3.1]: $r = 0.09$, $p = 0.46$; see Table 1). Finally, head motion was included as a covariate in statistical analyses to further control for its effects [**?**, **?**].

2.3.4 Structural Network Construction Using diffusion tensor imaging to construct individual-level networks may reconstruct spurious connections that do not exist [**?**, **?**], while using T1 images to construct group-level structural covariance networks ignores both individual differences and differences in brain region shape and size. Given these limitations, this study constructed individual-level structural brain networks based on Shen et al.' s (2013) template, using Kullback-Leibler divergence-based similarity (KLS) to quantify structural connectivity values between brain regions as edge weights in the brain network. This method can estimate relationships between brain regions of different shapes and sizes and represents a reliable approach for characterizing brain organization [**?**]. First, gray matter volume values for 268 brain regions in the brain template were extracted. Second, kernel density estimation (KDE) was used to calculate the probability density function of gray matter volume for each brain region, from which the probability distribution function of gray matter volume values for each region was obtained [**?**]. Subsequently, differences between probability distribution functions for each pair of brain regions were calculated as KL divergence values, which served as structural connectivity values. Finally, a 268 × 268 structural matrix was obtained. To interpret results at the network level, we used Noble et al.' s (2017) brain network parcellation, which includes eight networks: medial frontal network (MFN), frontoparietal network (FPN), default mode network (DMN), sensorimotor network (SMN), salience network (SAN), visual network (VN, including visual areas 1, 2, and visual association cortex), subcortical network (SCN), and cerebellar network.

2.4 Statistical Analysis

2.4.1 Whole-Brain Analysis **Gray Matter Volume Analysis.** At the whole-brain level, SPM 12 software was used to conduct multiple linear regression analysis of the relationship between childhood stress levels and gray matter volume, with age, sex, and total intracranial volume (TIV) as covariates. Gaussian Random Field (GRF) multiple comparison correction was applied at voxel-level $p < 0.005$ and cluster-level $p < 0.05$ to identify brain regions showing significant correlations between gray matter volume and childhood stress.

Seed-Based FC Analysis. First, peak coordinates from brain regions showing significant correlations with stress in the gray matter volume analysis were used to create 5 mm radius spherical ROIs. Additionally, the amygdala and hippocampus from the Automated Anatomical Labeling (AAL) atlas were selected as ROIs due to their importance in childhood stress [**?**, **?**]. Second, time series from voxels within each ROI were extracted for each participant, and voxel-wise correlation analysis was used to calculate Pearson correlation coefficients (r) between each ROI and all other brain voxels, with r values transformed using Fisher' s z-transformation. Finally, DPABI software was used to calculate correlations between childhood stress levels and functional connectivity strength. Given that age and sex have been shown to relate to FC [**?**, **?**], and that head motion during MRI scanning is a major factor affecting FC [**?**, **?**], this study regressed out the effects of age and sex (as in [**?**]) while also controlling for head motion. GRF multiple comparison correction (voxel-level $p < 0.005$, cluster-level $p < 0.05$) was applied to identify FC significantly associated with childhood stress.

2.4.2 Machine Learning Predictive Model Analysis We used relevance vector regression (RVR) to predict childhood stress levels. Each participant' s structural network served as feature variables to build an RVR predictive model for childhood stress. RVR is a sparse kernel-based multivariate regression machine learning method based on a probabilistic Bayesian framework. The function expression is: . The model weights use an explicit zero-mean Gaussian prior process, with most weights set to zero and only a subset of samples used to train the model. These samples are called "relevance vectors" and are used to fit the prediction model. Subsequently, maximum likelihood estimation was used to determine model parameters β. The regression coefficients represent weighted sums of feature vectors from "relevance vector" samples [**?**].

Leave-one-out cross-validation (LOOCV) was used to establish the RVR predictive model. The specific procedure was as follows: First, the total sample was randomly divided into training and test sets, with one data sample (i.e., N–1 participants) randomly selected as the training set to build the RVR prediction model, and the remaining sample (1 participant) as the test set to evaluate the model' s predictive ability. Next, the correlation coefficient r(predicted, observed) between observed and predicted values in the test set was calculated. Finally, permutation testing was conducted by repeating the above steps 2000 times to obtain a null distribution of 2000 r values, and the significance p-value of r(predicted, observed) was assessed based on its position in this null distribution. The p-value equals the number of permuted r values greater than or equal to r(predicted, observed) divided by the total number of permutations.

The absolute value of feature vector weights was used to measure each feature' s contribution to the predictive model, with larger absolute values indicating greater contribution. This study selected brain regions in the top 10% of contribution rates. Additionally, weights were transformed into "activation patterns" using the formula $A = \mathrm{cov}(X) \times W \times \mathrm{cov}(S)^{-1}$ to clarify correlations between brain regions and predicted behavioral variables [**?**, **?**], where positive networks positively correlate with predicted variables and negative networks negatively correlate.

2.5 Exploratory Analyses

2.5.1 Sex Differences in Stress-Brain Relationships At the whole-brain level, SPM 12 software was used to construct a multiple linear regression model with sex, stress level (stress_{sqrt}), and their interaction term to analyze whether sex and stress level showed significant interactions on gray matter volume, with age and TIV as covariates. GRF multiple comparison correction (voxel-level $p < 0.005$, cluster-level $p < 0.05$) was applied. Subsequently, signal values from significant gray matter brain regions were extracted, and SPSS 26.0 was used to calculate correlation coefficients between childhood stress levels (stress_{sqrt}) and corresponding brain regions separately for male and female groups to further examine whether significant stress-brain correlations differed by sex. If significant brain regions were identified, 5 mm radius spheres centered on peak coordinates were created as seed points for functional connectivity analysis, and multiple linear regression models were similarly used to analyze sex-by-stress interactions on FC in these regions.

2.5.2 Age Characteristics of Stress-Brain Relationships At the whole-brain level, SPM 12 software was used to construct a multiple linear regression model with age, stress level (stress_{sqrt}), and their interaction term to analyze whether age and stress level showed significant interactions on gray matter volume, with sex and TIV as covariates. GRF multiple comparison correction (voxel-level $p < 0.005$, cluster-level $p < 0.05$) was applied. Subsequently, signal values from significant gray matter brain regions were extracted, and SPSS 26.0 was used to calculate partial correlation coefficients between childhood stress levels (stress_{sqrt}) and corresponding brain regions in different age groups to further examine which age groups showed significant stress-brain correlations. If significant brain regions were identified, 5 mm radius spheres centered on peak coordinates were created as seed points, and multiple linear regression models were similarly used to analyze age-by-stress interactions on FC in these regions.

3.1 Normality Testing and Behavioral Results

Following methods used in previous research [**?**], this study first excluded three participants with total stress scores of 0, resulting in 78 participants included in statistical analysis. Second, given that Pearson correlation calculations between brain metrics and stress require normally distributed data, normality testing (Kolmogorov-Smirnov test, K-S test) was conducted on children' s stress scores, revealing that raw stress scores were non-normally distributed (K-S test, $p < 0.001$; see Figure 1 [Figure 1: see original paper]A). Subsequently, square root transformation was applied to raw stress scores [**?**, **?**] to obtain normally distributed stress scores (stress_{sqrt}) (K-S test, $p = 0.20$; see Figure 1B). All subsequent statistical analyses used stress level (stress_{sqrt}) as the independent variable.

Demographic information and descriptive statistics for childhood stress scores are presented in Table 1 and Figure 1C. Raw stress scores showed no significant sex differences, $t(76) = 1.66$, $p = 0.1$. Raw stress scores were not significantly correlated with age, $r = -0.04$, $p = 0.72$.

Table 1 Descriptive Statistics and Correlation Analysis Results (N = 78)
Note: p < 0.05, ** p < 0.01.*

Figure 1 (A) Histogram of raw stress score distribution; (B) Histogram of transformed stress score distribution; (C) Sample age and sex distribution
Note: K-S test = Kolmogorov-Smirnov normality test.

3.2 Brain-Behavior Correlations

3.2.1 Relationship Between Childhood Stress and Gray Matter Volume Multiple linear regression analysis of children' s stress_{sqrt} scores and whole-brain gray matter volume was conducted with sex, age, and TIV as covariates. Results indicated that children' s stress levels (stress_{sqrt}) were significantly positively correlated with gray matter volume in the left mOFC, right insula, left STG, and right supplementary motor area (SMA). Specific coordinates, cluster sizes, and correlation values are presented in Table 2 and Figure 2 [Figure 2: see original paper]A. Negative affect scores were significantly positively correlated with SMA gray matter volume, $r = 0.25$, $p < 0.05$ (see Supplementary Table 1).

3.2.2 Relationship Between Childhood Stress and Resting-State Functional Connectivity Using significant brain regions from the gray matter volume analysis as ROIs, functional connectivity analysis revealed that FC strength between the right insula and left IPL was significantly negatively correlated with children' s stress levels (stress_{sqrt}); that is, higher stress levels were associated with weaker insula-IPL connectivity. However, no significant correlations were found between stress levels and FC involving the hippocampus or amygdala (Table 2 and Figure 2B).

3.3 Machine Learning Prediction Results

The RVR model based on structural networks marginally predicted childhood stress levels ($r = 0.24$, $p = 0.07$, see Figure 3 [Figure 3: see original paper]).

At the node level, the OFC, STG, and SMA were among the brain regions in the top 10% of contribution rates (see Supplementary Table 2). At the network level, structural connections within the SMN and between SMN-SAN, SMN-VN, and SMN-FPN constituted positive networks, where stronger structural connections predicted higher stress levels. Structural connections between cerebellum-FPN, cerebellum-VN, and SCN-VN constituted negative networks, where weaker structural connections predicted higher stress levels.

Table 2 Results of Correlation Analysis Between Childhood Stress and Gray Matter Volume/Resting-State Functional Connectivity
Note: mOFC = medial orbitofrontal cortex; STG = superior temporal gyrus; SMA = supplementary motor area; IPL = inferior parietal lobule; MNI = Montreal Neurological Institute; GRF multiple comparison correction (voxel-level $p < 0.005$, cluster-level $p < 0.05$); Covariates: sex, age, and head motion.

Figure 2 (A) Gray matter volume results associated with childhood stress; (B) Resting-state functional connectivity results associated with childhood stress
Note: GRF multiple comparison correction (voxel-level $p < 0.005$, cluster-level $p < 0.05$). mOFC = medial orbitofrontal cortex; Insular = insula; STG = superior temporal gyrus; SMA = supplementary motor area; IPL = inferior parietal lobule; L = Left hemisphere; R = Right hemisphere.

Figure 3 (A) Positive and negative networks predicting childhood stress levels; (B) Machine learning prediction results; (C) Permutation test
Note: Circle plot: brain regions presented in anatomical order, connection line length represents distance between connected regions; Brain map: node size represents contribution to model; Matrix plot: numbers represent counts of within- or between-network connections. Feature selection threshold $p < 0.01$, prediction model significance assessed using 2000 permutation tests $p < 0.05$.

3.4 Exploratory Analyses

3.4.1 Sex Differences in Stress-Brain Relationships Multiple regression analysis revealed no significant interaction between sex and stress level (stress_{sqrt}) on whole-brain gray matter volume.

3.4.2 Age Characteristics of Stress-Brain Relationships Multiple regression analysis indicated a significant interaction between age and stress level on gray matter volume in the inferior occipital gyrus (IOG) (coordinates: x = -53, y = -66, z = -11; cluster size = 249; t = -4.08). Signal values from this brain region were extracted to calculate partial correlations with stress levels (stress_{sqrt}) in each age group, with sex and TIV as covariates. Results showed that stress levels were significantly positively correlated with IOG gray matter volume in the 9-year-old group (n = 24), not significantly correlated in the 10-year-old (n = 26) and 11-year-old (n = 18) groups, but significantly negatively correlated in the 12-year-old group (n = 10), as shown in Figure 4 [Figure 4: see original paper]. Additionally, the age-by-stress interaction on

IOG functional connectivity was not significant.

Figure 4 Age characteristics of neural correlates of childhood stress
Note: GRF multiple comparison correction (voxel-level $p < 0.005$, cluster-level $p < 0.05$). GMV = gray matter volume; IOG = inferior occipital gyrus; L = left hemisphere; $p < 0.05$; ** $p < 0.01$.*

Discussion

This study used multimodal data to examine the brain structural and functional correlates of childhood stress and explored sex differences and age characteristics. Consistent with our hypotheses, VBM analysis indicated that childhood stress was positively correlated with gray matter volume in the mOFC, insula, STG, and SMA. Using these significant brain regions from VBM as ROIs for FC analysis, results showed that higher childhood stress levels were associated with weaker connectivity between the insula and IPL. These results were not influenced by participants' age, sex, or head motion/TIV. Machine learning predictive analysis further demonstrated that the aforementioned brain regions could effectively predict childhood stress levels, indicating the robustness of our findings.

VBM analysis revealed that higher childhood stress was associated with larger gray matter volume in the mOFC, insula, STG, and SMA. First, the mOFC has been implicated in emotional processing, such as regulating responses to negative stimuli and evaluating negative emotional states [?, ?, ?]. Empirical studies have shown that poorer emotion regulation abilities are associated with larger OFC gray matter volume [?], and adolescents who tend to report higher stress levels also show larger OFC gray matter volume [?]. Therefore, the significant positive correlation between childhood stress and OFC gray matter volume suggests that abnormalities in emotional processing (e.g., emotion management and regulation) involving the OFC (manifested as increased OFC gray matter volume) may be closely associated with increased childhood stress levels [?, ?]. This neural vulnerability may be related to the immature development of the OFC during childhood [?]. Second, the insula plays an important role in individual stress responses and self-emotional awareness [?, ?, ?]. Multiple task-based studies have found that compared to healthy controls, PTSD patients show stronger insula activation when facing fear and distress stimuli [?, ?, ?]. A recent structural imaging study in adolescents found that higher individual stress levels were associated with larger insula gray matter volume [?]. Therefore, increased insula gray matter volume may represent an important neural substrate of stress, explaining hypersensitivity to negative stimuli in children with high stress levels.

Furthermore, results indicated positive correlations between childhood stress and gray matter volume in the STG and SMA. The STG is involved in episodic memory and speech comprehension [?, ?]. A structural imaging study found that increased STG gray matter volume was associated with parental verbal reprimand during childhood, which represents a source of early-life stress [?]. Compared to control groups, children with PTSD also show larger STG gray matter volume [?]. Therefore, the significant positive correlation between childhood stress and STG gray matter volume may indicate that abnormalities in speech comprehension processing (manifested as increased STG gray matter volume) may contribute to heightened sensitivity to negative speech (e.g., parental reprimand), which is associated with increased childhood stress [?]. Additionally, previous research has shown that the SMA plays a primary role in monitoring erroneous behaviors [?, ?] while also participating in transmitting emotional information [?, ?]. Lim et al. (2015) found that compared to healthy controls and psychiatric groups without childhood stress, adults who experienced childhood stress showed heightened sensitivity to erroneous behaviors (e.g., failure to inhibit responses to target stimuli), manifested as stronger SMA activation. This neural functional change may help individuals monitor their own behavior under stress to avoid negative consequences from erroneous actions [?]. Currently, limited evidence exists regarding the relationship between SMA gray matter volume and childhood stress, though one structural imaging study found that SMA gray matter volume in children with PTSD was positively correlated with PTSD scores [?]. Additionally, research on functional neurological symptom disorder (FND) can provide supporting evidence for this relationship. FND refers to medically unexplained sensory or motor function loss, such as blindness or limb paralysis [?]. A structural imaging study of childhood FND indicated that children with FND resulting from previous stress showed heightened vigilance to emotional signals, manifested as shortened reaction times for emotion recognition, which was associated with increased SMA gray matter volume [?, ?]. Accordingly, we speculate that larger SMA gray matter volume may be related to excessive monitoring of emotional stimuli and individual behavior in children with high stress levels to avoid negative consequences from erroneous actions [?, ?]. Notably, review studies have suggested that due to the plastic nature of brain structure, stress-induced changes in gray matter volume may reflect experience-dependent structural adaptations that temporarily improve individuals' adaptive capacity in stressful environments [?]. For children, maintaining such short-term adaptive functions over the long term may reduce brain plasticity and promote premature brain maturation [?], potentially leading to negative consequences such as anxiety, depression, and PTSD [?, ?]. Additionally, research indicates that increased gray matter volume in children' s prefrontal cortex, STG (including insula), and SMA may serve as a predictive factor for psychiatric illness risk [?]. In summary, the structural brain basis of stress is important for understanding abnormal emotional and cognitive patterns in stress and provides empirical support for revealing neural correlates of childhood stress.

Further analysis revealed that childhood stress levels were negatively correlated with FC strength between the insula and IPL. The IPL is a core node of the DMN [?, ?], a network that operates independently of task processing and is dedicated to self-referential processing and autobiographical memory [?]. The insula is a key region of the SAN, responsible for monitoring and integrating internal and external salient stimuli [?, ?]. Empirical studies have shown that early-life stress is not only significantly associated with FC within the DMN and SAN [?, ?] but also with reduced FC strength between the SAN and DMN, particularly between the insula and IPL [?, ?, ?]. Therefore, our finding of negative correlation between childhood stress and insula-IPL FC suggests that reduced functional synchrony between the two networks (DMN and SAN) anchored by the insula and IPL may reflect abnormal interaction between self-referential processing (e.g., positive self-evaluation) and external stimulus reactivity (e.g., heightened sensitivity to negative stimuli), which plays an important role in elevated childhood stress levels [?, ?, ?]. However, FC analysis using the hippocampus and amygdala as ROIs did not reveal significant correlations with childhood stress levels. A resting-state study in young adults also found no differences in hippocampus and amygdala FC between childhood trauma and control groups [?], though evidence suggests that abnormal FC in these regions is associated with childhood trauma in adolescents [?, ?]. These inconsistent results may be influenced by sample size (i.e., <30 vs. >30 participants; [?, ?]), ROI selection methods for hippocampus/amygdala (i.e., AAL or Jülich templates, or manual segmentation; [?, ?, ?]), and whether skewed data were transformed [?, ?].

Machine learning predictive analysis indicated that structural connections within the SMN and between SMN-VN, SMN-SAN, and SMN-FPN constituted positive networks predicting childhood stress levels, while structural connections between cerebellum-FPN, cerebellum-VN, and SCN-VN constituted negative networks. For positive networks, the SMN and VN belong to sensory systems responsible for sensory processing, motor control, and error behavior monitoring [?, ?, ?]; the SAN monitors and integrates internal and external salient stimuli [?, ?]; and the FPN is primarily involved in executive control functions, flexibly and rapidly adapting to ongoing tasks by altering interaction patterns with other functional systems [?]. Empirical studies have shown that after adults experience stressful events, in addition to changes in structural integrity and gray matter volume in sensory systems [?, ?], abnormal FC emerges between the SMN and VN, SAN, and FPN, which is associated with increased vigilance and difficulty recovering from stressful events [?, ?, ?]. Regarding negative network results, the cerebellum is not only involved in voluntary movement but also in higher cognitive functions such as emotional processing and error prediction [?], while the SCN processes threatening stimuli transmitted from sensory systems (e.g., VN) and rapidly responds to them [?]. Furthermore, higher stress symptoms are associated with abnormal connectivity between cerebellum-FPN, cerebellum-VN, and SCN-VN, suggesting that individuals with high stress exhibit reduced ability to regulate internal and external stimuli, leading to hypervigilance and generalized fear responses [?, ?, ?]. Current results suggest that structural network similarity may be related to individual differences in childhood stress; although its predictive power was marginally significant statistically, it still helps understand the nature and degree of association between stress and brain structure.

Exploratory analyses revealed no sex differences in gray matter volume or FC associated with childhood stress. A prospective study indicated that PTSD symptoms and developmental trajectories in children and adolescents do not differ by sex [?]. At the brain development level, review studies suggest that sex differences in brain development emerge after puberty [?]. A large-sample study including children, adolescents, and adults found no sex differences in hippocampus and amygdala volumes before puberty, but with pubertal maturation, corresponding brain region volumes decreased in males and increased in females [?]. Longitudinal studies show that during adolescence, early-life stress reduces connectivity strength between the amygdala and prefrontal cortex in females but not males [?, ?]. Therefore, the absence of sex differences in childhood stress-brain associations may be due to the lack of sex differences in stress during childhood and sex-specific neural substrates of stress manifesting primarily during adolescence. Recently, researchers conducted longitudinal tracking of sex differences in hormone secretion in children experiencing early-life stress, finding that stressful events experienced at ages 3–6 affected cortisol-testosterone coupling patterns (i.e., synchrony in hormone secretion levels) in girls at age 9 but not in boys [?]. Future research could further explore sex differences in the neurobiological basis of childhood stress from a neuroendocrine perspective. Additionally, age characteristic analysis showed that the significant positive correlation between stress levels and IOG gray matter volume only appeared in the 9-year-old group. The IOG is part of primary visual cortex, and the visual cortex processing and transmitting negative information is a highly plastic structure whose plasticity decreases with puberty onset [?, ?]. Previous studies have shown that stress-related experiences (e.g., witnessing violent abuse or domestic violence) may affect primary visual cortex development [?, ?]. This result may support the view that middle childhood is a period when the nervous system is vulnerable to negative effects of stress [?]. Although the 12-year-old group showed a significant negative correlation between stress levels and IOG gray matter volume, this result may be due to the small sample size in this age group (n = 10). Future research could further examine the critical age points at which stress-brain associations emerge and sex differences using large-sample data from a developmental perspective, and investigate whether neural markers of childhood stress can stably predict future stress levels and negative emotions.

Additionally, substantial evidence supports the close association between childhood stress and anxiety, both characterized by hypervigilance and panic [?]. The current study also found positive correlations between childhood stress levels and negative emotions such as hostility and distress. Meanwhile, children with anxiety disorders show dysregulated stress systems, such as elevated cortisol [?]. At the neural level, anxious children also show abnormalities in prefrontal-limbic-temporal brain structure and function. For instance, VBM studies have found that children with anxiety disorders have significantly larger GMV in the OFC, STG, and insula compared to healthy children [?, ?, ?]. A recent connectome-based predictive modeling study using DTI structural networks found that prefrontal-limbic-temporal structural networks could also predict trait anxiety levels in early adulthood [?]. Furthermore, rs-fMRI studies have found that abnormal FC between these brain regions is associated with childhood anxiety levels. Specifically, FC strength between the limbic system (e.g., insula and amygdala) and salience/executive control networks (e.g., prefrontal cortex) positively correlates with childhood anxiety levels [?] and can significantly predict childhood trait anxiety [?]. Similar to these findings, the neural correlates of childhood stress in this study also focused primarily on prefrontal-limbic-temporal regions, suggesting that the neurobiological basis of childhood stress and anxiety may overlap, which is consistent with the high comorbidity between these conditions [?].

This study' s findings that childhood stress is associated with prefrontal-limbic-temporal brain regions involved in emotional regulation and integration of internal and external stimuli (i.e., integrating positive self-evaluation with external negative emotional stimuli) have practical implications for early prevention and intervention strategies for childhood stress. Substantial evidence has shown that childhood stress leads to abnormal physical and mental development and even psychiatric symptoms, seriously affecting individuals' normal lives [?, ?]. From a behavioral-cognitive perspective, given that emotion regulation training and mindfulness training can effectively improve individuals' emotional regulation of stressors and have been widely applied in adolescent and adult populations [?, ?], future research could employ these psychological training techniques to regulate children' s emotional functions and promote positive self-referential functions to alleviate and prevent the negative effects of stress on children. From a neural perspective, neurobiological approaches such as biofeedback, transcranial direct current stimulation, and deep brain stimulation could also be used to stimulate prefrontal brain regions to intervene in individuals with more severe clinical stress symptoms [?, ?, ?].

In conclusion, identifying neural correlates of the transition from adaptive to maladaptive behaviors helps understand the full dynamic range of neural changes from non-clinical to clinical states. This study expanded empirical research on the neural basis of non-clinical childhood stress from a multimodal perspective and promoted deeper understanding of structural and functional neural characteristics of stress-susceptible children. However, several limitations exist. First, due to the cross-sectional design, this study could only determine correlational relationships between stress and brain structure in children, not causal relationships. It remains unclear whether higher stress levels lead to larger gray matter volume in children or whether larger gray matter volume leads to higher stress levels. Future research could employ prospective longitudinal cohort designs with finer age group divisions to address these questions. Second, it is unclear what components gray matter volume changes correspond to, which may relate to changes in cell size, growth or atrophy of neurons or glial cells, and synaptogenesis [?]. Therefore, future research could use white matter fiber tracts and other indicators to understand the neurobiological basis of childhood stress from a more microscopic perspective. Third, this study measured childhood stress levels only through children’ s self-report questionnaires. However, important factors related to stress levels may affect neural correlates of childhood stress, such as intensity, duration, number of stress events, and interactions between different stressors [**?**]. Although the questionnaire used in this study could measure both stress level and number of stress events, the small sample size prevented exploration of interactions between these variables. Future research could examine brain differences between children with high stress events-low stress levels and those with low stress events-high stress levels. Finally, neural regions and psychological functions do not appear to have one-to-one correspondence [**?**], so alternative interpretations of current results are possible. Future research needs to further demonstrate whether the childhood stress-brain associations found in this study are best explained by functions such as emotion regulation and cognitive control.

Conclusion

This study is the first to use multimodal data combined with machine learning to investigate the neural basis of childhood stress. Results showed that childhood stress was positively correlated with gray matter volume in the mOFC, insula, STG, and SMA involved in emotional function, and negatively correlated with insula-IPL FC involved in internal-external information integration. These results were not influenced by participants’ age, sex, head motion, or TIV, demonstrating specificity of findings. Machine learning predictive analysis showed that the aforementioned brain regions could effectively predict childhood stress levels, demonstrating robustness of results. The findings reveal that abnormal emotional function and internal-external information integration are associated with increased childhood stress levels. This study not only expands current empirical evidence on the neural basis of childhood stress but also provides strong support for developing early prevention strategies and intervention approaches for childhood stress.

References

References are preserved exactly as provided in the original text.

Supplementary Tables

Supplementary Table 1 Results of Correlation Analysis Between Negative Affect and Stress Levels/Brain Metrics (N = 78)
Note: mOFC = medial orbitofrontal cortex; Insular = insula; STG = superior temporal gyrus; SMA = supplementary motor area; IPL = inferior parietal lobule; ^{1}Correlation coefficient marginally significant p = 0.06; $p < 0.05$; ** $p < 0.01$.*

Supplementary Table 2 Brain Regions in Top 10% of Contribution Rates for Predicting Childhood Stress Levels

Note: Feature selection threshold $p < 0.01$, prediction model significance assessed using 2000 permutation tests $p < 0.05$.

Note: Figure translations are in progress. See original paper for figures.