Gene - Environment Interaction: A TPH2 polymorphism and early life stress shape brain architecture and anxious avoidant behavior

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Running title

Early life stress and TPH2 genetics interact to shape the brain and anxious avoidance

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Abstract

Serotonin regulates developmental and experience-dependent neuroplasticity in limbic-prefrontal systems engaged in emotional reactivity and regulation. Tryptophan hydroxylase 2 (*TPH2*) regulates central serotonergic biosynthesis rates and transgenic animal models suggest that *TPH2*-associated changes in serotonergic signaling mediate the impact of early life stress (ELS) shaping a phenotype characterized by anxious avoidance. The present study employed an imaging genomics approach that capitalized on individual differences in a *TPH2* polymorphism (703G/T; rs4570625) to determine whether differences in serotonergic signaling modulate the effects of ELS on brain structure and function and punishment sensitivity in humans (n = 252). Higher ELS exposure was associated with increased gray matter volumes in a circuitry spanning thalamic-limbic-prefrontal regions and decreased intrinsic communication in limbic-prefrontal circuits selectively in TT carriers. On the phenotype level, the genotype moderated the association between higher ELS exposure and higher punishment sensitivity. In TT carriers, the association between higher ELS exposure and punishment sensitivity was critically mediated by increased thalamic-limbic-prefrontal volumes. The present findings suggest that ELS shapes the neural organization of the thalamic-limbic-prefrontal circuits in interaction with individual variations in *TPH2* to promote a phenotype characterized by facilitated threat avoidance, thus promoting early adaptation to an adverse environment.

Keywords amygdala, early life stress, punishment sensitivity, frontal cortex, serotonin

Introduction

Aversive experiences during early developmental periods, such as childhood neglect or maltreatment, have been associated with strongly increased prevalence rates for psychiatric disorders and emotional dysregulations in adulthood. Converging evidence from animal and human studies indicates that early life stress (ELS) during sensitive periods leads to lasting changes in the structural and functional organization of brain systems engaged in emotional reactivity and regulation (Nemeroff 2016). ELS exposure has been associated with reduced volumes in limbic systems engaged in threat and stress reactivity, particularly the amygdala and hippocampal formation, as well as frontal regions engaged in implicit emotion regulation such as the anterior cingulate (ACC) and adjacent ventromedial prefrontal cortex (vmPFC) (overview in Strüber et al. 2014 and Teicher et al. 2016).

Conceptually, these findings have been interpreted in the context of two overarching hypotheses. The prevailing 'diathesis-stress' perspective proposes that ELS leads to stress-related brain damage thus directly promoting the vulnerability for later psychopathology (e.g. Lupien et al. 2009). The recently emerging 'neural adaptation' perspective proposes that ELS-associated changes in limbic and prefrontal systems reflect experience-dependent adaptations that facilitate detection and avoidance of threat thus promoting survival in a malevolent environment (Rutter 2012; Teicher *et al.* 2016). In line with the latter hypothesis, a growing number of studies reported facilitated threat detection (Pollak and Tolley-Schell 2003; Masten et al. 2008) and exaggerated amygdala response to threatening stimuli in limbic regions of individuals following ELS (McCrory et al. 2011; Dannlowski et al. 2012) which may mediate anxious avoidant behavior (Fonzo et al. 2016).

Considerable variations in the long term sequelae of ELS have been reported. For example, a recent longitudinal study reporting that the majority of adults with severe ELS demonstrated cognitive impairments and emotional dysregulations even after timely transfer to supporting environments (Sonuga-Barke et al. 2017). On the other hand, a significant minority remained unaffected suggesting that innate factors such as genetic variations influence the susceptibility and resilience to the negative sequelae of ELS in later life (Songu-Barke et al., 2017). Initial studies that combined a candidate gene approach with neuroimaging reported that specific single-nucleotide polymorphisms (SNPs) of genes associated with neuroplasticity and stress reactivity, including brain derived neurotrophic factor, glucocorticoid and oxytocin receptor sensitivity, confer greater sensitivity to the detrimental impact of ELS, e.g. pronounced reductions of limbic volumes (Dannlowski et al. 2016; Tozzi et al. 2016; van Velzen et al. 2016). Interestingly, two initial studies reported that specific allelic combinations associated with differences in central dopamine and serotonergic functioning exhibited ELS-associated changes in hippocampal and amygdala activation that may reflect improved threat learning and implicit emotion regulation following early aversive experiences (Hermann et al. 2012; van Rooij et al. 2017).

Given the regulatory role of serotonergic neurotransmission in both synaptogenesis and dendritic maturation during sensitive periods as well as emotional learning and regulation (Gaspar et al. 2003; Vitalis et al. 2007), innate differences in central serotonergic signaling may represent a promising target system that mediates susceptibility and resilience to ELS. Support for this hypothesis comes from animal models demonstrating that experimental modulation of serotonin signaling during early developmental periods induces neuroplastic

adaptations in limbic-prefrontal systems that determine altered threat responsivity and regulation in adulthood (Suri et al. 2015; Soiza-Reilly et al. 2019).

Central serotonin biosynthesis is regulated by the rate-limiting enzyme tryptophan hydroxylase (*TPH*), with the second isoform *TPH2* being exclusively expressed in central serotonin neurons (Walther et al. 2003; Zhang et al. 2004). Individual differences in the *TPH*2 encoding gene have been linked to variations in both, central serotonergic neurotransmission, including serotonin (5-HT) synthesis rates in limbic and prefrontal regions (Ottenhof et al. 2018) and phenotypic variations in traits associated with emotional reactivity and regulation (Herrmann et al. 2006; Gutknecht et al. 2007; Reuter et al. 2007; Canli et al. 2008).

A growing number of transgenic animal models capitalized on the selective effects of the *TPH2* isoform on central serotonergic signaling to determine interactions between *TPH2*-associated congenital serotonergic deficiency (in a mouse model with a mutated *TPH2* knock-in human polymorphism resulting in reduced brain 5-HT levels) and ELS. Findings suggest that serotonergic deficiency mediates the impact of ELS on emotional behavior in adulthood, particularly increased anxious behavior (Sachs et al. 2013; Sachs et al. 2015), indicating that the gene x environment interactions are partly mediated by serotonergic modulation of synaptic plasticity in limbic-prefrontal circuits that underpin emotional learning (Lesch and Waider 2012). Genetic studies in humans reported that *TPH2* SNP rs4570625 (-703 G/T) variations modulate the impact of ELS on emotional behavior, such that T-allele carriers exhibited increased attention to social threat signals in infanthood (Forssman et al. 2014) and elevated stress-reactivity in adulthood (Mandelli et al. 2012). Despite accumulating evidence suggesting that *TPH2* genetics interact with ELS to shape a phenotype characterized by

anxious behavior, the brain systems that mediate this association have not been determined in humans.

To determine interactive effects of ELS and the *TPH2* SNP rs4570625 (-703 G/T) polymorphism on brain structure and function and their relationship to anxious-avoidant behavior in adulthood a sample of healthy subjects (n = 252) underwent genotyping of the *TPH2* rs4570625 and acquisition of brain structural and functional magnetic resonance imaging (MRI). ELS exposure was retrospectively assessed with the Childhood Trauma Questionnaire (CTQ; Bernstein et al. 2003) and anxious behavior on the phenotype level was assessed using a scale developed by Torrubia (Torrubia et al 2001), which is based on Gray's personality model (Gray, 1987) that described two motivational systems, the Behavioral Inhibition System (BIS) and the Behavioral Activation System (BAS), that control aversive and appetitive behavior, respectively. The BIS appears particularly suitable given that it strongly overlaps with trait anxiety and specifically measures individual differences in the reactivity towards stimuli that are associated with a high probability of punishment and shapes anxious and avoidant behavior (see also revised reinforcement sensitivity theory, Corr et al. 2004). Across species the BIS has been associated with the septo-hippocamal system, with more recent conceptualizations additionally emphasizing the role of the amygdala (Gray and McNaughton 2000; Adrián-Ventura et al. 2019) and – in humans - medial prefrontal regions engaged in the assessment of distal threat and emotion regulation (Comte et al. 2015; Fung et al. 2019).

Based on previous findings suggesting that (a) ELS is associated with brain structural and functional changes in limbic-frontal circuits (Teicher et al. 2016) and that (b) *TPH2*

genetic variations, especially the rs4570625 “G” allele, are shown in a recent meta-analysis to be associated with mood disorders, schizophrenia and suicide presumably by regulating brain 5HT levels, albeit with little direct evidence for the functional role of these SNPs (Ottenhof *et al.* 2018) and that modulation of brain serotonin levels is thought to mediate experience-dependent synaptic plasticity in these circuits (Lesch and Waider 2012), we hypothesized (1) that the impact of ELS on the structural and functional organization of limbic-prefrontal circuit varies as a function of genetic differences in serotonergic transmission related to individual differences in the *TPH2* rs4570625 polymorphism, and (2) that ELS-associated neuroplastic changes in this circuitry determine variations in anxious avoidant behavior as assessed by trait punishment sensitivity.

Methods and Materials

Participants

A total of $N = 252$ healthy subjects participated in the study. All subjects were recruited from the student population of the University of Electronic Science and Technology in Chengdu, China. Participants that reported current or a history of medical, physical, neurological, or psychiatric disorders or regular use of nicotine, alcohol, illicit substances, medication as well as MRI contraindications were not enrolled in the study (see flow diagram, Supplementary Figure 1). The study was approved by the local ethics committee at the University of Electronic Science and Technology of China (UESTC) and study protocols were in accordance with the latest revision of the Declaration of Helsinki. Written informed consent

was obtained from all participants, after providing comprehensive information about procedures and aims of the study.

Childhood experience, perceived stress and punishment sensitivity

Levels of stress exposure during childhood were assessed using the CTQ (Bernstein *et al.* 2003) which consists of 25 retrospective self-report items spanning five types of negative childhood experiences. Following previous studies that also examined long-term consequences of ELS and interactions with genetics on brain structure and function, the CTQ total score was used (Dannlowski *et al.* 2012; Dannlowski *et al.* 2016). Additionally, we measured current stress using the Perceived Stress Scale (PSS) which assesses stress levels during the past month (Cohen et al. 1983). The Sensitivity to Punishment scale (SPS) from the sensitivity to punishment and sensitivity to reward questionnaire (SPSRQ) was administered to assess individual differences in trait behavioral inhibition (Torrubia et al. 2001). Individuals with high punishment sensitivity are expected to experience high levels of anxiety and to avoid potential dangers (Gray 1987).

Cronbach's α scores showed a good internal consistency of the scales in the present sample (0.847 for CTQ; 0.834 for PSS; 0.844 for SPS). The Shapiro-Wilk test indicated that CTQ scores in the present sample were non-normally distributed ($p < 0.001$), and hence the interaction of *TPH2* rs4570625and ELS on neutral indices were determined using non-parametric approaches.

Genotyping

Genomic DNA was purified from buccal cells using a MagNA Pure 96 robot (Roche Diagnostics, Mannheim). The sample probes were designed by TIB MolBiol (Berlin, Germany). Genotyping was conducted by real-time polymerase chain reaction (RT-PCR) on a Cobas Z 480 Light Cycler (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions.

MRI data acquisition

MRI data acquisition was conducted on a 3.0 Tesla GE MR750 system (General Electric Medical System, Milwaukee, WI, USA). T1-weighted high-resolution anatomical images were acquired with a spoiled gradient echo pulse sequence, repetition time (TR) = 5.9 ms, echo time (TE) = minimum, flip angle = 9°, field of view (FOV) = 256 × 256 mm, acquisition matrix = 256 × 256, thickness = 1 mm, number of slice = 156. For the resting state, a total of 210 functional volumes were acquired using a T2*-weighted Echo Planar Imaging (EPI) sequence (TR = 2000 ms, TE = 30 ms, FOV = 240 × 240 mm, flip angle = 90°, image matrix = 64 × 64, thickness/gap = 3.4/0.6mm, 39 axial slices with an interleaved ascending order). During the resting-state acquisition, participants were instructed to lie still and to fixate a white cross centered on a black background while not falling asleep. In post-MRI interviews, none of the subjects reported having fallen asleep during the scan. OptoActive MRI headphones (http://www.optoacoustics.com/) were used to reduce acoustic noise exposure during MRI acquisition (Roozen et al. 2008).

Brain structure: data processing and analysis of genotype x ELS effects

T1-weighted anatomical images were initially visually inspected to verify absence of anatomical abnormalities and image quality, and next manually reoriented to the anterior commissure – posterior commissure (AC-PC). The structural images were subsequently preprocessed using SPM12 (Statistical Parametric Mapping, http://www.fil.ion.ucl.ac.uk/spm/). The brain volumes were first segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) using the new unified segmentation approach (Ashburner and Friston 2005; Malone et al. 2015). Second, a group-specific template based on all participants was created using DARTEL algorithm (Ashburner 2007). Next, the segmented GM and WM images were iteratively registered via the fast diffeomorphic registration DARTEL algorithm to warp the GM and WM partitions on to the template and subsequently non-linearly normalized to MNI space. Finally, gray matter volumes were spatially smoothed with a Gaussian 8 mm FWHM (full width at half maximum) kernel. Total intracranial volume (TIV) was estimated and used as a covariate on the second level.

Due to the non-normal distribution of CTQ scores, non-parametric permutation tests were employed to examine the interaction between *TPH2* rs4570625genotype and ELS on brain structure at a voxel-wise whole-brain level using PALM (Permutation Analysis of Linear Models) (Winkler et al. 2014). To this end, the individual modulated GM images were entered into a single full factorial model, with genotype group as between-subject factor (TT vs. TG vs. GG). CTQ scores were entered as covariate (dimensional model), including the crucial interaction term of CTQ scores and genotype group. Age, gender, education and TIV were additionally included as nuisance regressors in the design matrix. All covariates were

mean-centered across participants. Corrections for multiple comparisons were conducted using permutation-based inferences (10,000 permutations) and significant clusters were determined using a threshold of $p_{FWE} < 0.05$ with multiple comparison correction by threshold-free cluster enhancement (TFCE) within a gray matter mask (spm grey.nii > 0.3).

Resting-state fMRI analysis

To examine whether the regional brain structural alterations were accompanied by functional communication differences between the nodes, resting state data was additionally acquired. Resting-state functional time-series were preprocessed using SPM12. For each subject, the first ten volumes were discarded to allow for T1 equilibration effect and allow active noise cancelling by the headphones. The remaining functional images were slice-time corrected and realigned to the first image to correct for head motion. The EPI images were then co-registered to the T1-weighted structural images, normalized to Montreal Neurological Institute (MNI) standard space using the segmentation parameters from the structural images and interpolated to 3 × 3 × 3 mm voxel size. Normalized images were finally spatially smoothed with a 6 mm FWHM. Next, 24 head movement parameters (i.e., 6 head motion parameters, 6 head motion parameters one time point before, and the 12 corresponding squared items) (Friston et al. 1996) along with mean signals from WM and CSF were removed from the data through linear regression. Finally, the influences of low-frequency drift and high-frequency noise were restricted by a band-pass filter (0.01–0.1 Hz). For the rsfMRI analyses, three subjects were excluded due to excessive head motion (exclusion criterion> 3 mm and/or 3 degrees, n = 2) or missed rsfMRI data (n = 1). Based on the results

of the voxel-based morphometry (VBM) analyses, we further explored whether regions showing *TPH2* genotype and ELS effects on brain structure additionally exhibit altered intrinsic functional interactions with other brain regions. To this end, regions identified by the VBM analysis served as seed regions of interest (ROI) to determine intrinsic functional connectivity maps for each subject. Next, *TPH2* genotype – ELS interactions on the intrinsic networks were determined using concordant non-parametric models as for the VBM data.

Functional connectivity analyses were performed using the Resting-State fMRI Data Analysis Toolkit (REST; http://www.restfmri.net). 5-mm spheres centered at the peak coordinates of significant clusters in the VBM analyses served as seed regions to create seed-to-whole brain intrinsic connectivity maps. To this end, the individual mean time series of each seed ROI was calculated and next correlation coefficients between the seed time series and other voxels were calculated to obtain r maps for each participant. Fisher z score transformations were employed to generate z-FC maps. Finally, z-FC maps were subjected to second-level random-effects group analyses. Consistent with the brain structural analyses, a single full factorial model was conducted using PALM with genotype group (TT vs GT vs GG) as between-subjects factor. CTQ scores were entered as covariate, including the interaction term of genotype and CTQ. Age, gender, education and mean framewise displacement (Van Dijk et al. 2012) were included as nuisance regressors. All covariates were mean-centered across participants. Significance thresholds were set at $p_{\text{FWE}} < 0.05$ after TFCE correction. In line with a previous study (Birn et al. 2017), relative effects of ELS and current stress on brain structure and function were further determined by recomputing the analyses and substituting the CTQ scores with the PSS scores as an index of current stress.

Associations with anxious avoidant behavior on the phenotype level

In a next step, associations between anxious avoidant behavior on the phenotype level, ELS exposure and brain structure or function (extracted gray matter estimates and functional connectivity values from 5mm-radius spheres centered at the peak coordinates of respective interaction clusters) were examined. We first explored the potential moderation effect of *TPH2* rs4570625genotype on the association of CTQ with sensitivity to punishment on the phenotype level using a moderation analysis as implemented in the SPSS 21 PROCESS macro (Preacher and Hayes 2004). More specifically, the moderation analysis tested whether the interaction between the predictor variable (CTQ scores) and the moderator variable (genotype) could predict the dependent variable (SPS scores), including gender as covariable. Next we explored associations between ELS-associated brain structural or functional changes and the behavioral phenotype using non-parametric correlation analyses as implemented in PALM (10,000 permutations). Finally, a mediation analysis was employed to determine whether brain structural changes mediated the effects of ELS on an anxious avoidant phenotype (punishment sensitivity) within the genotype groups (PROCESS macro, bootstrapping approach with 10,000 permutations, employing bias-corrected 95% confidence intervals (CIs) to determine the significance of indirect effects (Preacher and Hayes 2008).

Results

***TPH2* genotyping**

A total of 229 right-handed Han Chinese participants for whom structural MRI scans and complete ELS data were available were included (n = 115 females; mean age of complete sample: M = 21.55 ± 2.31 years, range 18-29 years). Genotyping for *TPH2* rs4570625 yielded n = 71 TT homozygotes versus n = 107 GT heterozygotes versus n = 51 GG homozygotes. Allele frequencies did not deviate from those expected according to the Hardy-Weinberg Equilibrium ($\chi^2_{(1)} = 0.735$, $p = 0.391$) and were in the expected distribution for Han Chinese populations (see ncbi.nlm.nih.gov). Previous studies on the *TPH2* rs4570625 were mostly conducted in Caucasian populations with a rare occurrence of the T-allele as compared to East Asian populations. The present distributions in Chinese allowed us to compare GT and TT carriers. Table 1 presents sociodemographic and questionnaire data dependent on *TPH2* rs4570625 genotype. Importantly, the genotype groups did not differ in demographic, psychopathology or CTQ measures (all ps > 0.36). There is a significant difference between genotype groups in PSS ($F_{(2, 226)} = 4.89$, $p = 0.008$, partial η2 = 0.041). Post hoc test revealed that PSS in TT homozygotes was significantly higher than in GT heterozygotes ($t_{(176)} = 3.10$, $p = 0.002$, Cohen's d = 0.47).

Interactive effects of *TPH2* x ELS on brain structure

No significant main effects of ELS (independent of genotype) and genotype (independent of CTQ scores) were found. Examining interactions of ELS x genotype revealed effects in a broad thalamic-limbic-frontal network, including a cluster spanning the bilateral thalamus, parahippocampal gyrus, hippocampus, and amygdala (k = 2609, $p_{\text{FWE with TFCE}} < 0.05$), a cluster spanning the bilateral ventromedial frontal cortex (vmPFC) (k = 1575, $p_{\text{FWE with TFCE}} <$

0.05), and additional clusters located in the left dorsolateral prefrontal cortex (DLPFC) (k = 201, $p_{\text{FWE with TFCE}} < 0.05$), right (k = 335, $p_{\text{FWE with TFCE}} < 0.05$) as well as left (k = 181, $p_{\text{FWE with TFCE}} < 0.05$) dorsal anterior cingulate cortex (dACC) (Figure 1, table 2). Post-hoc analyses by means of extracted parameter estimates from the significant interaction clusters revealed pronounced effects of ELS on gray matter volume in TT homozygotes, whereas both GT heterozygotes and GG homozygotes did not show significant effects after multiple correction. In the TT homozygotes, higher exposure to aversive experiences during early life was specifically associated with increased gray matter volumes in the bilateral thalamic-limbic cluster as well as the left dACC and dlPFC (all *p*s < 0.05 after Bonferroni correction, Figure 1, Table 3). The correlation coefficients were significantly different between TT homozygotes and GT heterozygotes ($p_{\text{corrected}} < 0.01$) (table 3). For the thalamic-limbic cluster the correlation coefficients were additionally significantly different between GG homozygotes and GT heterozygotes ($p_{\text{corrected}} < 0.05$) (Table 3). No significant main effect of current life stress (independent of genotype) and no significant interaction effects of current life stress and *TPH2* genotype on brain structure were observed.

Interactive effects of *TPH2* x ELS on intrinsic network communication

To explore whether the determined regions exhibit genotype x ELS effects with respect to the functional communication between the identified regions, a concordant analysis for the intrinsic connectivity maps of these regions was conducted. ELS and *TPH2* rs4570625 interactively impacted functional connectivity strengths between the left dACC and left DLPFC (MNI_{xyz} = [-6, 42, 27], k = 24, $p_{\text{FWE with TFCE}} < 0.05$), with subsequent post-hoc tests

demonstrating that the interaction was driven by pronounced effects of ELS in the TT homozygotes, whereas both GT and GG groups did not show significant associations after multiple correction (TT: $r = -0.43$, $p_{corrected} = 0.0021$; GT: $r = 0.11$, $p = 0.27$; GG: $r = 0.20$, $p = 0.18$; centered at $MNI_{xyz} = [-6, 42, 27]$; Fisher's Z: $z_{TT\ vs\ GT} = 3.61$, $p < 0.001$; $z_{TT\ vs\ GG} = 3.47$, $p = 0.001$; $z_{GT\ vs\ GG} = 0$, $p = 1$,) (Figure 2). Given previously reported genotype-dependent effects of ELS on amygdala activity (White et al. 2012), we additionally employed amygdala-focused analyses (small-volume correction, SVC) using the structurally defined amygdala mask from the Automated Anatomical Labelling (AAL) (Tzourio-Mazoyer et al. 2002) atlas. Small volume correction revealed interactive effects on the functional connectivity of the left dACC and left amygdala ($MNI_{xyz} = [-21, 0, -12]$, $k = 2$, $p_{FWE} < 0.05$) (Figure 2). The interaction was driven by a pronounced effect of ELS on the strengths of functional connectivity between left dACC and left amygdala in TT homozygotes, whereas both GT heterozygotes and GG homozygotes did not show significant ELS effects (TT: $r = -0.34$, $p_{corrected} = 0.032$, GT: $r = 0.28$, $p_{corrected} = 0.055$; GG: $r = -0.14$, $p = 0.35$; centered at $MNI_{xyz} = [-21, 0, -12]$; Fisher's Z: $z_{TT\ vs\ GT} = 3.93$, $p < 0.001$; $z_{TT\ vs\ GG} = 1.136$, $p = 0.26$; $z_{GT\ vs\ GG} = 2.31$, $p = 0.021$) (Figure 2). No significant interactive effects were observed with respect to the other seed regions determined by the brain structural analyses. No significant main effect of current life stress (independent of genotype) and no significant interaction effects of current life stress and TPH2 genotype on intrinsic connectivity were observed.

Associations with anxious avoidant behavior on the phenotype level

To explore the potential moderation effect of genotype on the association between ELS and punishment sensitivity on the phenotype level, a moderation model was applied. For visualization of the results the moderator variable was additionally split into three levels (low, moderate, high ELS exposure) using the Johnson-Neyman approach. A significant moderation effect ($R^2 = 0.05$, $F_{(4, 224)} = 3.12$, $p = 0.016$) was observed, reflecting that the interaction between CTQ and *TPH2* rs4570625significantly predicts punishment sensitivity ($B = 0.094$, $SE = 0.044$, $t_{(224)} = 2.15$, $p = 0.03$). Genotype significantly moderated the association between ELS and punishment sensitivity, with post hoc tests indicating that higher ELS was significantly associated with higher punishment sensitivity selectively in the TT carriers ($t_{(224)} = 2.59$, $p = 0.01$, Figure 3). Examining associations between gray matter volume or functional connectivity and sensitivity to punishment in the genotype groups using non-parametric correlation analysis revealed that specifically in TT homozygotes sensitivity to punishment was significantly positively associated with volumes of the thalamic-limbic region, right vmPFC and left DLPFC (right vmPFC: $r = 0.359$, $p_{\text{corrected}} = 0.006$; left DLPFC: $r = 0.387$, $p_{\text{corrected}} = 0.008$; bilateral thalamic-limbic: $r = 0.370$, $p_{\text{corrected}} =0.026$) (Table 4 and Figure 4). There was no significant association of sensitivity to punishment and the functional connectivity of left dACC (all *p*s > 0.05) after multiple comparison correction.

Next, based on the results of the associations with sensitivity to punishment, the cortical volume solely was entered in the mediation analysis, which tested whether brain volumetric variations mediate the association between higher ELS and elevated punishment sensitivity within the genotype groups. The mediation model reached significance in the TT group only, implying that a considerable proportion of variation in sensitivity to punishment may be

explained by the level of ELS exposure in the TT group. Moreover, a significant overall indirect effect was observed (mediation by brain volume; indirect effect ratio, 66.75%; effect size a×b = 0.1096, 95% confidence interval = [0.0247-0.2212]; $p < 0.05$), suggesting that gray matter volumes in the thalamic-limbic-prefrontal network significantly mediated the influence of early life experience on sensitivity to punishment solely in TT carriers (details see Supplementary Table 1).

Discussion

Previous animal models and initial findings in humans suggest that variations in serotonergic signaling associated with individual differences *TPH2* polymorphisms mediate the effects of early adverse experiences on emotional dysregulations (Mandelli *et al.* 2012; Sachs *et al.* 2013; Forssman *et al.* 2014; Sachs *et al.* 2015). ELS and *TPH2* rs4570625 genotype interact to shape the structural and functional architecture of thalamic-limbic-prefrontal circuits. Specifically, in TT homozygotes higher levels of adverse childhood experiences were associated with increased volumes of the hippocampal-amygdala complex, the thalamus and frontal regions engaged in emotion reactivity and regulation, including the vmPFC, dACC and DLPFC. On the network level, aversive experiences and *TPH2* rs4570625 genotype exhibited interactive effects on the functional connectivity of the left dACC with the left DLPFC and left amygdala, with TT homozygotes exhibiting a decreased connectivity with increasing levels of aversive experience while no associations were observed in the G-carrier groups. On the phenotypic level, *TPH2* genotype significantly moderated the impact of ELS on punishment sensitivity. Within the TT homozygotes higher levels of sensitivity to

punishment were associated with both, higher levels of aversive childhood experiences and limbic and frontal volumes. An additional mediation analysis furthermore suggests that increased brain volumes in this circuitry critically mediate the impact of aversive childhood experiences on sensitivity to punishment.

Most previous studies examining associations between childhood aversive experiences and brain structure in adulthood reported that higher levels of aversive childhood experience are associated with decreased brain volumes in limbic and prefrontal regions (see e.g. overview in Teicher et al. 2016). When we stratify exposure to ELS by *TPH2* genotype we also observed a negative association between ELS and brain volumes, however solely in the pooled G-carrier group (see Supplementary Table 2). Animal models suggest that prolonged stress during sensitive developmental periods detrimentally impacts dendritic growth in limbic and prefrontal regions (Radley et al. 2008; Yang et al. 2015) that could partially explain lower volumes observed on the macro-anatomic level (Brunson et al. 2005; Ivy AS et al. 2010; Molet et al. 2015). The few previous studies that examined whether polymorphisms modulate the impact of ELS on brain structure in humans observed that specific allelic variants coding brain-derived neurotrophic growth factor and oxytocinergic pathways pathway genes exhibit pronounced volumetric decreases following ELS (Dannlowski *et al.* 2016; van Velzen *et al.* 2016). These results in accordance with the diathesis-stress perspective, have been interpreted as genetic vulnerability factors in partially mediating aversive environmental influences.

In this study, the opposite association between higher ELS and increased brain volumes observed in the *TPH2* rs4570625TT homozygotes contrasts with previous reports on a genetic

susceptibility for pronounced ELS-associated brain structural deficits. While some studies that combined the low-frequency *TPH2* rs4570625 T-carrier groups initially revealed increased psychopathological risk (Gao et al. 2012), anxiety-associated traits (Gutknecht *et al.* 2007) and detrimental effects of ELS (Mandelli *et al.* 2012; Forssman *et al.* 2014), accumulating evidence indicates that TT homozygotes differ from both other variants in terms of lower anxiety-related behavior and psychopathological risk (Reuter *et al.* 2007; Lehto et al. 2015; Laas et al., 2017; Ottenhof *et al.* 2018). While reduced gray matter volumes in limbic-prefrontal regions have been consistently reported in psychiatric disorders characterized by exaggerated anxiety and deficient emotion regulation (van Tol et al. 2010; Bora et al. 2012; Ahmed-Leitao et al. 2016), associations between emotional functioning and limbic-prefrontal volumes in healthy subjects remain less clear, such that elevated anxiety has been associated with both, volumetric increases as well as decreases in this circuitry (e.g. Modi et al. 2019; however, see Günther et al. 2018). On the other hand, a growing number of studies consistently reported positive associations between aversive learning and volumetric indices in this circuit (Hartley et al. 2011; Cacciaglia et al. 2015; Winkelmann et al. 2016) and trauma exposure in adulthood has been associated with volumetric increases of the amygdala and concomitantly facilitated aversive learning (Cacciaglia et al. 2017). In the present study, both, childhood aversive experience and thalamic-limbic-prefrontal volumes were positively associated with higher punishment sensitivity in the TT homozygotes. According to the reinforcement sensitivity theory, individual differences in trait punishment sensitivity are neutrally underpinned by the BIS, a brain-behavioral system that facilitates the formation of aversive motivation by orchestrating behavior in response to conditioned aversive events

(Gray 1987; Gray and McNaughton 2000; Corr 2004) and interacts with associative learning to facilitate aversive learning and future avoidance of threats (Reynolds et al. 2018). Increased punishment sensitivity on the phenotype level is considered to reflect an underlying hyperactive BIS system and has been consistently associated with better threat detection and facilitated aversive and avoidance learning (Avila and Parcet 2000, 2002). The identified network exhibiting genotype x early life stress interaction effects highly overlaps with the neural circuits engaged in these functional domains, specifically the acquisition and extinction of conditioned threat responses (Milad and Quirk 2012; Fullana et al. 2016). Within this circuitry, the amygdala, hippocampus and dACC detect and encode threat probability of stimuli in the environment (e.g. Rodrigues et al. 2009) and are regulated by prefrontal-thalamic-limbic cicruits that subserve implicit and explicit regulation of the innate or acquired threat response (Kalisch 2009; Ochsner et al. 2012; Ramanathan et al. 2018). Serotonin plays an important role in these aversive learning mechanisms (Bauer 2015) and underpins neuroplasticity in limbic-prefrontal circuits (Lesch and Waider 2012). Mice with a knockin mutilated *TPH2* polymorphism (rs4570625) resulting in serotonin deficiency exhibit a phenotype characterized by facilitated threat reactivity and threat acquisition that are mediated by neural morphology in limbic regions, particularly the hippocampal-amygdalar complex (Nietzer et al. 2011; Waider et al. 2017; Waider et al. 2019).

Although the neurobiological implications of the *TPH2* rs4570625 polymorphism are not fully understood and the T-variant has been associated with increased as well as decreased central serotonergic transmission in limbic-prefrontal circuits (Zhang *et al.* 2004; Lin et al. 2007; Scheuch et al. 2007; Ottenhof *et al.* 2018; however see Lin et al., 2007 in a Han

population), the present findings may reflect that the *TPH2* rs6570625 polymorphism, perhaps reflecting individual differences in 5HT neurotransmission, potentially mediate the impact of ELS on experience-dependent neuroplastic changes in thalamic-limbic-prefrontal circuits engaged in aversive learning. We further suggest the notion that this polymorphism may shape a phenotype with an enhanced capability to detect and avoid potentially threatening stimuli and thus potentially facilitating survival in a malevolent environment (Castellà and Pérez 2004). This interpretation of early-life stress induced adaptive changes on the neural and phenotype level harmonizes the experience-dependent adaptation hypothesis suggesting that aversive experiences during sensitive developmental periods shape neural adaptations to promote the avoidance of threats in the environment (Teicher et al., 2016).

On the network level, ELS-associated volumetric increases observed in TT carriers were accompanied by decreased functional communication of the dACC with both, the DLPFC and the amygdala. Deficient intrinsic functional interaction within this circuit has been increasingly reported following ELS and has been associated with increased emotional dysregulations and threat reactivity on the behavioral level (Cisler et al. 2013; Philip et al. 2014; Kaiser et al. 2018). Deficient recruitment of the dACC and functional communication within the dACC-DLPFC-amygdala circuitry have been associated with impaired voluntary emotion regulation (Burghy et al. 2012; Zilverstand et al. 2017; Zimmermann et al. 2017; Kim et al. 2019). In this context, the present findings may suggest that the increased regional-specific volumes of the aversive learning circuit in TT carriers come at the expense of less regulatory control within this circuitry, shifting the systems towards higher sensitivity for threatening stimuli in the environment rather than internal emotion regulation.

Together, the present findings suggest that ELS shapes the neural organization of the thalamic-limbic-prefrontal circuits in interaction with individual genetic variations in modulating in part serotonergic neurotransmission. The identified network strongly overlaps with the circuitry engaged in aversive learning and avoidant behavior. Previous studies suggest that serotonin facilitates aversive learning via promoting neuroplasticity in thalamic-limbic-prefrontal circuits (Nietzer *et al.* 2011; Waider *et al.* 2017; Waider *et al.* 2019). Within this context the present findings suggest that genetic differences in serotonergic signaling associated with a specific *TPH2* polymorphism determine the impact of early life aversive experiences on the structural and functional architecture of the brain and shape a phenotype adapted to avoid threat in a malevolent environment.

Strengths and limitations

By capitalizing on the regulatory role of a common *TPH2* SNP on central serotonergic neurotransmission in combination with MRI and trait assessments in a representative sample of healthy subjects, the present study allowed us to determine the role of polymorphic gene differences presumably affecting serotonergic signaling on adaptation to early life stress on the neural and phenotype level. Owing to the low prevalence of the T-allele in Caucasian populations, previous studies combined T-carriers (Canli et al. 2005; Markett et al. 2016), whereas the higher frequency of the T-variant in Asian populations enabled us to additionally examine characteristics of the TT-homozygote variant. Nevertheless, the current findings need to be tempered by the following limitations: (1) Early life stress was retrospectively assessed; however, importantly the lack of significant differences of self-reported ELS exposure

between the genotype group argues against genotype-dependent differences in retrospective aversive experiences recall. (2) Delayed and age-dependent effects of ELS on brain structure have been reported (Andersen and Teicher 2004) and the present cross-sectional approach does not allow to determine genotype-dependent different trajectories of the ELS-associated adaptations. (3) Different effects of early life stress during differentially sensitive developmental periods were reported (Andersen et al. 2008; Pechtel et al. 2014). However, the employed retrospective assessment does not allow us to further determine genotype-dependent differences at the onset and duration over the course of stress exposure during childhood. Single SNPs only explain a small fraction of the variance in complex phenotypes, including anxiety (Montag and Reuter 2014), and a recent meta-analysis suggests that interactions between single allele variants of the serotonergic 5-HTTLPR and ELS in depression are not robust (Culverhouse et al. 2018). However, the imaging genomics strategy implemented in the current study has been suggested to be a more powerful approach that allows a more robust determination of brain-based phenotypes (Meyer-Lindenberg and Weinberger 2006) that is highly informative towards understanding the interactive effects of ELS and genetic variations on complex behavioral traits.

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Tables and legends

Table 1. Sample Characteristics (N = 229) Stratified According to *TPH2* rs4570625

	TT (n = 71)	GT (n = 107)	GG (n = 51)	*p* Value
Age, Years	21.55 ± 2.48	21.46 ± 2.24	21.76 ± 2.27	0.74[a]
Gender (M/F)	32/39	56/51	26/25	0.63[b]
Education, Years	15.55 ± 2.09	15.69 ± 1.84	15.78 ± 1.84	0.79[a]
CTQ	38.08 ± 9.22	37.92 ± 10.65	38.45 ± 9.10	0.95[a]
PSS	26.18 ± 5.51	23.61 ± 5.38	24.55 ± 5.21	0.008[a]
SPS	11.11 ± 4.81	10.21 ± 4.49	10.96 ± 3.79	0.363[a]

Mean and standard deviations (± SD) are displayed. Abbreviations: CTQ, Childhood Trauma Questionnaire; F, female; M, male; STAI, State-Trait Anxiety Inventory; PSS, Perceived Stress Scale; SPS, Sensitivity to Punishment scale. [a] *F* tests, [b] $\chi 2$ tests

Table 2. Interaction between *TPH2* genotype and ELS on brain structure (whole-brain)

Brain Area	Peak located at MNI			TFCE	Cluster Size k	$p_{\text{FWE with TFCE}}$
L dACC	-1.5	12	28.5	932.25	181	0.045
L DLPFC	-34.5	31.5	34.5	1008.65	201	0.034
B thalamic -limbic	19.5	-25.5	12	1238.10	2609	0.015
B vmPFC	1.5	15	-7.5	1068.18	1575	0.027
R dACC	6	33	24	956.25	335	0.042

Abbreviations: TFCE, threshold-free cluster enhancement; dACC, dorsal anterior cingulate cortex; vmPFC, ventromedial frontal cortex; DLPFC, dorsolateral prefrontal cortex; thalamic -limbic, parahippocampus gyrus/ hippocampus/thalamus/amygdala; L, left; R, right; B, bilateral; GMV, gray matter volume.

Table 3. Correlation between GMV and CTQ and correlation differences between genotypes

Brain Area	MNI coordinates			Genotype[a]			*Z* value[a]		
				GG	TG	TT	GG *vs* TG	GG *vs* TT	TG *vs* TT
L dACC	-1.5	12	28.5	0.00	-0.03	0.47***	0.19	2.71	3.48**
L DLPFC	-34.5	31.5	34.5	0.08	-0.27	0.49***	2.08	2.38	5.22***
B thalamic -limbic	19.5	-25.5	12	0.37	-0.15	0.47***	3.11*	0.65	4.26***
B vmPFC	1.5	15	-7.5	-0.03	-0.25	0.35	1.27	2.07	3.93**
R dACC	6	33	24	-0.12	-0.18	0.35	0.33	2.59	3.49**

Mean gray matter volumes were extracted from a 5-mm sphere centered at the peak coordinates of the respective clusters identified in the VBM analyses. Results were corrected for multiple comparisons using Bonferroni correction [(a)]. Abbreviations: dACC, dorsal anterior cingulate cortex; vmPFC, ventromedial frontal cortex; DLPFC, dorsolateral prefrontal cortex; thalamic-limbic, parahippocampus gyrus/ hippocampus/thalamus /amygdala; L, left; R, right; GMV, gray matter volume; CTQ, Childhood Trauma Questionnaire. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 4. Associations between gray matter volume and sensitivity to punishment

Brain Area	MNI coordinates			Genotype[a]			*Z* value[b]		
				GG	TG	TT	GG *vs* TG	GG *vs* TT	TG *vs* TT
L dACC	-1.5	12	28.5	-0.14	0.02	0.20	0.90	1.83	1.21
L DLPFC	-34.5	31.5	34.5	-0.12	-0.02	0.39**	0.62	2.83**	2.72**
B thalamic -limbic	19.5	-25.5	12	0.07	0.15	0.37*	0.46	1.68	1.51
B vmPFC	1.5	15	-7.5	0.07	0.06	0.36**	0.02	1.65	2.01*
R dACC	6	33	24	0.06	-0.05	0.24	0.65	0.96	1.89

Gray matter volumes extracted from regions demonstrating significant environment x genetic interactions and associations with punishment sensitivity. Positive associations were specifically observed in the TT homozygotes. Abbreviations: dACC, dorsal anterior cingulate cortex; vmPFC, ventromedial frontal cortex; DLPFC, dorsolateral prefrontal cortex; thalamic-limbic, parahippocampus gyrus/ hippocampus/thalamus/amygdala; L, left; R, right; B, bilateral; GMV, grey matter volume; SPS, sensitivity to punishment scale.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; [a]multiple comparisons corrected with Bonferroni correction; [b]no Bonferroni-correction applied

Figures and legends

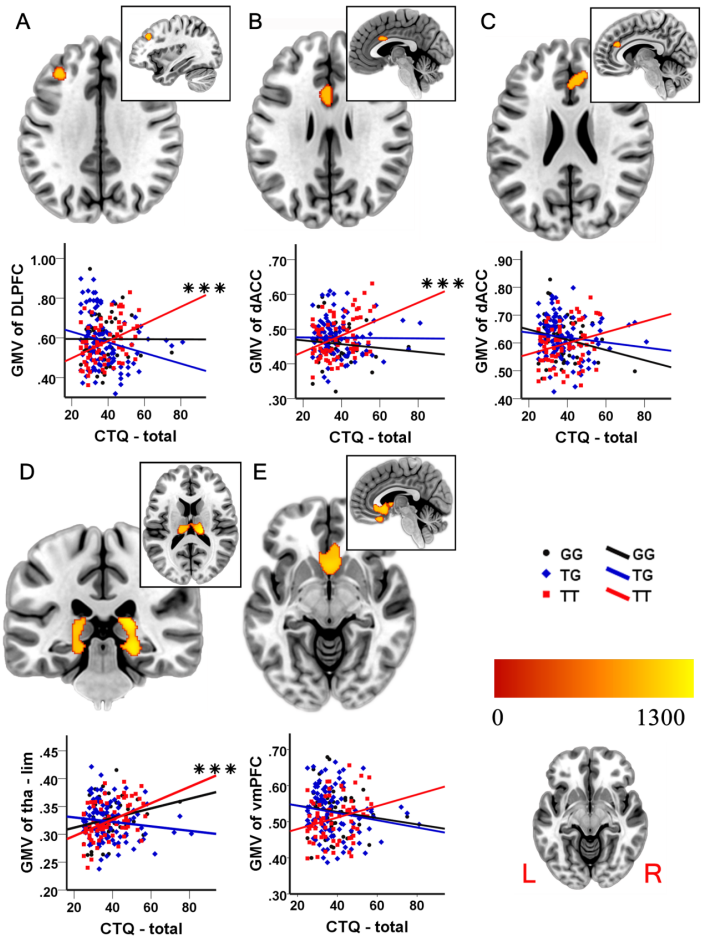

Figure 1. Interaction of *TPH2* rs4570625 and ELS on grey matter structure and relationship between grey matter volume and Childhood-Trauma -Questionnaire (CTQ) scores. Regions showing the effect of ELS on gray matter volume as a function of *TPH2* genotype (n = 77 TT homozygotes versus n = 112 TG heterozygotes versus n = 53 homozygotes). Analysis depicts the interaction term (color bar = TFCE value) for the separate clusters showing a significant interaction effects between genotype and early life stress exposure (2A-2E). Statistical significance was thresholded at $p_{\text{FWE with TFCE}} < 0.05$. dACC, dorsal anterior cingulate cortex; vmPFC, ventromedial frontal cortex; DLPFC, dorsolateral prefrontal cortex; thalamic-limbic, parahippocampus gyrus/ hippocampus/thalamus; *** $p < 0.001$, multiple comparisons using Bonferroni correction.

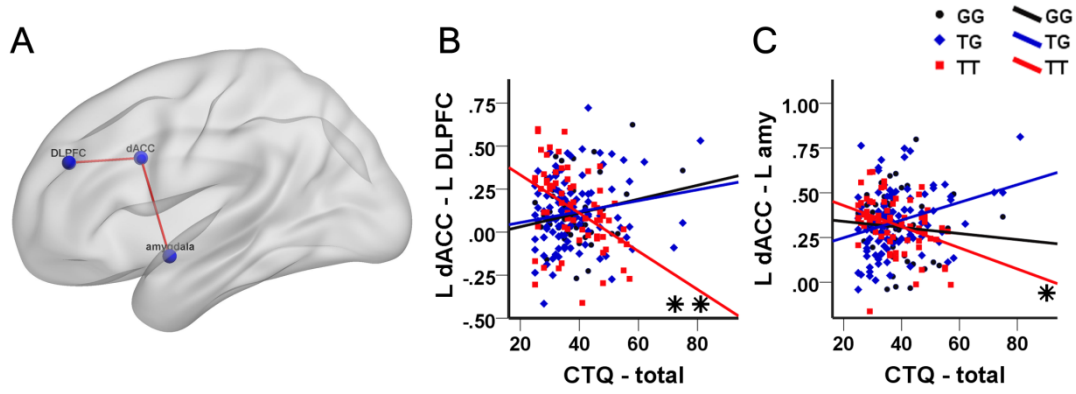

Figure 2. Interaction of *TPH2* rs4570625 and ELS on the functional connectivity strength of the left dorsal anterior cingulate cortex (dACC). Regions showing the effect of ELS (Childhood Trauma Questionnaire [CTQ] scores) on the functional connectivity strength between the left dACC and dorsolateral prefrontal cortex (DLPFC) and amygdala as a function of *TPH2* genotype (n = 69 TT homozygotes (red) versus n = 106 GT heterozygotes (blue) versus n = 51 GG homozygotes (black)) (3A). Associations between extracted parameter estimates and CTQ total scores in the separate genotype groups for the left dACC – left DLPFC pathway (3B) and the left dACC – left amygdala pathway (3C). Statistical significance was thresholded at $p < 0.05$ with TFCE (for the DLPFC at whole-brain level; for amygdala, small volume correction was used). dACC, dorsal anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; amy, amygdala; * $p < 0.05$; ** $p < 0.01$, multiple comparisons using Bonferroni correction.

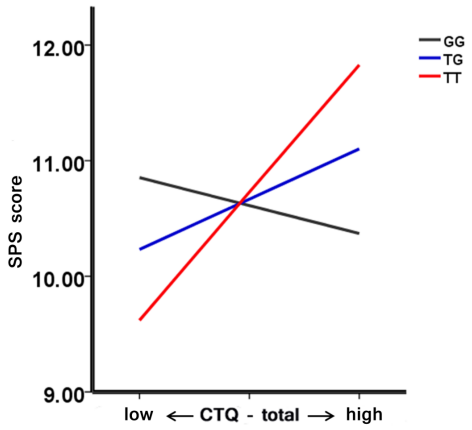

Figure 3. Moderation effect of genotype on the association between sensitivity to punishment scale (SPS) and Childhood Trauma Questionnaire (CTQ) scores. For visualization the moderator variable (CTQ) was split into three levels using the Johnson-Neyman approach (low, medium, high CTQ scores). The moderation effect was significant ($t_{(224)} = 2.59$, $p = 0.01$), indicating that the CTQ scores were positively correlated with the SPS only in TT homozygotes group. SPS, sensitivity to punishment scale; CTQ, Childhood Trauma Questionnaire.

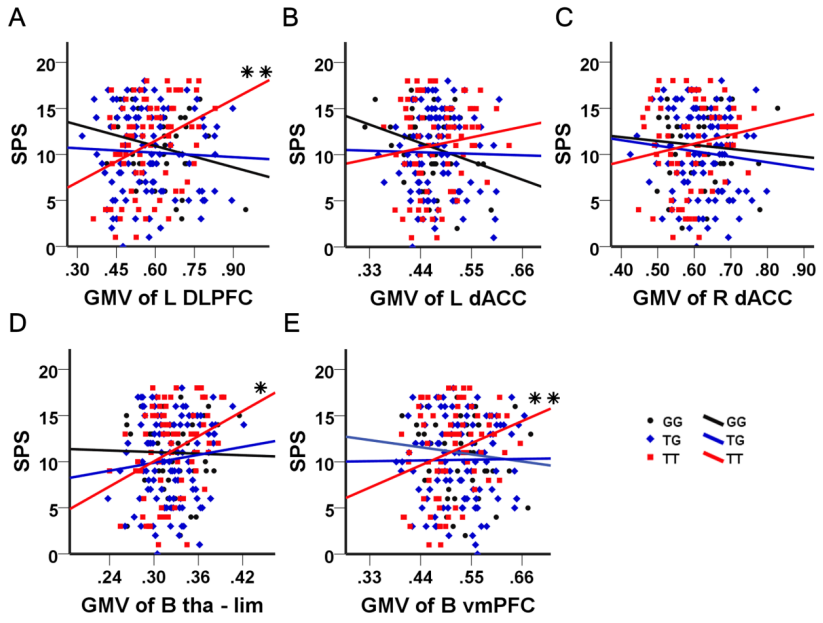

Figure 4. Relationship between gray matter volume and sensitivity to punishment scale (SPS) scores for TT homozygotes (red), TG heterozygotes (blue) and GG homozygotes (black) in the regions showing significant genotype x early life stress effects (5A-D). GMV, gray matter volume; dACC, dorsal anterior cingulate cortex; vmPFC, ventromedial frontal cortex; DLPFC, dorsolateral prefrontal cortex; thalamic-limbic, parahippocampus gyrus/ hippocampus/thalamus; L, left; R, right; B, bilateral; * $p < 0.05$; ** $p < 0.01$, multiple comparisons using Bonferroni correction.