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ORIGINAL CONTRIBUTION

Serotonergic influence on depressive symptoms and trait anxiety is mediated by negative life events and frontal activation in children and adolescents

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Abstract

Depression and anxiety are common in childhood and adolescence. Even though cardinal symptoms differ, there is a considerable overlap regarding the pathogenic influence of serotonergic innervation, negative life experience, disturbed emotion perception/affect regulation, and impaired neural functioning in the fronto-limbic circuit. In this study, we examined the effect of the *5-HTT*LPR/rs25531 genotype on depressive symptoms and trait anxiety under the consideration of the amount of negative life events in healthy children and adolescents ($N = 389$). In a subsample of 49 subjects, we performed fMRI to add fronto-limbic brain activation as a second interacting factor. Across all subjects, negative life events moderated the influence of the *5-HTT*LPR/rs25531 genotype on both depressive symptoms and trait anxiety. In the fMRI subsample, *5-HTT*LPR/rs25531 $S + S/L_G + S/L_A + L_GL_A + L_GL_G$ genotype-associated left middle frontal gyrus (MFG) activation mediated the influence of *5-HTT*LPR/rs25531 genotype on depressive symptoms, however, only in combination with negative life events. Genetic influence on trait anxiety was predominantly mediated by negative life events; only L_AL_A genotype-specific activation in the right MFG worked as a mediator in combination with negative life events. The present findings hint towards distinct mechanisms mediating the influence of *5-HTT*LPR/rs25531 genotype on depressive symptoms and anxiety, with negative life events playing a crucial role in both phenotypes. With regard to depressive symptoms, however, this influence was only visible in combination with MFG activation, whereas, in anxiety, it was independent of brain activation.

Keywords fMRI · Anxiety · Depression · Negative life events · *5-HTT*LPR/rs25531

Introduction

Depression and anxiety are common in childhood and adolescence. Each disorder is associated with substantial functional impairment and both disorders often co-occur [1, 2]. Even though cardinal symptoms differ, there is a considerable overlap regarding the influence of (a) serotonergic transmission [3, 4], and (b) negative life events [5, 6], (c) disturbed emotion perception/affect regulation [7, 8], and (d) impaired neural functioning in fronto-limbic circuits [9, 10].

In detail, the serotonin transporter gene (*5-HTT*) has been reported as one candidate gene in the pathogenesis of affective disorders. The *5-HTT* gene-linked polymorphic region (*5-HTT*LPR) with a low-expressing short allele (S) and a high-expressing long allele (L) modifies gene function. The S allele is associated with reduced serotonin (5-HT) binding, lower *5-HTT* mRNA concentrations and diminished 5-HT

Katharina Kneer and Julia Reinhard have contributed equally to this work and should, therefore, both be considered first authors.

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reuptake as compared to the L allele [11]. The closely related single-nucleotide polymorphism rs25531 A/G further modifies serotonin transporter expression with the L_AL_A genotype conferring highest activity, followed by L_AL_G and L_A/S [12]. The low-expressing S allele had been linked to the risk for depression (for review, [13, 14]). Caspi and colleagues (2003) showed that individuals carrying the *5-HTT*LPR S allele were more often diagnosed with major depression and had higher depressive symptomatology compared to individuals with the L allele [13]. However, this relation was dependent on whether the individual experienced traumatic life events, in that the *5-HTT*LPR1 S allele rendered an individual more prone to develop depression after experiencing a form of trauma and/or stress [15]. Healthy S allele carriers displayed higher levels of trait anxiety compared to non-carriers [16]. The S allele was associated with different measures of fear and anxiety, e.g., harm avoidance, sensitivity to stress and emotionality in general [15–18], while in interaction with critical life events experienced in childhood, subjects carrying the more active LL or L_AL_A genotype, respectively, showed increased anxiety sensitivity [19]. Patients suffering from PTSD showed a higher frequency of the SS genotype compared to a control group, while in patients with anxiety disorders, particularly panic disorder, not clear association could be discerned [20, 21]. In turn, the L_AL_A genotype strongly interacted with environmental parameters, such as childhood trauma [22], bullying at work [23], and self-efficacy [24] in anxiety-related phenotypes. In sum, findings in anxiety-related phenotypes suggest a less conclusive and potentially more indirect impact of *5-HTT*LPR/rs25531 gene variation compared to depression.

In addition to genetically determined serotoninergic neurotransmission, both disorders share a common neural network, i.e., fronto-limbic/mediotemporal pathways associated with emotional processing including the perception of emotion (limbic bottom-up processing) and affect regulation in terms of frontal top-down control [25–27]. For example, in depression, increased activation in the amygdala [27–29] and in prefrontal regions [30, 31] was found when processing faces with fearful expression. Furthermore, increased amygdala activation was observed in *5-HTT*LPR S allele carriers compared to carriers of the *5-HTT*LPR/rs25531 L_AL_A genotype (e.g., [30]). Likewise, in anxious adolescents and adolescent patients with anxiety disorders, heightened amygdala response to fearful as well and angry faces was reported [25, 32, 33], which seemed to be dependent on symptom severity and self-reported anxiety levels [25, 27]. In accordance with findings in depression, in anxiety phenotypes, the S allele was associated with an exaggerated neuronal reactivity in the amygdala. In the prefrontal cortex (PFC), however, activation was discerned to differ between patients with anxiety and depression when performing an emotional task: while patients with depression tended to present increased frontal activation or to activate additional regions within the PFC, patients with anxiety disorders showed decreased activation [34–36].

In the present study, we examined the effect of the *5-HTT*LPR/rs25531 gene variation ($S + S/L_G + S/L_A + L_GL_A + L_GL_G$ vs. L_AL_A genotypes) on depressive symptoms and trait anxiety using a gene–environment-interaction (G X E) approach in a sample of 389 healthy children and adolescents. We applied mediation and moderation analyses to determine how negative life events shaped the influence of the *5-HTT*LPR/rs25531 genotype on the respective phenotype. In addition, a subsample of 49 subjects underwent fMRI scanning. In this effort, we addressed emotional processing on the behavioral and neural level using the well-established emotional face matching task by Hariri et al. [37, 38] to induce activation in a fronto-mediotemporal network. Genotype-specific multiple regressions were performed to identify those regions, which were related to depressive symptoms or trait anxiety, respectively. In a second step, the interplay between *5-HTT*LPR/rs25531 genotype, negative life events, brain activation (in the fMRI subsample), and depressive symptoms/anxiety traits was statistically determined using mediation and moderation analyses as suggested by Hayes [39]. Both statistical procedures address the relation or hierarchy between several variables, one variable is the independent variable X which holds a certain impact of a second variable Y. If this influence is direct but influenced by a third (or more) variable(s) M, it corresponds to the model of a moderation with the variable(s) M operating as moderators. Is the influence of X on Y indirect via a third (or more) variable(s) M it reflects a mediation model with M mediating the influence X has on Y. In addition, control variables such as age and sex can be added into the model.

Regarding the affective phenotype, individuals carrying the $S + S/L_G + S/L_A + L_GL_A + L_GL_G$ genotypes were hypothesized to have higher depressive symptom scores compared to L_AL_A genotype carriers. On brain level, which was analyzed in an explorative approach, we expected to find task-induced brain activation predominantly in fronto-mediotemporal pathways. We hypothesized that amygdala activation varied as a function of both depressive symptoms and trait anxiety across all genotypes. Frontal activation, however, was supposed to increase with depressive symptoms and decrease with trait anxiety and to be further enhanced in carriers of the $S + S/L_G + S/L_A + L_GL_A + L_GL_G$ genotypes as compared to L_AL_A genotype. Regarding the mediation and moderation analyses, we expected to find both significant gene X brain and gene X environment interactions: based on the literature reviewed above, we expected to find a direct influence of *5-HTT*LPR/rs25531 genotype on depressive symptoms, accompanied by a moderating effect of negative life events (negLE). Trait anxiety, however, was assumed

to be influenced indirectly by the *5-HTT*LPR/rs25531 with negLE constituting a significant mediator.

Materials and methods

Participants

In total, we examined 460 children and adolescents between the age of 8 and 15 years. Children were partly incorporated in a prior study of our research group [40]. Based on missing psychometric data and blood samples, a final data set of 389 participants could be analyzed ($M_{age} = 10.3 \pm 1.5$ years, females: 193). Participants were recruited from local schools in the greater region of Würzburg, Germany within the Collaborative Research Center SFB-TRR-58/subprojects Z02 and C02 funded by the German Research Foundation (DFG). For a first clarification of general inclusion/exclusion criteria, all participants and/or parents were interviewed about the participant's (Caucasian) descent, (right) handedness, fluency in German, presence of any current mental/neurological disorder, family history of mental disorders, and intake of psychoactive medication via telephone by experienced child and adolescent psychologists and psychotherapists working at the Department of Child and Adolescent Psychiatry in Würzburg. At the examination day, participants underwent a full and detailed clinical and neuropsychological screening to ascertain all relevant psychometric data used in the present analyses and to further exclude manifest or lifetime DSM-IV axis I disorder, severe medical conditions, and an IQ < 85 using German versions of the Diagnostic Interview for Mental Disorders for Children and Adolescents (Kinder-DIPS; [41]), the State-Trait Anxiety Inventory for Children (STAIC-T; [42]), and the Depression Inventory for Children and Adolescents (DIKJ; [43]). To measure negative life events (negLE), the German *Zürcher Life-Event List* (ZLEL; [44]) was used. The general IQ score was ascertained using the German version of the Culture Fair Intelligence Test 2 (CFT-20R; [45]), and in the fMRI subsample normal physical development was assessed by means of the Tanner stages [46, 47] in combination with landmarks of puberty (i.e., menarche, pubic hair growth, or voice change). For genotyping, EDTA-blood samples were collected from all participants. A subsample of 50 children and adolescents ($M_{age} = 11.5 \pm 1.5$ years, females: 17) underwent additional fMRI measurements. As one volunteer refused to provide a blood sample for genotyping, the final sample consisted of $N = 49$. Participants as well as their parents/legal guardian gave written informed consent for the participation in the experiment prior to testing. All procedures of this study were in accordance with the Declaration of Helsinki in its latest version and approved by the medical ethics committee of the University of Würzburg, Germany.

Genotyping

Genomic DNA was extracted from whole-blood samples. The total sample and the fMRI subsample were genotyped for the *5-HTT*LPR as well as the related single-nucleotide polymorphism rs25531 according to previously published protocols [24]. Fulfillment of Hardy–Weinberg criteria was determined by the online program DeFinetti (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). For statistical analyses, genotype groups were defined as "L_AL_A" vs. all other genotypes, summarized as "$S + S/L_G + S/L_A + L_GL_A + L_GL_G$ genotype" genotypes.

Task

We administered the emotional face matching task by [38]. Participants were instructed to match a target stimulus (i.e., an emotional face or a geometric shape presented in the upper row of the screen) to one of two stimuli (presented in the lower row of the screen). Participants were asked to indicate which shape or face in the lower row corresponded to the target by pressing a button with their index finger either with the right hand or the left hand, depending on the location of their answer (right face or left face or shape in the lower row).

The task was constructed in a block design with shape matching blocks and emotional face matching blocks being presented in an alternating order. Each block started with a brief introduction statement announcing the condition (shape vs. face) for 2 s. Blocks consisted of six trials, respectively, with a trial duration of 2.9 s. Each trial started with a 400 ms stimulus presentation, followed by a response time of 2.5 s. Inter-trial intervals varied between 1.5 and 5.5 s. The task comprised 9 blocks (5 shape matching and 4 face matching blocks) with inter-block intervals and lasted in total 6.2 min. Prior to scanning, participants successfully completed a training trial to insure the understanding of the instructions.

MRI data acquisition

The scanning was performed on a 3.0 T TIM Trio Scanner (Siemens, Erlangen, Germany). Whole-brain T2*-weighted BOLD images were recorded with a gradient echo-planar imaging sequence (repetition time (TR) = 2000 ms, echo time (TE) = 30 ms, 33 slices, 3 mm thickness, field of view = 192 mm, flip angle = 90°, and 187 volumes). In addition, anatomical images were obtained, using an isotropic high-resolution T1-weighted 3D structural MR images (magnetization prepared rapid gradient echo (MPRAGE), 176 slices, TR = 2300 ms,

TE = 2.95 ms, FoV = 270 mm, flip angle 9°, and slice thickness 1.20 mm).

MRI data processing

Data processing was performed using the Statistical Parametric Mapping Software Package (SPM12, Wellcome Department of Imaging Neuroscience, London, UK, Wellcome Trust Centre for Neuroimaging; http://www.fil.ion.ucl.ac.uk/spm/). The functional images were realigned to the first functional volume as well as unwarped. A spatial normalization into a standard stereotactic space (Montreal neurological Institute, MNI) was conducted, data were resampled to isotropic 2*2*2 mm^3 voxel size and smoothed with a Gaussian kernel of 8 mm FWHM (full width at half maximum). Statistical analysis on the individual first level was based on the general linear model (GLM) approach. Model specification included the definition of experimental conditions "faces" and "shapes". For each condition, block onset times were determined at the time when the block instruction was presented. In addition to the experimental conditions, "realignment parameters" (six regressors containing movement in three spatial and three rotational axes) were specified as nuisance regressors. On the single subject level, the contrast of interest was 'face > shapes' to identify brain activation associated with emotional face matching only, corrected for matching processing. Resulting contrast images entered statistical group analyses.

Statistical analysis

Main statistical analyses of this study were mediation and moderation analyses across all subjects as well as the fMRI subsample. In case of the fMRI subsample, brain activation analyses had to be performed in an earlier step to identify regions of fronto-limbic activation. In detail, we followed the described work flow:

a. A priori differences between *5-HTT*LPR/rs25531 genotype groups ("$S+S/L_G+S/L_A+L_GL_A+L_GL_G$ genotype" vs. "L_AL_A") of trait anxiety, depressive symptoms, and negative life events as well as fMRI-specific behavioral data were determined using two sample t tests (for details, see Table 1). In addition, correlations of phenotype variables were performed to test the interrelation between variables. Finally, a priori effects of sex and age were tested. For the total sample ($n = 389$), a correction for five comparisons was performed (sex, age, STAI-C,

Table 1 Sample description stratified for *5-HTT*LPR/rs25531 genotype groups

	L_AL_A genotype	$S+S/L_G+S/L_A+L_GL_A+L_GL_G$ genotypes	Statistics
N	98	291	
Sex (m/f)	45/53	151/140	$X^2 = 1.0$, $p = 0.350$
Age (SD)	9.9 (1.6)	9.8 (1.4)	$T_{(2,387)} = 0.7$, $p = 0.932$
Phenotypes/life events			
Trait anxiety [STAIC-T]	30.0 (5.9)	29.3 (6.6)	$T_{(2,387)} = 0.8$, $p = 0.410$
Depression severity [DIKJ]	5.4 (4.0)	6.6 (5.4)	$T_{(2,387)} = 1.9$, $p = 0.052$
Negative life events [negLE]	5.1 (3.0)	5.0 (3.5)	$T_{(2,387)} = 0.1$, $p = 0.932$
fMRI subsample			
N	17	32	
Sex (m/f)	12/5	19/13	$X^2 = 0.6$, $p = 0.541$
Age (SD)	12.0 (1.4)	11.2 (1.5)	$T_{(2,47)} = 1.8$, $p = 0.076$
Phenotypes/life events			
Trait anxiety [STAIC-T]	27.8 (6.1)	27.6 (5.7)	$T_{(2,47)} = 0.2$, $p = 0.876$
Depression severity [DIKJ]	2.4 (2.7)	5.8 (5.4)	$T_{(2,47)} = 2.3$, $p = 0.029$
Negative life events [negLE]	2.9 (2.7)	7.0 (5.5)	$T_{(2,47)} = 2.8^*$, $p = 0.007$
Behavioral performance emotional face match task			
Accuracy [overall, %correct]	97.2 (2.8)	96.0 (4.3)	$T_{(2,47)} = 1.0$, $p = 0.313$
Accuracy [faceMatch, %correct]	98.9 (2.1)	98.7 (2.3)	$T_{(2,47)} = 0.2$, $p = 0.864$
Reaction time [overall, ms]	1218 (325)	1267 (223)	$T_{(2,47)} = 0.6$, $p = 0.543$
Reaction time [faceMatch, ms]	1324 (295)	1382 (247)	$T_{(2,47)} = 0.7$, $p = 0.479$

For the total sample ($n = 389$), a correction for five comparisons was performed, resulting in a corrected p threshold of $q^* = 0.01$; for the fMRI subsample ($n = 49$), a correction for nine comparisons was performed, resulting in a corrected p threshold of $q^* = 0.007$

$^*p < q^*$

DIKJ, and negLE); for the fMRI subsample, a correction for nine comparisons was performed (+4 behavioral parameters, see Table 1).

b. *Identification of fronto-limbic activation* For the detection of the activated brain network in the fMRI subsample, multiple regression analyses were executed with the independent factors 'depressive symptoms' (DIKJ) and 'trait anxiety' (STAIC-T), contrast images were defined as dependent variables and age as nuisance variable. Multiple regressions were performed separately for genotype groups ("L_AL_A" and "$S + S/L_G + S/L_A + L_GL_A + L_GL_G$ genotype"). All fMRI analyses were performed in a region of interest (ROI)-based approach only focusing on brain regions in the fronto-mediotemporal pathway (using masks of the AAL atlas [Automated Anatomical labeling, [48]), Frontal_Mid_L/R, Front_Mid_orb_L/R, Front_Inf_oper_L/R, Front_Inf_otri_L/R, Front_Inf_orb_L/R, Front_Med_orb_L/R, Hippocampus:L/R and Amygdala_L/R as implemented in the Wake Forest University PICKATLAS toolbox (http://www.fmri.wfubmc.edu)]. Whole-brain analyses were not performed. Correction for multiple comparisons was performed on voxel level using $p_{FDR\text{-}corr} < 0.05$.

c. After identification of regions associated with the influence of *5-HTT*LPR/rs25531 genotype on depressive symptoms and trait anxiety, respectively, we performed mediation and moderation analyses to address the underlying mechanisms of (a) whether the genotype influenced depressive symptoms and trait anxiety (directly or indirectly) and (b) whether negative life events and/or associated brain activation served as potential mediators/moderators. In detail, we performed mediation and moderation analyses to reveal the structure of the gene X environment X brain interaction on depressive symptoms and trait anxiety. As independent factor Y, we defined the *5-HTT*LPR/rs25531 genotype group ("$S + S/L_G + S/L_A + L_GL_A + L_GL_G$ genotype" vs. "L_AL_A"), and the dependent variable X was either the DIKJ or STAIC-T score. Mediating variables were negative life events (M1) and, in the fMRI subsample, brain activation (M2, i.e., contrast estimates of significantly activated clusters). Age and sex were included in all models as nuisance variables (for models using brain activation as independent variable and without genotype, see table s1). For the whole sample, 4 models (2 mediation and 2 moderation models), for the subsample 18 models for DIKJ (9 mediation/moderation analyses varying in the localization of brain activation), and 12 models for STAIC-T (6 mediation and 6 moderation models varying in the region of neural activation) were tested. Moderation analyses were corrected for 9/6 tests. In mediation analyses, bootstrapping of 10,000 iterations was implemented to ensure effect robustness. Mediation and moderation analyses were performed using the PROCESS software for SPSS by Hayes [39] (see Fig. 1). Post hoc power analyses have been performed, as sample size/statistical power has been proven to be crucial for mediation/moderation analyses [49]. To determine the statistical power for mediation, the online tool MedPower was used (https://davidakenny.shinyapps.io/MedPower/) using partial r coefficients of effect on X on M (path a), effect of M on Y (path b), direct effect of X on X (path c′), and alpha = 0.05. For moderations, G*Power (http://gpower.hhu.de/) was applied using the parameters: *F* tests—Linear multiple regression: Fixed model, R^2 deviation from zero, analysis: Post Hoc: compute achieved power, Input: Effect size $f^2 = 0.15$ α err prob = 0.05 Power ($1 - \beta$ err prob) = 0.80 Number of predictors = 3. For all analyses, a power ≥ 0.80 was generally considered desirable [49].

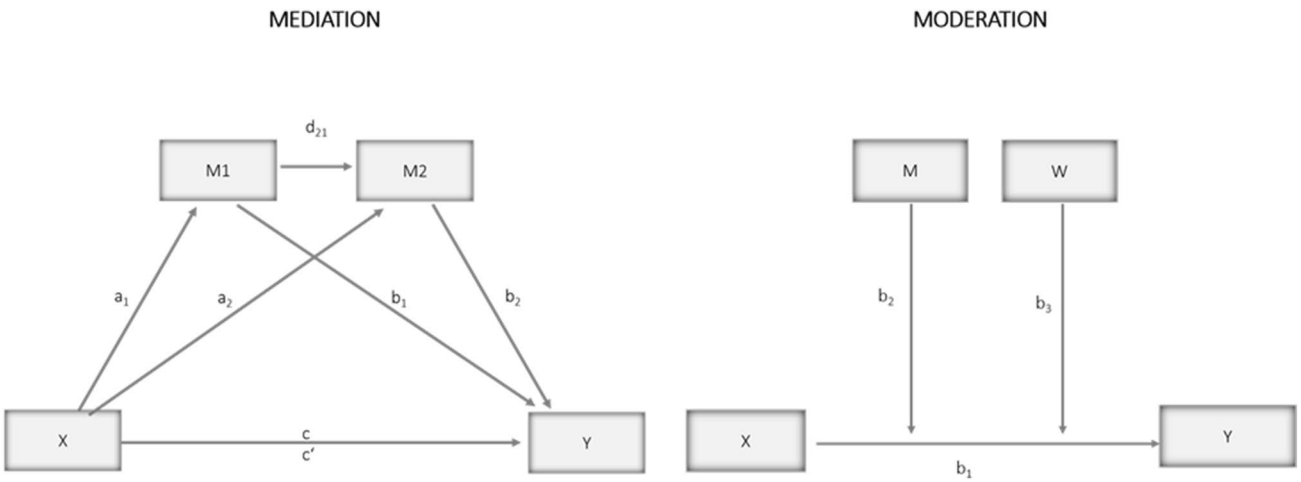

Fig. 1 Structure of mediation and moderation models including the path definitions

All fMRI analyses were performed in a region of interest (ROI)-based approach focusing on brain regions in the fronto-mediotemporal pathway [using masks of the AAL atlas (Automated Anatomical labeling, [48]), Frontal_Mid_L/R, Front_Mid_orb_L/R, Front_Inf_oper_L/R, Front_Inf_otri_L/R, Front_Inf_orb_L/R, Front_Med_orb_L/R, Hippocampus:L/R and Amygdala_L/R as implemented in the Wake Forest University PICKATLAS toolbox (http://www.fmri.wfubmc.edu)].

All results were corrected for multiple comparisons using the false discovery rate as suggested by Benjamini and Hochberg [50] with $P_{corr} < 0.05$. As the number of multiple comparisons varied between analyses, the corrected threshold (q^*) was reported in the respective analyses.

Results

Characteristics of the total sample

In the total sample, average IQ was 108 (SD: 14), the mean number of negLE was 5.0 (SD: 3.4), and dimensional phenotypes for depression and anxiety did not meet clinical criteria ($M_{DIKJ} = 6.3 \pm 5.1$, $M_{STAIC\text{-}T} = 29.5 \pm 6.5$). Correlation analyses revealed that DIKJ and STAIC-T were significantly correlated ($R = 0.525$, $p = 0.000$), and that both DIKJ and STAIC-T were associated with negLE ($R_{DIKJ} = 0.162$, $p = 0.001$; $R_{STAIC\text{-}T} = 0.178$, $p = 0.000$). Neither DIKJ, nor STAIC-T nor negLE were related to age ($R_{DIKJ} = -0.011$, $p = 0.822$, $R_{STAIC\text{-}T} = 0.033$, $p = 0.522$, $R_{negLE} = 0.057$, $p = 0.389$, $n = 389$, p corrected for 5 comparisons revealed $q^* = 0.001$). STAIC-T differed between sexes ($T(2,387) = 4.8$, $p = 0.000$), but DIKJ and negLE did not. There was no significant difference between *5-HTT*LPR/rs25531 genotypes in age, sex distribution, DIKJ, STAIC-T, and negLE (see Table 1). Hardy–Weinberg criteria were fulfilled for *5-HTT*LPR genotype distribution (LL = 106, SL = 208, SS = 75; $p = 0.46$) as well as for the triallelic model ($L_AL_A = 97$, $L_GL_A/SLA = 197$, $L_GL_G/SL_G/SS = 95$; $p = 0.50$).

Gene X environment interactions in the total sample

Moderation analyses revealed for both, depressive symptoms and trait anxiety, a trend-wise significant moderation of negLE on the influence of the *5-HTT*LPR/rs25531 genotype. However, whereas regarding depressive symptoms, both the direct path between *5-HTT*LPR/rs25531 genotype and DIKJ and the influence of negLE on DIKJ were significant, trait anxiety was only significantly moderated by negLE. The direct path did not pass the threshold of significance. In addition, interaction effects differed between both measures. Regarding depressive symptoms, the moderating effect was significantly higher in carriers of the $S+S/L_G+S/L_A+L_GL_A+L_GL_G$ genotype, when the number of negative life events was low-to-average. In subjects with a high number of negative life events, however, the moderating influence of negLE did no longer vary between *5-HTT*LPR/rs25531 genotypes (see also Table 2; Fig. 2). In trait anxiety, however, the moderating effect of negLE was descriptively but not significantly higher in L_AL_A genotype carriers. Here, the difference between genotype increased with the number of negLE (see also Table 2; Fig. 3).

Table 2 Significant moderation of *5-HTT*LPR/rs25531 genotype effects on depression severity [DIKJ] and trait anxiety [STAIC-T] with the moderator *M* = negative life events [negLE], $n = 389$

Model		*R*-sq	MSE		*F*		*Df*		*p*
5HTT → DIKJ		0.06	24.9		6.3*		385.4		0.0001
5HTT → STAIC-T		0.10	38.2		8.1*		385.4		0.0000
Step	Variables		Path	Coeff.	SE	*p*	95% CI		
							LL	UL	
5HTT → DIKJ									
1 (X → Y)	*5-HTT*, DIKJ		b_1	2.3	0.82	0.00*	− 3.8	− 0.72	
2 (M → Y)	negLE, DIKJ		b_2	0.2	0.08	0.01*	− 0.3	− 0.04	
3 (X*M → Y)	*5-HTT**LE → DIKJ		b_3	0.3	0.15	0.06	− 0.6	0.01	
5HTT → STAIC-T									
1 (X → Y)	*5-HTT*, STAIC-T		b_1	3.9	2.6	0.13	− 1.2	9.0	
2 (M → Y)	LE, STAIC-T		b_2	3.6	0.7	0.00*	2.2	5.1	
3 (X*M → Y)	*5-HTT**LE → STAIC-T		b_3	− 2.8	1.4	0.06	− 5.6	0.1	

A correction for two comparisons was performed resulting for DIKJ and STAIC-T in a corrected $p(q^*) = 0.025$

*$p < q^*$

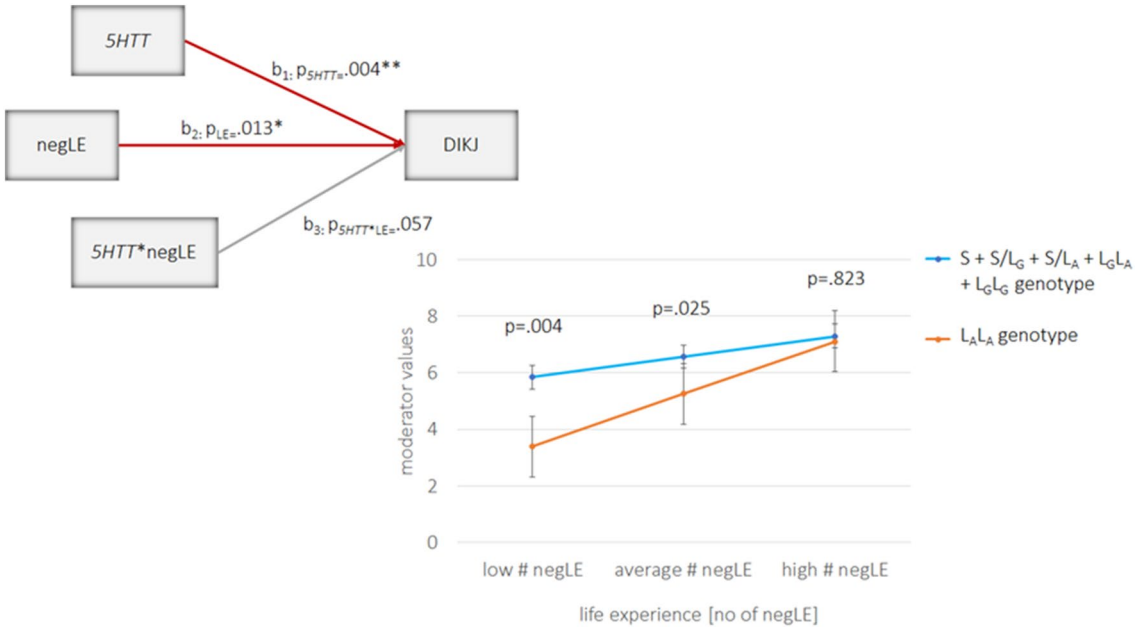

Fig. 2 DIKJ-related moderation model (left) with the p values indicating significant or not significant paths. On the right, the line plot reveals the moderation of the effect of *5HTTLPR*/rs25531 genotype (X) on depression symptoms/DIKJ (Y) by negative life events/negLE (M) in the total sample of $N = 389$ healthy children and adolescents. Moderator values represent the conditional indirect effect of *5HTTLPR*/rs25531 genotype on depressive symptoms at values of the moderator negLE. Error bars represent 1SD

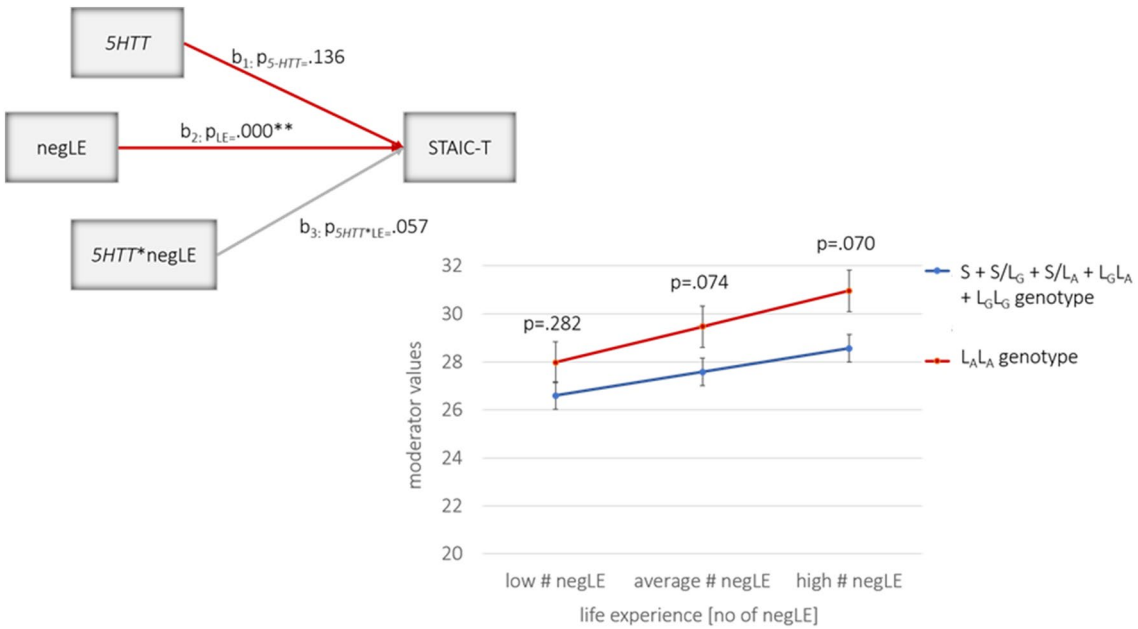

Fig. 3 STAIC-related moderation model (left) with the *p* values indicating significant or not significant paths. On the right, the line plot reveals the interaction between *5-HTT*LPR/rs25531 genotype and the number of negative life events in the total sample of $N = 389$ healthy children and adolescents. Moderator values represent the conditional indirect effect of *5HTTLPR*/rs25531 genotype on trait anxiety at values of the moderator negLE. Error bars represent 1SD

Characteristics of the fMRI subsample

In the fMRI subsample, average IQ was 109 (SD: 15), mean age was 11.5 years (SD: 1.5), mean Tanner stage was 2.1 (SD: 0.9), the mean number of negLE was 5.8 (SD: 5.2), and the dimensional phenotypes for depressive symptoms and trait anxiety did not meet clinical criteria ($M_{DIKJ} = 4.7 \pm 4.9$, $M_{STAIC\text{-}T} = 27.5 \pm 5.8$). Hardy–Weinberg criteria were fulfilled for *5-HTT*LPR genotype distribution (LL = 17, SL = 26, SS = 6; $p = 0.41$) as well as for the triallelic model ($L_AL_A = 17$, L_GL_A/SLA = 19, L_GL_G/SL_G/SS = 13; $p = 0.13$). The overall task accuracy, reaction time. as well as depressive symptoms significantly correlated with age (accuracy: $R_{overall} = 0.378$*, $p = 0.011$; $R_{faceMatch} = 0.002$, $p = 0.992$; reaction times: $R_{overall} = -0.424$*, $p = 0.004$; $R_{faceMatch} = -0.372$*, $p = 0.012$; $R_{STAIC\text{-}T} = -0.172$, $p = 0.252$, $R_{DIKJ} = -0.354$*, $p = 0.022$, $R_{LE,\ pos} = -0.216$, $p = 0.153$, $R_{LE,\ neg} = -0.305$, $p = 0.042$, $q^* = 0.025$). There were no sex differences, neither regarding behavioral performance nor phenotype parameters.

Comparisons revealed that carriers of the $S + S/L_G + S/L_A + L_GL_A + L_GL_G$ genotype had experienced a significantly higher number of negative life events and scored higher (trend to significance) on the DIKJ compared to carriers of the L_AL_A genotype. *5-HTT*LPR/rs25531 genotype groups did not significantly influence any behavioral parameter (see Table 1).

Genotype group-specific multiple regression analyses revealed a positive correlation between depressive symptoms and activation in frontal areas, i.e., bilaterally in the middle frontal gyrus (MFG) and the anterior cingulate cortex (ACC) as well as in the left HC in carriers of the L_AL_A genotype. Trait anxiety was positively correlated with activation bilaterally in the prefrontal cortex, i.e., right and left MFG, and the right IFG pars opercularis in the L_AL_A genotype (Table 3).

Like carriers of the L_AL_A genotype, brain activation correlated positively with depressive symptoms in the left HC and bilaterally in the MFG in $S + S/L_G + S/L_A + L_GL_A + L_GL_G$ genotype carriers. However, activation clusters did not overlap between genotype groups. Trait anxiety, in return, was negatively correlated with activation in the right MFG, similar to the cluster correlating with depressive symptoms, and left amygdala in the $S + S/L_G + S/L_A + L_GL_A + L_GL_G$ genotype group (Table 3).

Gene X environment interactions in the fMRI subsample

With regard to depressive symptoms, we found that the $S + S/L_G + S/L_A + L_GL_A + L_GL_G$ genotype-associated left MFG activation mediated the influence of the *5-HTT*LPR/rs25531 genotype on depressive symptoms, however, only in combination with negative life events (see Table 4; Fig. 4). In addition, coefficients of path a, the influence of the *5-HTT*LPR/rs25531 genotype on negLE, were negative, showing that the influence was driven by the $S + S/L_G + S/L_A + L_GL_A + L_GL_G$ genotypes. Genetic influence on trait anxiety was predominantly mediated by negative life events; only L_AL_A genotype-specific activation in the right MFG regions functioned as mediator in combination with negative life events (see Tables 5, 6; Fig. 5). Like in

Table 3 *5-HTT*LPR/rs25531 genotype group-specific multiple regressions on fMRI data

Contrast	L_AL_A genotype						$S + S/L_G + S/L_A + L_GL_A + L_GL_G$ genotypes					
	k	*X*	*y*	*z*	*T*	Brain region	*k*	*x*	*y*	*z*	*T*	Brain region
DIKJ(+)	33	− 18	− 18	− 18	9.9	Left HC	15	− 10	− 38	6	4.6	Left HC
	15	− 14	− 2	− 14	6.0	Left HC	10	32	− 26	− 10	3.9	Right HC
	59	− 42	50	18	6.3	Left MFG	84	− 28	26	50	4.4	Left MFG
	12	36	44	34	5.2	Right MFG	96	32	26	48	4.2	Right MFG
	11	10	44	18	4.8	ACC	24	48	22	38	4.1	
DIKJ(−)	n.s.						n.s.					
STAIC-T(+)	101	40	44	2	5.5	Right IFG, pars opercularis	n.s.					
	30	42	30	40	5.5	Right MFG						
	12	− 36	46	14	5.1	Left MFG						
	29	36	46	36	5.0	Right MFG						
STAIC-T(−)	n.s.						22	− 24	− 22	− 14	3.9	Left amygdala
							10	50	22	38	3.5	Right MFG

DIKJ Depression Inventory for Children and Adolescents, *STAIC-T* Trait scale of the State/Trait Anxiety Inventory for Children, (+) positive correlation, (−) negative correlation, *HC* hippocampus, *MFG* middle frontal gyrus, *ACC* anterior cingulate cortex, *IFG* inferior frontal gyrus

Results are reported on a significance level of $p < 0.001$, $k = 10$vxls uncorrected on voxel level

Table 4 Significant mediation of *5-HTT*LPR/rs25531 genotype effects on depression severity with the mediators M1 = negative life events and M2 = S + S/L_G + S/L_A + L_GL_A + L_GL_G genotype-specific left MFG activation

Step	Variables	Path	Coeff.	SE	*p*	95% CI		Power
						LL	UL	
1 (X → Y)	*5-HTT*, DIKJ	c	− 2.8	1.5	0.07	− 0.2	5.85	0.93
2a (X → M1)	*5-HTT*, LE	a_1	− 3.3	1.6	0.05*	0.1	6.47	0.80
2b (X → M2)	*5-HTT*, lMFG	a_2	− 0.2	0.2	0.26	− 0.1	0.49	0.22
3 (X + M1/M2 → Y)	*5-HTT* → DIKJ	c′	− 1.1	1.4	0.44	− 1.7	3.9	0.86
	LE/brain → DIKJ							
Indirect effects								
(X → M1 → Y)		a_1 b_1	− 0.9	0.7[a]		− 0.2[b]	2.6[b]	0.80
(X → M2 → Y)		a_2 b_2	− 0.9	0.9[a]		− 0.1[b]	3.6[b]	0.22
(X → M1 → M2 → Y)		a_1 d_{21} b_2	− 1.7	1.1[a]		0.1[b]	4.6[b]	

c = total effect, a, b = indirect effect, c′ = direct effect

*$p < .05$

[a]Bootstrapping standard error

[b]Bootstrapping confidence interval, alpha for all power calculations set to 0.050. Effects (a, b, and c′) are Betas estimated from partial correlations

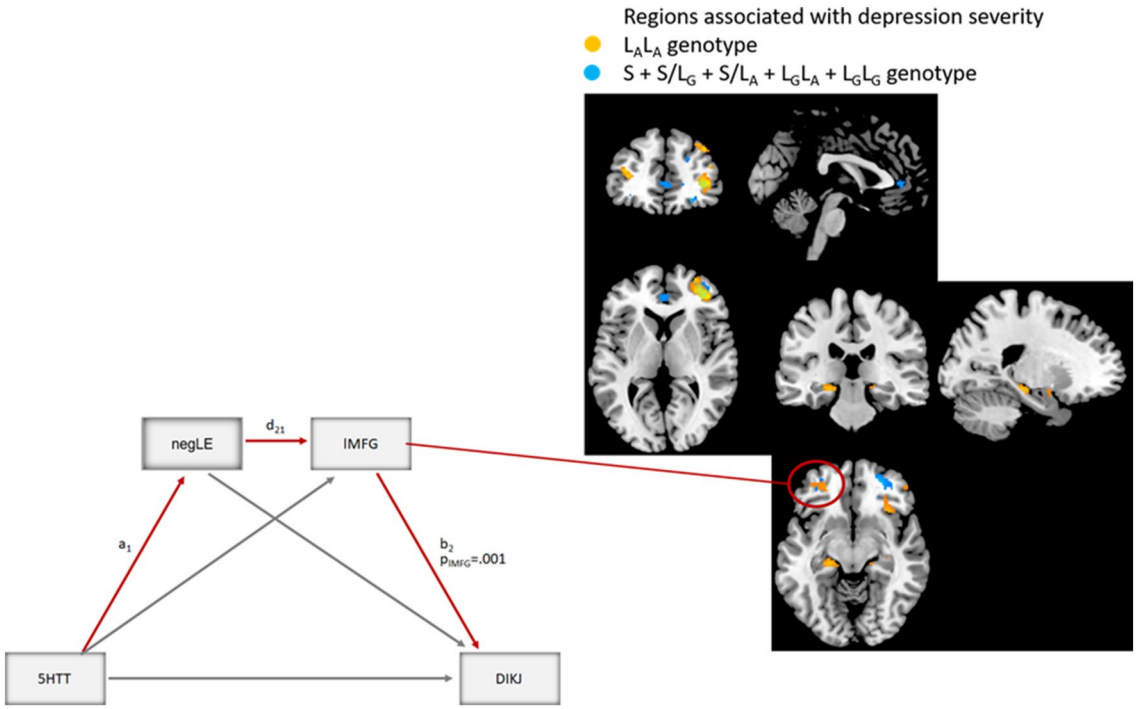

Fig. 4 Activation of brain regions, which significantly correlated with depression severity, stratified for *5-HTT*LPR/rs25531 genotype groups in the fMRI subsample ($N = 49$). On the left, the moderation model is presented showing the significant indirect influence of negative life events (negLE, moderator #1) and brain activation (moderator #2), indicated by red arrows. Gray arrows show not significant paths

depressive symptoms, coefficients of path a were negative, reflecting a priori differences in negLE between genotype groups. Post hoc analyses of statistical power were determined for each path (for details, please see Tables 4, 5, 6).

Discussion

In this study, we examined the influence of *5-HTT*LPR/rs25531 genotype on two different but highly overlapping

Table 5 *5-HTT*LPR/rs25531 L_AL_A genotype-specific mediation for trait anxiety

Path	Right IFG (40, 44, 2)						Right MFG (42, 30, 40)					
	Coeff.	SE	*p*	95% CI		Power	Coeff.	SE	*p*	95% CI		Power
				LL	UL					LL	UL	
c	− 0.9	1.9	0.66	− 4.8	3.1	0.73	0.9	1.9	0.66	− 4.8	3.1	0.73
a_1	− 3.3	1.6	0.05*	0.1	6.4	0.80	− 3.3	1.6	0.05*	0.1	6.5	0.80
a_2	0.2	0.1	0.10	− 0.5	0.1	0.56	− 0.1	0.2	0.60	− 0.2	0.4	0.08
c′	2.1	1.9	0.28	− 5.9	1.8	0.22	2.8	1.8	0.13	− 6.5	0.9	0.21
a_1 b_1	− 1.7	1.0[a]		0.1[b]	4.0[b]	0.78	− 1.8	1.0[a]		0.2[b]	4.3[b]	0.77
a_2 b_2	0.5	0.7[a]		− 2.6[b]	0.3[b]	0.54	− 0.1	0.3[a]		− 0.1[b]	1.2[b]	0.08
a_1 d_{21} b_2	− 1.2	1.2[a]		− 0.9[b]	3.7[b]		− 2.0	1.1[a]		0.2[b]	4.6[b]	
Path	Left MFG (− 36, 46, 14)						Right MFG (36, 46, 36)					
	Coeff.	SE	*p*	95% CI		Power	Coeff.	SE	*p*	95% CI		Power
				LL	UL					LL	UL	
c	− 0.9	1.9	0.66	− 4.8	3.1	0.73	− 0.9	1.9	0.66	− 4.7	3.1	0.76
a_1	− 3.3	1.6	0.05*	0.1	6.4	0.80	− 3.3	1.6	0.05*	0.1	6.5	0.80
a_2	0.2	0.1	0.29	− 0.2	0.1	0.26	− 0.1	0.1	0.76	− 0.2	0.3	0.09
c′	2.4	1.8	0.20	− 6.2	1.4	0.22	2.9	1.7	0.10	− 6.4	0.5	0.21
a_1 b_1	− 1.8	1.0[a]		0.2[b]	4.2[b]	0.77	− 1.9	0.9[a]		0.3[b]	4.0[b]	0.79
a_2 b_2	0.3	0.4[a]		− 1.6[b]	0.2[b]	0.25	− 0.2	0.7[a]		− 1.0[b]	2.0[b]	0.09
a_1 d_{21} b_2	− 1.6	1.1[a]		− 0.3[b]	4.1[b]		− 2.1	1.2[a]		0.1[b]	5.0[b]	

c = total effect, a, b = indirect effect, c′ = direct effect

*$p < .05$

[a]Bootstrapping standard error

[b]Bootstrapping confidence interval, alpha for all power calculations set to 0.050. Effects (a, b, and c′) are Betas estimated from partial correlations

Table 6 *5-HTT*LPR/rs25531 $S + S/L_G + S/L_A + L_GL_A + L_GL_G$ genotype-specific mediation for trait anxiety

Path	Left amygdala (− 24, − 22, − 14)						Right MFG (50, 22, 38)					
	Coeff.	SE	*p*	95% CI		Power	Coeff.	SE	*p*	95% CI		Power
				LL	UL					LL	UL	
c	− 0.9	1.9	0.66	− 4.8	3.1	0.73	− 0.9	1.9	0.66	− 4.8	3.1	**0.72**
2.a_1	3.3	1.6	0.05*	0.1	6.4	0.80	3.3	1.6	0.05*	0.1	6.4	0.80
a_2	− 0.1	0.1	0.9	− 0.2	0.2	0.24	0.1	0.2	0.6	− 0.3	0.5	0.27
c′	− 2.5	1.8	0.18	− 6.2	1.2	0.21	− 2.6	1.9	0.17	− 6.4	1.2	0.22
a_1 b_1	1.6	0.9[a]		.07[b]	3.8[b]	0.77	1.8	1.0[a]		0.1[b]	4.2[b]	0.76
a_2 b_2	0.1	0.3[a]		− 0.4[b]	1.0[b]	0.07	− 0.1	0.2[a]		− 1.0[b]	0.2[b]	0.09
a_1 d_{21} b_2	1.6	1.0[a]		− 0.1[b]	3.9[b]		1.7	1.1[a]		− 0.2[b]	4.1[b]	

c = total effect, a, b = indirect effect, c′ = direct effect

*$p < .05$

[a]Bootstrapping standard error

[b]Bootstrapping confidence interval, alpha for all power calculations set to 0.050. Effects (a, b, and c′) are Betas estimated from partial correlations

affective phenotypes, i.e., depressive symptoms and trait anxiety, in healthy children and adolescents to delineate common features as well as specificities. As mediating and moderating factors, negative life events and fronto-limbic activation were hypothesized to have a strong impact on the developing brain. Results will be discussed first regarding $S + S/L_G + S/L_A + L_GL_A + L_GL_G$ genotype-specific effects and then regarding an L_AL_A genotype-specific effect.

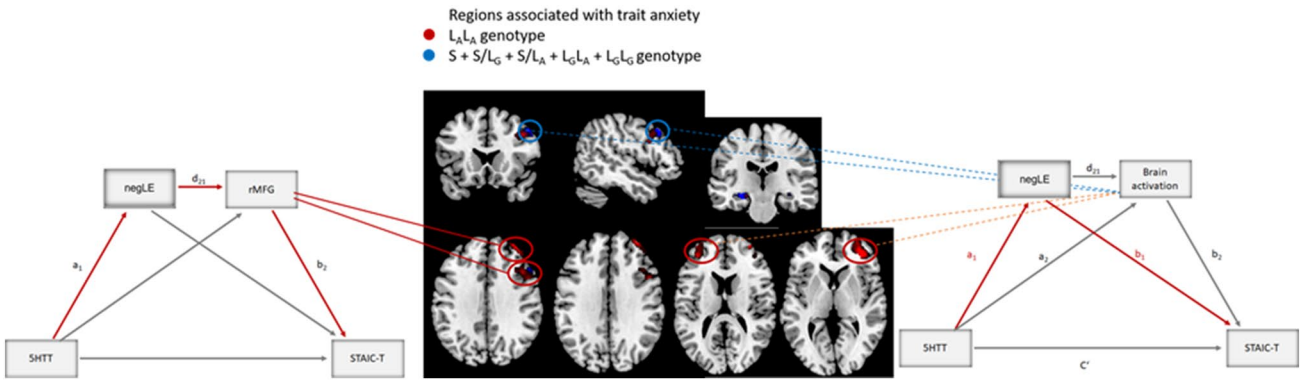

Fig. 5 Activation of brain regions, which significantly correlated with trait anxiety, stratified for *5-HTT*LPR/rs25531 genotype groups in the fMRI subsample ($N=49$). On the left, the moderation model is presented showing the significant indirect influence of negative life events (negLE, moderator #1) and brain activation (moderator #2). On the right, the moderation mode of the significant indirect influence of negative life events is depicted. In both diagrams, red arrows represent significant paths and gray arrows not significant ones

In line with our hypotheses, in the total sample, *5-HTT*LPR/rs25531 genotype had a direct influence on depressive symptoms the way that the $S + S/L_G + S/L_A + L_GL_A + L_GL_G$ genotype was associated with higher depression symptoms. This influence was moderated by negative life experience, when the number of negLE was low-to-average. However, in subjects with negative life experience above average, the interaction was no longer significant, as in both genotype groups, the influence of negLE on depressive symptoms was of comparable strength. The strong influence of the $S + S/L_G + S/L_A + L_GL_A + L_GL_G$ genotype on depressive symptoms has been reported in numerous studies before [14, 18], as well as the moderating effect of negative life experience. However, the presently observed leveling effect has not been reported before. Even though these results seem to be plausible in the context of earlier findings from adults, the present finding needs replication and validation in larger samples as well as in adults, possibly also taking into account an extended gene X environment X coping approach to address the potentially buffering effect of protective influences on G X E risk constellation (cf [23]). In contrast to our hypotheses, negative life events in the total sample served as a moderator on the influence of the *5-HTT*LPR/rs25531 genotype on trait anxiety. However, analyses revealed that, in line with earlier findings supporting the crucial role of negLE in anxiety [5], only the path between negLE and STAIC-T was significant, the direct path from genotype to trait anxiety remained insignificant across all subjects (i.e., subjects with a low/average/high number of negLE). In the fMRI subsample, a mediating effect of negLE became significant.

$S + S/L_G + S/L_A + L_GL_A + L_GL_G$ genotype-specific results

In line with our hypotheses, in both samples, $S + S/L_G + S/L_A + L_GL_A + L_GL_G$ genotype carriers displayed higher depressive symptom scores. The fact, that there was no effect on trait anxiety might be due to the age distribution of the samples and the different onsets of depression and anxiety. The mean developmental stage in the fMRI subsample which can be considered representative for the total sample was Tanner Stage 2, which is at the beginning of puberty and in the transition to adolescence. Thus, a stronger effect regarding depressive symptoms might be due to the imbalance between the onsets of depression and anxiety: whereas depression is more prevalent in adolescence [1], anxiety has an earlier age of onset than depression (e.g., [51]) and is overall more prevalent in childhood [52] compared to adolescence. In addition, the present failure to detect a significant impact of *5-HTT*LPR/rs25531 genotype on trait anxiety reflects the inconsistent body of previous evidence regarding a specific *5-HTT*LPR/rs25531 risk-genotype constellation in anxiety-related phenotypes (see "Introduction"). In addition, in the fMRI sample, $S + S/L_G + S/L_A + L_GL_A + L_GL_G$ genotype carriers had a significantly higher number of negative life events compared to L_AL_A genotype carriers. As this finding did not apply to the total sample, it might not be representative and underlines the necessity of larger samples sizes for imaging-genetic approaches [49].

On the neural level, carriers of the $S + S/L_G + S/L_A + L_GL_A + L_GL_G$ genotype showed a significant increase in middle frontal activation with depressive symptoms, reflecting a potential compensatory mechanism as described in earlier studies [30, 31]. In addition, increased

hippocampal activation as presently observed in $S+S/L_G+S/L_A+L_GL_A+L_GL_G$ genotype carriers has previously been reported in depressive patients [53], predominantly when processing negative stimuli [54, 55]. Hippocampal alterations have also been discussed 'to mediate the effect of long-term stressful life events on depression risk rather than constituting a disease marker' [56]. Findings of significant associations between traumatic life events in childhood and impaired emotion regulation [57] with reduced hippocampal volumes have been reported in healthy adults [58, 59] and adolescents with and without depression [60]. In our study, however, we did not discern a significant influence of life events on brain activation. Mediation analyses revealed a significant direct influence of *5-HTT*LPR/rs25531 genotype on life events and an indirect influence on depressive symptoms via life events and MFG activation, supporting the mediating effect of life events in the context of depression, but not its relation to brain activation, particularly hippocampal function.

The present finding of a direct gene X negLE association in the total sample of n = 389, in that carriers of the *5-HTT*LPR/rs25531 $S+S/L_G+S/L_A+L_GL_A+L_GL_G$ genotypes experienced a higher number of life events as compared to carriers of the L_AL_A genotype is in line with earlier moderation analyses. However, earlier findings revealed an age-specific effect: while in adolescence (e.g., [13, 61–63]), life events seemed to mediate/moderate a serotonergic influence, and this was no longer the case in adulthood [64, 65]. Authors argued that the acquisition of (early) life events in adults consisted predominantly via interviews/questionnaires, where subjects had to report life history retrospectively. Thus, in an adult sample, these experiences had been made partially over decades ago and thus were potentially confounded by recall bias [66]. Finally, mediation analyses of the fMRI subsample revealed that *5-HTT*LPR/rs25531 genotype constellation did not directly influence depressive symptoms, but via MFG activation patterns as well as life events. This notion is strengthened by earlier animal as well as human studies [67–71] implicating frontal involvement, partly mediated by environmental factors. For example, Bartollomucci et al. reported that heterozygous *5-Htt* knockout mice which were exposed to stress in childhood showed lower levels of serotonin metabolism in the frontal cortex [71].

Regarding anxiety, MFG activation was decreased in $S+S/L_G+S/L_A+L_GL_A+L_GL_G$ carriers as hypothesized. Likewise, activation in the amygdala was found to be decreased which seems to be counterintuitive (e.g., [72]). A reduction in amygdala response has been described during processing of neutral/positive scenes [73], neutral words dependent on *5-HTT*LPR/rs25531 genotype [74], as well as—independently of *5-HTT*LPR/rs25531 genotype—in fear conditioning during the extinction phase [75]. Thus, it is possible that $S+S/L_G+S/L_A+L_GL_A+L_GL_G$ genotype carriers in our sample did not perceive the stimuli as 'notably' negative or developed a tolerance for the face expressions presented.

L_AL_A genotype-specific results

In the total sample, L_AL_A genotype carriers demonstrated a higher moderation strength of negative life events on trait anxiety as compared to carriers of the $S+S/L_G+S/L_A+L_GL_A+L_GL_G$ genotypes. In addition, moderation increased with the number of negative life events, supporting the close interrelation between these three variables as described, e.g., in the concept of 'plasticity genes' [76]. 'Plasticity genes' is an extensional concept of the term 'risk allele' by taking not only the biological/genetic risk into account but also the (associated) altered susceptibility for environmental influences. Based on the findings of differential 's' and 'l' allele functioning when interacting with stressful life events also in our sample, it is a promising concept. In the fMRI subsample, the L_AL_A genotype was associated with increased brain activation in relation to both, depressive symptoms and trait anxiety. Regarding the depression-related phenotype, L_AL_A genotype carriers showed an increase in ACC activation in addition to prefrontal and hippocampal areas. The ACC has previously been associated with affective traits [77], self-reported depressive symptoms [78], and affective processing [79, 80], along with altered glutamatergic and GABAergic neurotransmission in depression [81, 82]. The fact that not only serotonin but also other neurotransmitter systems play crucial roles in depression has been reviewed before (e.g., [83–85]). Against the hypothesis, L_AL_A genotype carriers showed a stronger activation of the PFC associated with trait anxiety. Similar results have been reported in a PET study showing increased metabolism for the *5-HTT*LPR L/L genotype in the left middle frontal gyrus [86]. Independently of *5-HTT*LPR/rs25531 genotype constellation, correlations between frontal activation and trait anxiety have been described in volunteers performing tasks of working memory [87], inhibitory control [88], as well as at rest [89]. However, the overall body of literature is very inconsistent. In the present sample comprising children and adolescents, the ongoing frontal maturation might have constituted an additional relevant factor, as frontal specialization is still poor during this age range [90].

Finally, the findings emerging from mediation analyses revealed that life events were significant mediators of the influence of *5-HTT*LPR/rs25531 genotype constellation on trait anxiety across all regions of interest. This finding extends previous results. For example, Klauke et al. reported an interaction with childhood negative life events in healthy subjects carrying the more active *5-HTT*LPR L/L *5-HTT*LPR/rs25531 L_AL_A genotype, respectively

[19]. Likewise, negative life events have previously been reported as important mediator for anxiety disorders [3, 91] as well as anxious behavior (startle response: [92] and exploratory behavior: [93]). Interaction with frontal activation, however, has not been described before. However, in the study by Pagliaccio et al., the interplay between genes involved in the hypothalamus–pituitary–adrenal stress axis, life events, and fronto-amygdala activation [94] has been addressed in school-aged children identifying a weakening effect of genetic risk and stress exposure on fronto-amygdalar connectivity. In this study, we examined two samples which differed with regard to sample size and the availability of fronto-limbic activation data. Therefore, a comparison between the results is not possible without limitations. However, we interpret these results as complementary, since a priori differences, such as depressive symptoms, varied between genotype groups in a similar fashion.

The differences across genotypes regarding the number of negative life events in the fMRI subsample, in return, might be relativized, as they could not be observed in the total sample. The significant difference in negLE in the fMRI sample might have overestimated the influence of negative life events in the mediation, and possibly, at the expense of fronto-limbic influence. Similarly, trait anxiety differed between sexes in the total group, but not in the fMRI subsample. Numerous studies reported regarding sex-specific serotonergic modulation in anxiety as well as depression (for reviews, [95, 96]), even at young ages (e.g., [97]). Thus, an influence of sex on our models could not be generally excluded, particularly since in the smaller fMRI sample, differences might have been obscured by the small sample size. Thus, and to harmonize statistical models, we included sex as nuisance variable in all models, risking the overestimation of sex in the fMRI sample. Thus, results emerging from this study have to be considered preliminary and warrant replication in larger, well-characterized samples. Furthermore, we did not consider developmental effects, even though gene-by-development interactions (for review, [97]), i.e., the variation of genetic influence over lifetime is a crucial question and significant for future research. Instead, we used age as nuisance variable to focus on age-independent effects. The reason for this approach was the small sample size and age distribution in groups stratified for genotype. Furthermore, and in contrast to other studies, we defined genotype as independent factor and not as mediator in our mediation analyses. Based on the fact that, in contrast to all our other parameters, genotype is a fixed and stable variable, and we followed the suggestions by Hayes for the definition of biological process models [98].

Conclusions

In sum, the present findings hint towards distinct mechanisms underlying the influence of *5-HTT*LPR/rs25531 genotype constellation on depressive symptoms and trait anxiety with negative life events playing a crucial role in both depression and anxiety. Regarding depressive symptoms, however, this influence was only visible in combination with MFG activation, whereas regarding anxiety, it was independent of brain activation.

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Compliance with ethical standards

Conflict of interest All authors declared no conflict of interests.

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