Effect of oxytocin receptor gene variants and stressful experiences on ventral striatal activity and risk for social-affective problems

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Keywords:

Oxytocin, genetics, functional magnetic resonance imaging, ventral striatum, amygdala, social behavior

Abstract: 250 words

Word length: 3,941

Tables: 2

Figures: 5

Supplementary Materials: 9

Abstract

Background: Common variants in the oxytocin receptor-gene (*OXTR*) are known to influence social and affective behaviour, and to moderate the effect of adverse experiences on risk for social-affective problems. Whereas human functional neuroimaging studies have reported that oxytocin effects on social behaviour and emotional states are mediated by amygdala function, animal models indicate that oxytocin-receptors in the ventral striatum modulate sensitivity to social reinforcers. This study aimed to comprehensively investigate *OXTR*-dependent brain mechanisms associated with social-affective problems.

Methods: In a sample of 1,445 adolescents we tested the effect of 23-tagging SNPs across the *OXTR* region and stressful life events (SLE) on fMRI-BOLD activity in the ventral striatum (VS) and amygdala to animated angry faces. SNPs for which gene-wide significant effects on brain function were found were then carried forward to examine associations with social-affective problems.

Results: A gene-wide significant effect of rs237915 showed that adolescents with minor CC-genotype had significantly lower VS activity than CT/TT-carriers. Significant or nominally significant GxE effects on emotional problems (in girls) and peer problems (in boys) revealed a strong increase in clinical symptoms as a function of SLEs in CT/TT-carriers but not CC-homozygotes. However, in low-SLE environments, CC-homozygotes had more emotional problems (girls) and peer problems (boys). Among CC-homozygotes, reduced VS activity was related to more peer problems.

Conclusions: These findings suggest that a common *OXTR*-variant affects brain responsiveness to negative social cues, and that in “risk-carriers” reduced sensitivity is simultaneously associated with more social-affective problems in “favourable environments” and greater resilience against stressful experiences.

Introduction

The neuropeptide oxytocin (OT) is an important regulator of social behaviour and affective states (1). It has been shown to influence social attachment, eye contact (2) and empathy (3) among others, and has been implicated in several disorders involving social-affective dysfunctions, including autism spectrum disorder (4), anxiety disorders (5) and depression (6). OT is synthesized in the paraventricular nuclei of the hypothalamus and acts peripherally as a pituitary hormone, and centrally as a neuromodulator. Centrally released, OT-neurons project to several brain regions involved in social-affective processing, including the nucleus accumbens in the ventral striatum and the amygdala (7). However, OT effects are to a significant extent dependent on expression and function of oxytocin receptors (8). Hence, genetic variations in the oxytocin receptor gene (*OXTR)* have become particularly interesting candidates for the understanding of individual differences in social-affective behaviour and dysfunctions. The human *OXTR* is located on chromosome 3p25, contains four exons and three introns, and encodes a polypeptide belonging to the G-protein-coupled receptor family. Genetic studies of single nucleotide polymorphisms (SNPs) in the *OXTR* revealed associations with several social-affective phenotypes, including empathy (9), maternal sensitivity (10), risk for affective symptoms (11), attachment insecurity (12) and depression (13).

Furthermore, recent studies have shown that common variants in the *OXTR* moderate the risk of emotional problems after the experience of early adversity (14)(15).

However, the intermediate neurobiological mechanisms underlying these behavioural observations are not fully understood. Several recent pharmacological functional magnetic resonance imaging (fMRI) studies investigated the effect of intranasal OT-administration on the amygdala, as the amygdala has dense OT-receptors and is involved in social and emotional processing, and threat-detection (16). They reported that OT-inhalation significantly attenuated amygdala response to

angry, fearful (17) and happy faces (18) and during fear conditioning (19). This suggested that oxytocin facilitates social-affective behaviour by reducing anxiety, neuroendocrine stress response, or more broadly arousal (16, 17) (but see (20) for the opposite effect in women). Recent gene-neuroimaging studies also showed *OXTR*-genotype effects on amygdala volume (21-23) and function (23). However, the directionality of the effect is not always consistent. For example, the gene-fMRI study observed that risk-carriers with lower social temperament had in fact lower amygdala response to fearful/ angry faces. Also reduced amygdala-activity found in autism (24) and conduct disorder (25) indicates that it is not necessarily associated with increased social behaviour. One reason for these inconsistencies may be moderating environmental factors as studies in rats (26) and mice (27) suggest that *OXTR*-receptor levels in limbic regions, including the amygdala, are affected by early stressful experiences.

Animal models with voles suggest a second neurobiological pathway by which oxytocin influences social behaviour (1). The comparison between high social-affiliative prairie voles and low social-affiliative montane voles has shown that OT-receptors in brain regions involved in reward and reinforcement processing, primarily the nucleus accumbens (NAcc) / ventral striatum but also the basal amygdala (28) are necessary for social-affiliative behaviour. High social-affiliative prairie voles, especially females, were also more affected by adverse environmental factors than low social-affiliative species, as manifested in decreased regional OT-receptor expression and long-lasting detrimental effects on social behaviour (29).

Hence, the present study tested two novel hypotheses. First, the results of the vole model shift the emphasis from the amygdala to the ventral striatum and suggest that *OXTR*-effects on social-affective behaviour may be modulated by individual differences in sensitivity to social reinforcers (30). Second, we tested the hypothesis

that *OXTR-v*ariants moderate the effect of stressful experiences on brain function and behaviour. We carried out a comprehensive analysis in 1,445 adolescents from the IMAGEN sample to investigate the effect of 23 tagging-SNPs covering the *OXTR* gene and stressful life events (SLEs) on fMRI BOLD activity in the ventral striatum and the amygdala to angry faces. We used an angry face paradigm, as in healthy individuals angry face expressions act as a social threat and as a potent negative social reinforcer, and evoke activity in the amygdala (31) as well as the ventral and dorsal striatum (32). SNPs for which gene-wide significant effects on brain function were found were then carried forward to test their main and interaction effects on emotional problems, peer problems, conduct problems and pro-social behaviour (33). Finally, we examined whether *OXTR*-effects on brain function contributed to behavioural differences.

Methods and Materials

Participants

The IMAGEN sample comprises approximately 2,000 adolescents aged 13-14 years who were recruited from local high schools and tested in eight assessment centres across Europe (see (34)). In the current study, 1,445 adolescents of European origins (697 boys (48.2%), 748 girls (51.8%), mean age 14.4 years (range: 12.8-15.6) were included. **Supplementary Table 1** lists excluded data-sets due to missing genotypic or phenotypic information, non-Caucasian ancestry or failure to meet various quality control criteria. The study was approved by local ethics research committees at each site. Parents and adolescents gave written consent and assent, respectively.

Genotyping.

DNA was extracted from whole blood samples (~10ml). *OXTR*-SNP information was drawn from the Illumina HumanHap610 and HumanHap660 Genotyping BeadChips.

In addition, two of the most frequently tested SNPs, rs2254298 and rs2268494 were genotyped by KBiosciences (Hoddesdon, UK) using taqman genotyping assays. SNPs with call rates of <98%, minor allele frequency < 1%, deviation from the Hardy-Weinberg equilibrium ($p \leq 1 \times 10^{-4}$), and non-autosomal SNPs were excluded. Identity-by-state similarity was used to estimate cryptic relatedness for each pair of individuals using PLINK software (35). Closely related individuals with Identity-by-descent (IBD > 0.1875) were eliminated from the analysis. Population stratification for the GWAs data was examined by principal component analysis (PCA) using EIGENSTRAT software (36) and the four HapMap populations as reference groups. Individuals with CEU-divergent ancestry were excluded. For each SNP, minor allele frequencies are shown in **Supplementary Table 2**. A linkage disequilibrium plot is provided in **Supplementary Figure 1**.

Functional Magnetic Resonance Imaging (fMRI).

fMRI data acquisition and processing. fMRI data was acquired in eight IMAGEN assessment centres with 3T MRI scanners (Siemens, Philips, General Electric, Bruker) using the same scanning protocol. Briefly, BOLD functional images were acquired with a gradient-echo, echo-planar imaging (EPI) sequence. For the Angry Faces Task, 160 volumes were collected per subject, each comprising 40 slices (slice thickness: 2.4 mm, 1 mm gap, matrix: 64^2) parallel to the anterior-commission/ posterior commission line. A short echo-time (TE=30 ms, TR=2.2s) was used to optimize reliably imaging of subcortical areas.

Image processing and analysis were performed using SPM 8 (Statistical Parametric Mapping, http://www.fil.ion.ucl.ac.uk/spm). Preprocessing was performed centrally (Neurospin, CEA) and comprised slice-timing correction, spatial realignment to the first volume, and non-linear warping of each EPI to an EPI-template. Images were then smoothed with a Gaussian kernel of 5-mm full-width at half-maximum.

At the individual subject level, for each experimental condition, each trial was convolved with the hemodynamic response function to form regressors. Estimated movement parameters (3 translations, 3 rotations, 3 quadratic and 3 cubic translations, and each 3 translations with a shift of ± 1 TR) were added to the design matrix. For each contrast, individual SPMs were then taken to second-level group analyses. Summary statistical maps were thresholded at $p < 0.05$ family-wise error (fwe)-corrected.

Angry Faces Task (37).

Participants passively viewed short (2-5 secs) black-and-white video clips of male and female faces facing the viewer. 5 blocks (à 18 secs) showed faces with animated anger expressions, 5 blocks showed faces with ambiguous expressions (e.g., raising eye-brows, nose-twitching). They were interleaved with 9 blocks of expanding-and-contracting concentric circles, which served as a control condition. All gene x neuroimaging analyses reported below focused on the Angry Faces vs. Control contrast.

Stressful life events (SLEs)

SLEs were measured using an adapted version of the Life Events questionnaire (LEQ) (38). The LEQ is a self-report measure that includes 39-items that probe for the occurrence of positive and negative life events over the entire life span, and over the past year. As we reasoned that stressful experiences may have a differential effect on brain function/ clinical symptoms than neutral/ positive experiences, we derived a separate subscale of specifically stressful life events ever experienced based on the quality judgements of N=1,239 adolescents who had experienced the event (see **Supplementary Table 3**).

Clinical symptoms

The Strength and Difficulties Questionnaire (SDQ) provides a dimensional assessment of social-affective symptoms including emotional problems (anxiety-depression), peer problems, conduct problems, and hyperkinetic symptoms, as well as prosocial behaviour (33). In the current study, continuous scores that combined self and parental reports were used. The sub-scale "hyperkinetic problems" was not considered, as a link with *OXTR*-variants was not *a priori* predicted.

Statistical analyses

Region of interest analysis (ROIs). Functional ROIs were created using the Marsbar toolbox (http://marsbar.sourceforge.net). For the amygdala, peak activation coordinates of the angry faces vs. control contrast (height threshold: p<.05 fwe-corrected) were used as sphere centres (left amygdala: 8 mm radius =-18, -7, -14; right amygdala: 8 mm radius + 21, -7, -14). Functional ROIs of the ventral striatum were defined based on a meta-analysis of reward processing (39) (left ventral striatum, 10 mm radius: -16, 12, -10; right ventral striatum: 10 mm radius: +12, 10, -8]. This is because due to the large sample size, the random-effects analysis revealed a very large cluster (12,000 voxels) with peaks in the posterior fusiform gyrus and the left and right amygdala (**Supplementary Table 4**). Although this cluster also included voxels in ventral and dorsal portions of the striatum, it was not easy to clearly delineate peak-coordinates. For each univariate analysis the mean contrast values within the ROI were used as the dependent measure.

Separate regression models were fitted to test for the effect of *OXTR*-SNP, SLEs and GxE interactions on each ROI. Sex and study centre were included as covariates of no interest. Handedness and age were not included as initial analyses revealed no effect on either ROI. Gene-wide significance was empirically determined using permutations that corrected for 23 SNPs (accounting for their LD structure), 4 ROIs, and the number of tests (main effects of SNPs, GxE interactions).

Whole brain regression analyses. For exploratory whole-brain analyses, the same multiple regression model was fitted as described for the ROI analyses. Voxel-wise height threshold was set at p<.001 (uncorrected). Statistically significant differences are reported as voxel-intensity T-values for clusters at p<.05 fwe-error corrected using Gaussian Random Field Theory.

Analysis of clinical symptoms. We used Poisson regression models to examine *OXTR*-genotype main and GxE interaction effects on emotional problems, conduct problems, peer problems, and prosocial behavior. Sex and study site were included as covariate of no interest. The empirical NULL distribution was determined using 100,000 permutations of the variables of interest. We report the more conservative p-values (p_{emp}) corresponding to the empirical NULL distribution as this was found to diverge from the theoretical NULL. Significance thresholds were Bonferroni-corrected for the number of tests per SNP (rs237915: genotype, GxE) and the 4 symptom scales.

Internal validation using bootstrapping. We used bootstrapping, a well-established statistical resampling procedure, to estimate the robustness of our main findings (40). The bootstrapping procedures used for general linear models and poisson models as well as additional simulations carried out are described in **Supplementary Methods 1**.

Results

Whole-brain analyses.

The angry faces vs. control contrast evoked strong activation in an extended cluster centering on the right posterior fusiform gyrus ($p_{fwe\text{-}corrected}$ <.0001) and the right and left amygdala, and extending to frontal and striatal regions, among others.

We also observed a separate significant cluster in more dorsal portions of the caudate ($p_{fwe\text{-}corrected}$ <.0001) (**Supplementary Table 4**).

Region of interest analyses

Region of interest analyses showed gene-wide significant effects of rs237915 and rs237893 in the left ventral striatum (rs237915: F(2,1438)=9.4, $p_{fwe\text{-}corrected}$ =.0009; rs237893: F(2, 1436)=7.68, $p_{fwe\text{-}corrected}$=.007) and of rs237915 in the right ventral striatum (F(2,1438)=8.3, $p_{fwe\text{-}corrected}$=.004) (**Table 1**). Minor allele homozygotes of both SNPs had reduced BOLD-responses in the ventral striatum (**Figure 1**). In addition, we found several nominally significant SNP effects in the left and right ventral striatum, including rs53576, rs35498753, rs35413809, and rs2301261 (**Table 1**). By contrast, SLEs did not significantly affect ventral striatal activity (all p>.05) and we observed no significant GxE interaction effects (all p>.05).

Analyses of the amygdala revealed no significant main effect of any SNP (all p>.05, **Table 1**), and no significant effect of SLEs (all p>.05), but a nominally significant interaction between rs35498753 and SLEs on left amygdala activity approaching gene-wide significance (F=(2,1438)=7.0, $p_{fwe\text{-}corrected=}$.06) (**Supplementary Figure 2**). Nominally significant interaction effects of rs11706648, rs11131149, and rs6777726 x SLEs in the left amygdala, and of rs35498753 x SLEs in the right amygdala were also found (**Table 1**).

We then carried forward rs237915 for subsequent analyses. Further analyses of rs237894 were omitted as this SNP is in partial LD with rs237915 (D'=1, r^2=0.45) (**Supplementary Figure 1**) and showed similar associations with VS activity.

Whole-brain regression analyses. Exploratory whole brain voxel-wise regression analyses of rs237915 confirmed genotype effects in the striatum (right caudate: $p_{fwe\text{-}corrected}$ =.001; right putamen: $p_{fwe\text{-}corrected}$=.001; left putamen: $p_{fwe\text{-}corrected}$ =.019). In addition, we found strong genotype effects in the right ($p_{fwe\text{-}corrected}$ =.003) and left

cingulate gyrus ($p_{fwe\text{-}corrected}$ <.0001), right thalamus ($p_{fwe\text{-}corrected}$.001), left inferior frontal gyrus ($p_{fwe\text{-}corrected}$ =.004), left lingual gyrus ($p_{fwe\text{-}corrected}$=.001), and right cerebellar regions ($p_{fwe\text{-}corrected}$ =.001) (see **Table 2**).

Gene-behaviour analyses.

Gene-behaviour analyses showed strong effects of stressful life events (SLEs) on emotional problems, conduct problems, and peer problems (all $p_{emp,\ fwe\text{-}corrected}$<.00001) and a significant interaction between rs237915-genotype x SLEs on emotional problems (χ^2= 23.6, $p_{emp,\ fwe\text{-}corrected}$=.035). Among CT and TT-carriers emotional problems significantly increased as a function of SLEs (CT: r(582)=.24, p=3.3 x 10^{-8}; TT: (r(730)=.26, p=2.1 x 10^{-12}). These very high p-values were partly due to the large sample sizes. By contrast, in adolescents with minor CC-genotype SLEs were not associated with more emotional problems (r(111)=-.07, p=.45) (**Figure 2A)**. The correlation coefficients were significantly different between CC-homozygotes and CT (Z_{obs}=2.3) and TT-carriers (Z_{obs}=2.6), respectively.

We then used linear combination of estimators (LINCOM) to further characterize the pattern of the interaction. LINCOM predicted that when adolescents experience 0-3 SLEs, CC-homozygotes have significantly more emotional problems than CT and TT-carriers but significantly fewer emotional problems after 6 or more SLEs (all p<.05).

A similar but non-significant trend towards an interaction between rs237915-genoytpe x SLEs on peer problems was also found (χ^2=10.38, $p_{emp,\ uncorrected}$=.09). Again, in CT and TT-carriers peer problems significantly increased as a function of SLEs (CT: r(582)=.15, p=.0003, TT r(730)=.19, p=3.8 x 10^{-7}) but not in the CC-homozygotes (r(111)=-.03, p=.75).

We observed strong sex differences across all clinical symptoms. Girls had significantly more emotional problems (F=142.64, p=.1.2 x 10^{-6}) but significantly fewer peer problems (F=8.6, p=.003) than the boys. This finding is in agreement with an extant literature on sex differences in anxiety/depression and antisocial behavior, including adolescence (41-43). Therefore, and because several sex-specific effects of *OXTR*-genotype on behavior (44) have been reported, we next stratified our sample by sex.

This revealed that the rs237915-genotype x SLE interaction effect on emotional problems was primarily driven by the girls (Girls: χ^2=23.9 $p_{emp,\ fwe\text{-}corrected}$=.019). **Figure 2B** (left panel) shows highly significant associations between SLEs and increased numbers of emotional problems in female CT (r(314)=.30, p=3.4 x 10^{-8}) and TT-carriers (r(376)=.27, p=6.3 x 10^{-8}) but not the girls with CC-genotype (r(42)=-.19, p=.20). Again, the correlation coefficients significantly differed between female CC vs. CT (Z_{obs}=3.1) and TT-genotype groups (Z_{obs}=2.8). LINCOM estimated female CC-homozygotes to have more emotional problems between 0-5 SLEs than CT/ TT carriers but fewer after 9+ SLEs (all p <.05). In boys, the GxE interaction was not significant (p>.05).

Among boys we found a nominally significant interaction between rs237915-genotype x SLEs on peer problems (χ^2=13.7, $p_{emp,\ uncorrected}$=.046). Whereas male TT-homozygotes had a significant increase in peer problems after SLEs, (r(351)=.18, p=.001) this was not true for those with CT (r(265)=.10, p=.11) and CC-genotype (r(66)=-.10, p=.38) (**Figure 2C**, right panel). Correlation coefficients were significantly different between CC and TT-homozygotes (Z_{obs}=2.15). LINCOM predicted that between 0-4 SLEs, male CC-homozygotes have significantly more but after 12+ SLEs significantly fewer peer problems than TT-carriers (all p <.05).

Brain-behaviour analyses.

Finally we tested whether rs237915-genotype differences in VS activity mediated risk for emotional or peer problems. In the entire sample no significant associations were found (all p>.43). However, in CC-carriers reduced left VS activity was significantly related to more peer problems (r(111)=-.20, p=.03) (**Figure 3**).

We then investigated whether reduced left VS activity per se modulates greater resilience against the effect of SLEs on peer problems. To this end we divided the sample in five quantile groups according to left VS activity. Per left VS quantile group we then examined rs237915-genotype differences in the association-strength (slope) between SLEs and peer problems. Findings showed that across quantile groups, slopes of CC-homozygotes were consistently lower than of CT and TT-carriers (**Supplementary Methods 2**).

To test the alternative hypothesis that the observed effect is *OXTR*-genotype specific we compared the slopes of CC-homozygotes vs. equally-sized surrogate samples drawn from the entire sample with similar distribution of left VS activity and other covariates (**Supplementary Methods 2**). Of 10,000 simulations, the slope of the CC-homozygotes was at the lower 5% tail (p=.015). This indicates that the modulatory effect of lower left VS activity on higher resilience against SLEs may be *OXTR*-genotype dependent (**Supplementary Figure 2)**.

Internal validation using bootstrapping

Bootstrapping was performed to estimate the robustness of our main findings. **Figure 4** shows histograms of 10,000 bootstrapped p-values for rs237915-genotype effects on VS activity and **Figure 5** for GxE effects on emotional and peer problems. The rank values of the original p-values indicate that all main findings were well within the 95% confidence interval. To indicate the likelihood that the bootstrap p-values of the underlying effect will be obtained in a similarly distributed data set we performed simulations and assessed those against reference scores (**Supplementary Methods 1**). A score of 50% indicates p-values of .05, a score of

80% indicates strong support of the original p-value or a more significant one, and a score of less than 20% indicates that the original p-value may in fact have been non-significant.

The effect of rs237915-genoytpe on left VS activity was reproduced in 97% and on right VS activity in 94%. In the entire sample, the rs237915-genotype x SLE effect on emotional problems was reproduced in 82.7% and in the girls in 86.3%. The rs237915 genotype x SLE effect on peer problems was 63.7%, which still indicates strong likelihood of obtaining p-values <.05.

Together, this demonstrates no evidence for rejecting the original p-values and indicates that for all main findings the likelihood of reproducing the same or more significant effects in a similarly distributed sample is high.

Discussion

This study comprehensively examined in a large adolescent sample the effect of 23 common variants in the *OXTR* gene and stressful life events on fMRI BOLD activity in the ventral striatum and the amygdala to animated angry faces, and risk for social-affective problems.

Our results suggest that the *OXTR-SNP* rs237915 moderates the effect of stressful experiences on social and emotional problems, and that this is partly mediated by genotype-differences in sensitivity to the reinforcement value of negative social cues. These conclusions are based on the following observations:

First, a gene-wide significant effect of rs237915 on ventral striatal activity bilaterally showed that adolescents with the minor CC-genotype had significantly lower ventral striatal activity than CT and TT-carriers. A similar effect of rs237893, which is in partial LD with rs237915, on left ventral striatal was also found. Whole-brain regression analyses of rs237915 confirmed genotype differences in striatal

regions (caudate, putamen) and also identified similar effects in the cingulate gyrus, angular gyrus, inferior frontal gyrus, thalamus and the cerebellum.

Second, we found interaction effects between rs237915-genotype and SLEs on emotional problems (in girls) and peer problems (in boys). Whereas CT and TT-carriers showed the well-documented increase in emotional problems and TT-carriers in peer problems as a function of stressful experiences, adolescents with CC-genotype did not. However, when the number of stressful experiences was low (i.e., "favourable environments") girls with CC-genotype had the highest rates of emotional problems and boys with CC-genotype highest rates of peer problems.

Third, we showed that in CC-homozygotes, lower ventral striatal activity was associated with greater resilience against the effect of SLEs on peer problems. The ventral striatum is a key region in the processing of social reinforcement (46), including "negative social reinforcers" such as the anger signals (32) used here. OT-neuromodulation in the VS is likely affected by OT-receptor densities (8). Taken together these findings suggest that *OXTR*-genotype may not directly influence better or worse social behavior but may primarily modulate responsiveness to social-emotional cues. In risk-allele carriers diminished responsiveness to negative social-emotional cue manifests in more social-affective problems in "favourable environments" but at the same time they are less affected by stressful life events. These findings are consistent with the pattern of GxE effects of two other *OXTR* SNPs (rs2254298, rs53576) on emotional problems (14, 15). They are also consistent with the "differential susceptibility" model (45), which suggests that it is evolutionary plausible for the same allelic variation to be associated with favourable outcomes in one environmental setting and to simultaneously carry higher risk for psychiatric disorders in another one.

The finding that the GXE effect on emotional problems was only significant in the girls and the GxE effect on peer problems in the boys does not necessary imply sex-specific mechanisms: We found that in the opposite sex the pattern of the

interactions was similar but weaker. This may be due to the respectively fewer symptom numbers, which is in agreement with previous reports of sex differences in internalizing/externalizing problems and disorders (41, 42) regardless of genotype-differences. However, we found no evidence that the GxE effect on emotional problems was modulated by ventral striatal activity; hence other mediating factors remain to be identified.

However, we could not replicate the previously reported effect of rs53576-genotype on amygdala activity (23). Instead rs53576, which is one of the most frequently investigated *OXTR*-SNPs in gene-behaviour studies (9, 47) was nominally significantly associated with ventral striatal activity. Several factors may have contributed to this negative finding, including study differences in stimuli used and participant characteristics (13-14 year-old boys and girls vs. mostly male adults). As persistent changes in brain development throughout puberty are well known (48), our findings from a large adolescent-cohort may not generalize to adults or younger children. However, we observed a trend towards a gene-wide significant interaction effect between rs35498753-genotype x SLEs on left amygdala activity. This finding was preliminary due to the low MAF but deserves further exploration.

Several limitations of this study should be considered: Our gene-neuroimaging analyses were based on a contrast comparing fMRI BOLD responses to angry faces vs. non-biological motion stimuli. The paradigm used here did not include a “neutral face” condition per se as the animated faces originally intended to display neutral expressions contained elements of ambiguity and also evoked strong activity in the amygdala (48) . Nevertheless, a similar contrast between angry/ fearful faces and non-social control stimuli has deliberately been used in several previous pharmacological OT studies (17) and one *OXTR* gene-neuroimaging study (23) because of its strong signal-to-noise ratio (49). Although we cannot rule out that

findings reflected broader *OXTR*-genotype effects on face recognition, the functional role of the ventral striatum in reward-reinforcement processing strongly indicates modulatory effects on the emotional-motivational value of the angry face expressions. Also future studies should include a happy face condition to examine *OXTR*-genotype effects on positive social reinforcers; a potentially important mechanism for social-affiliative development.

Finally, in our study stressful experiences were operationalized as the number of stressful life events ever experienced, and they were assessed by a self-report measure. Future research is needed to examine more specific interaction effects of stressful events experienced in early vs. later childhood, including maltreatment.

Taken together, based on a large adolescent gene-neuroimaging sample the present findings showed a robust effect of a common *OXTR*-SNP (rs237915) on ventral striatal activity to anger expressions. They further indicate a mechanism by which in “risk-carriers” reduced sensitivity to negative social-emotional cues is simultaneously associated with more social problems in ‘favourable environments’ and greater resilience against stressful life events.

Acknowledgements

This work was supported by the European Union-funded FP6 Integrated Project IMAGEN (Reinforcement- related behaviour in normal brain function and psychopathology) (LSHM-CT- 2007-037286), the FP7 projects ADAMS (Genomic variations underlying common neuropsychiatric diseases and disease related cognitive traits in different human populations) (242257), the German Ministry of Education and Research (BMBF grant # 01EV0711), the Swedish Research Council (FORMAS), the Innovative Medicine Initiative Project EU-AIMS (115300-2), as well as the United Kingdom National Institute for Health Research (NIHR) Biomedical Research Centre Mental Health and the Medical Research Council Programme Grant “Developmental pathways into adolescent substance abuse” (93558).

IMAGEN consortium: Steven Charles Rees Williams, Laurence John Reed, Charlotte Nymberg, Lauren Topper, Lindsey Smith, Catherine Morgan, Cybele Peng Wong, Simone Kühn, Ines Häke, Nicole Strache, Lisa Albrecht, Nikolay Ivanov, Jan Reuter, Carla Palafox, Christina Schilling, Katharina Lüdemann, Alexander Romanowski, Andreas Ströhle, Elisabeth Wolff, Michael Rapp, Rüdiger Brühl, Albrecht Ihlenfeld, Bernadeta Walaszek, Florian Schubert, Jennifer Jones, Edmund Lalor, Eithne McCabe, Aisling Ní Shiothcháin, Robert Whelan, Rainer Spanagel, Fernando Leonardi-Essmann, Wolfgang Sommer, Sabine Steiner, Sabine Vollstedt-Klein, Eva Stolzenburg, Christine Schmäl, Penny Gowland, Nadja Heym, Claire Lawrence, Craig Newman, Zdenka Pausova Amir Tahmasebi, Zdenka Pausova, Melkaye G. Melka, Thomas Hübner, Stephan Ripke, Eva Mennigen, Katrin Müller, Veronika Ziesch, Uli Bromberg, Stefan Meindl, Jan Peters, Sophia Schneider, Tahmine Fadai, Lisa Lueken , Juliana Yacubian , Jürgen Finsterbusch, Eric Artiges, Marie-Laure Paillère Martinot, Hervé Lemaitre, Nadège Bordas, Zuleima Bricaud, Fanny Gollier Briand, Jessica Massicotte, Ruben Miranda, Jani Penttilä, Nathalie Boddaert, Alexis

Barbot, Yannick Schwartz, Christophe Lalanne, Vincent Frouin, Virgile Fritsch, Jeff Dalley, Adam Mar, Trevor William Robbins, Naresh Subramaniam, David Theobald, Nicola Richmond, Mischa de Rover, Anna Molander, Emily Rose Jordan, Emma Robinson, Margarita Moreno, Mercedes Arroyo, Dai Stephens, Tamzin Ripley, Hans Crombag, Yolanda Pena, Mark Lathrop, Diana Zelenika, Simon Heath, Dirk Lanzerath, Bert Heinrichs, Tade Matthias Spranger, Beate Fuchs, Claudia Speiser, Arno Klaassen, Ivo Klaassen, Patrick Constant, Xavier Mignon, Tormod Thomsen, Stephan Zysset, Anders Vestboe, James Ireland, John Rogers

Financial Disclosures

Dr. Banaschewski has served as an advisor or consultant to Bristol- Myers Squibb, Desitin Arzneimittel, Eli Lilly, Medice, Novartis, Pfizer, Shire, UCB, and Vifor Pharma; he has received conference attendance support, conference support, or speaking fees from Eli Lilly, Janssen McNeil, Medice, Novartis, Shire, and UCB, and he is involved in clinical trials conducted by Eli Lilly, Novartis, and Shire; the present work is unrelated to these relationships. Dr. Gallinat has received research funding from the German Federal Ministry of Education and Research, AstraZeneca, Eli Lilly, Janssen-Cilag, and Bristol-Myers Squibb; he has received speaking fees from AstraZeneca, Janssen- Cilag, and Bristol-Myers Squibb. Dr Barker has received honoraria from General Electric for teaching on scanner programming courses. The other authors report no financial relationships with commercial interests.

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Figure legends

Figure 1. Effect of *OXTR*-SNPs rs237915 and rs237893 on left ventral striatal activity (Z-scores were used to account for the effect of study-site).

Figure 2. Interaction effect of rs237915-genotype and SLEs on emotional problems and peer problems. **A.** Entire sample, **B.** Emotional problems, split by sex, **C.** Peer problems, split by sex. Participant Ns, by rs237915-genotype group and sex: Girls: CC=46, CT=314 TT=379; boys: CC=69, CT=268, TT=354

Figure 3. Path diagram. **A.** Effect of rs237915-genotype on left VS activity. CC-homozygotes have reduced VS activity **B.** rs237915-genotype x SLE interaction effect on peer problems in boys. Male CC-carriers do not show an effect of SLEs on peer problems but have more peer problems than CT/TT carriers in low-SLE environments. **C.** CC-carriers show a significant correlation between lower left VS-activity and increased peer problems. Additional simulations (not shown) indicate that the modulatory effect of reduced VS activity on greater robustness against the effect of SLEs on peer problems is CC-genotype specific.

Figure 4. Histograms of p-value distributions for 10,000 bootstrap samples. **A**. rs237915-genotype effect on left and right VS. All original p-values were lying within the 95% confidence interval.

Figure 5. Histograms of p-value distributions for 10,000 bootstrap samples. Red arrows indicate significance threshold p=.05; black arrows indicate rank of the original p-value. **A.** Entire sample emotional problems, **B.** Entire sample peer problems, **C.** Girls emotional problems, **D.** Girls peer problems. **E.** Boys Emotional Problems, **F**. Boys Peer problems. For A, B, C, & F original p-values were within the 95% confidence interval. In contrast, the pattern shown in D & E strongly resembles uniform distributions, indicating no interaction effect.

Table

Table 1. Main and GxE interaction effects of each *OXTR* SNP on fMRI BOLD activity in the ventral striatum (VS) and amygdala (uncorrected p-values).

		Main effects				GxE			
OXTR SNP	Ns per genotype group	p-value left VS	p-value right VS	p-value left amygdala	p-value right amygdala	p-value left VS	p-value right VS	p-value left amygdala	p-value right amygdala
rs7632287	GG: 820, AG: 537, AA: 88	1.1E-01	1.9E-01	8.5E-01	2.0E-01	3.5E-01	6.5E-01	4.0E-01	4.8E-01
rs1042778	GG: 511, TG: 698, TT: 226	3.8E-01	2.5E-01	7.6E-01	4.1E-01	9.2E-01	7.6E-01	9.1E-01	8.7E-01
rs11706648	AA: 604, CA: 672, CC: 169	9.7E-01	6.3E-01	3.7E-01	3.8E-01	4.8E-01	1.9E-01	2.9E-02	4.1E-01
rs237887	AA: 509, GA: 711, GG:	2.3E-01	1.6E-01	7.7E-01	9.8E-01	6.0E-01	3.8E-01	1.4E-01	8.2E-01
rs2268490	CC: 1086, TC: 335, TT: 18	5.4E-01	6.0E-01	6.1E-01	8.0E-01	8.6E-01	8.2E-01	2.6E-01	6.1E-01
rs237888	TT: 1276, CT: 161, CC: 8	7.9E-01	9.3E-01	3.1E-01	6.0E-01	7.7E-01	9.3E-01	9.2E-01	2.6E-01
rs2268491	CC: 1151, TC: 281, TT: 13	7.4E-01	5.4E-01	8.2E-01	9.4E-01	4.6E-01	4.5E-01	2.1E-01	4.6E-01
rs2268492	CC: 704, TC: 601, TT: 137	9.3E-01	6.5E-01	8.5E-01	7.5E-01	9.8E-01	9.8E-01	2.9E-01	8.2E-01
rs2268494	TT: 1218, AT: 220, AA: 7	7.7E-01	7.4E-01	4.7E-01	1.8E-01	4.4E-01	5.8E-01	1.7E-01	1.5E-01
rs2254298	GG: 1144, AG: 288, AA: 13	8.3E-01	6.1E-01	7.9E-01	9.6E-01	4.3E-01	4.5E-01	1.7E-01	5.3E-01
rs237889	CC: 581, TC: 672, TT: 192	3.1E-01	2.3E-01	7.6E-01	6.8E-01	1.6E-01	9.2E-02	2.3E-01	9.6E-01
rs11131149	GG: 531, AG: 666, AA: 248	3.7E-01	3.1E-01	9.0E-01	9.9E-01	6.1E-01	4.2E-01	4.5E-02	6.4E-01
rs53576	GG: 690, AG: 603, AA: 152	1.8E-02	3.7E-02	2.0E-01	5.6E-01	2.8E-01	8.0E-01	9.2E-01	1.7E-01
rs35498753	TT: 1164, GT: 266, GG; 15	6.1E-03	2.5E-02	7.5E-01	9.3E-01	8.5E-01	4.8E-01	**9.2E-04**	1.5E-02
rs237893	AA: 452, GA: 430, GG: 56	1.5E-04	1.1E-03	3.6E-01	5.1E-01	6.5E-01	4.7E-01	5.0E-01	9.4E-01
rs11711703	AA: 959, GA: 694, GG: 297	6.4E-01	4.4E-01	8.6E-01	6.7E-01	3.3E-01	4.0E-01	4.4E-01	9.9E-01
rs4686302	CC: 1102, TC: 317, TT: 26	7.5E-01	8.9E-01	7.9E-01	3.2E-01	8.7E-01	9.3E-01	4.2E-01	2.4E-01
rs237915	CC: 115, CT: 590, TT: 740	**1.3E-05**	**5.8E-05**	8.4E-01	9.3E-01	5.5E-02	7.3E-02	9.5E-02	3.6E-01
rs35413809	GG: 1218, AG: 217, AA: 10	1.3E-02	5.1E-02	9.6E-01	7.6E-01	2.9E-01	2.5E-01	9.8E-02	1.6E-01
rs2301261	CC: 1223, TC: 213, TT: 9	2.5E-02	8.3E-02	9.8E-01	6.9E-01	3.6E-01	3.3E-01	9.1E-02	1.6E-01
rs3806675	GG: 542, AG: 675, AA: 228	1.1E-01	6.0E-02	7.5E-01	5.9E-01	3.0E-01	4.5E-01	1.0E+00	7.4E-01
rs1465386	GG: 1262, TG: 175 TT: 6	1.2E-02	6.7E-02	4.4E-01	7.7E-01	1.5E-01	1.6E-01	6.8E-02	1.8E-01
rs6777726	GG: 1277, AG: 162, AA:6	9.1E-02	2.2E-01	4.3E-01	5.8E-01	2.4E-01	2.0E-01	4.6E-02	1.1E-01

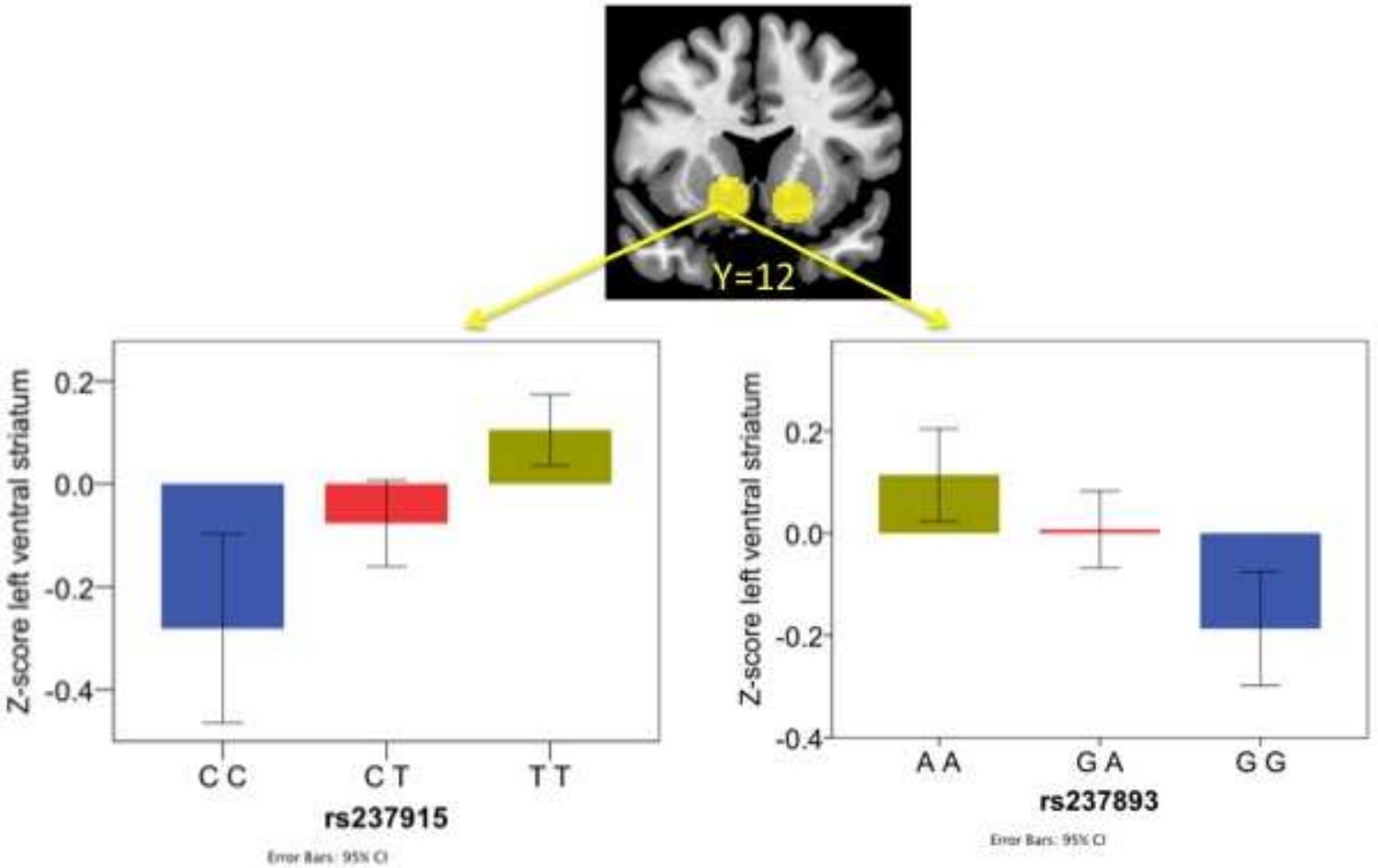
Table

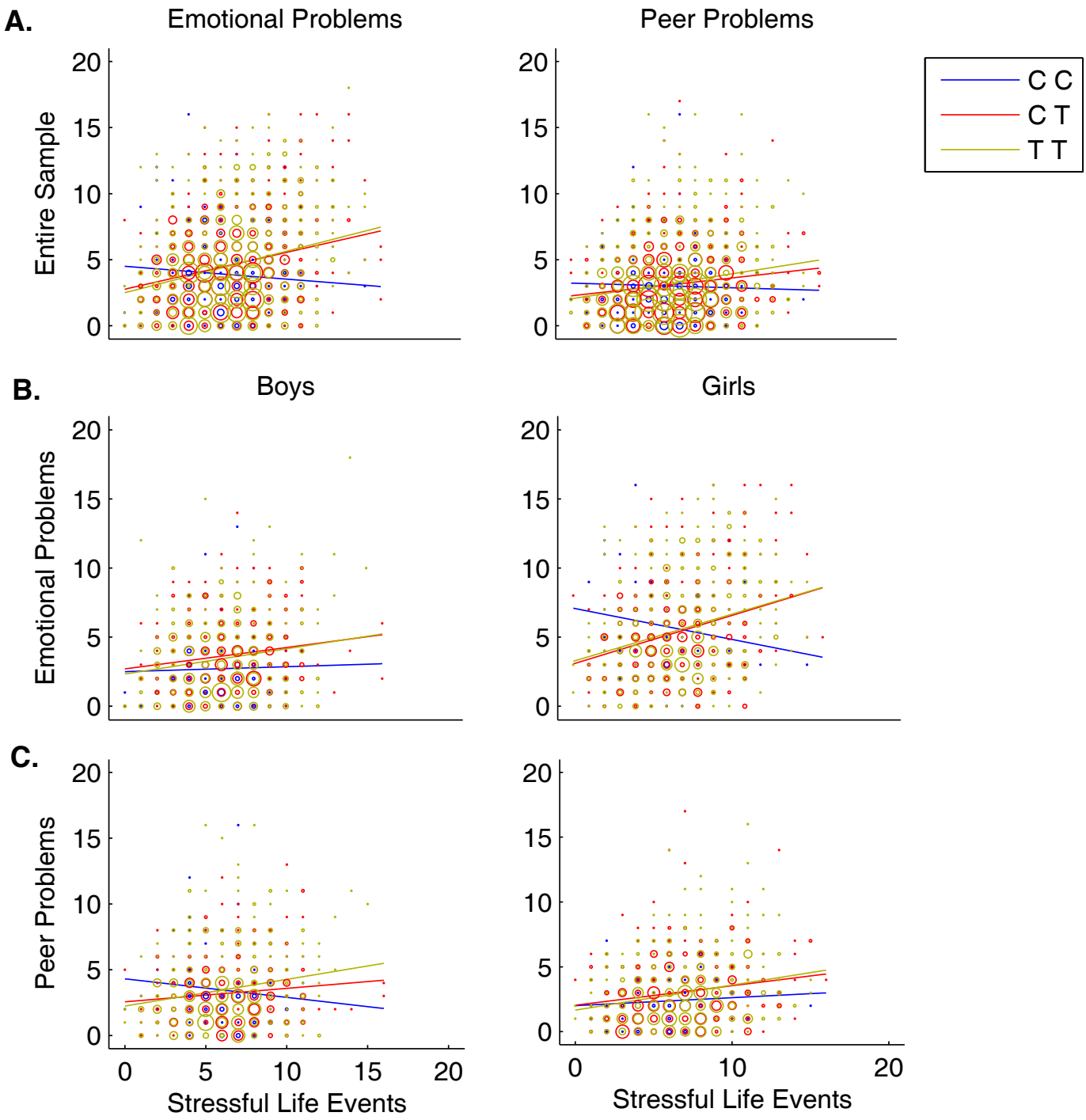
Table 2. Whole-brain voxel-wise regression analyses testing the additive effect of rs237915 on fMRI BOLD activity on the contrast Angry Faces vs. Control. Sex, handedness and study centre were included as covariates of no interest. (Height threshold, p<.001 uncorrected, extent threshold, *k*=10 voxels), p-values are reported at fwe-corrected cluster-level.

Brain area	Talairach Coordinates	T-value	Cluster size *k*	p-value fwe-corrected cluster level
R cingulate gyrus	3, -28, 25	5.32	64	0.003
L cingulate gyrus	-9, 14, 34	4.29	161	<0.0001
R cingulate gyrus	9, 23, 31	4.27		
R anterior cingulate gyrus	6, 35, 25	4.07		
Striatum				
R caudate	15, 17, -2	5.09	78	0.001
R putamen	33, -16, -2	4.45	74	0.001
L putamen	-15, 11, -8	3.97	46	0.019
L inferior frontal gyrus	-45, 14, 19	4.49	61	0.004
L lingual gyrus	-6, -97, -5	4.33	78	0.001
R cerebellum	6, -61, -23	4.08	44	0.024

Figure

Click here to download high resolution image




A.
Emotional Problems
Peer Problems
Entire Sample
B.
Boys
Girls
Emotional Problems
C.
Peer Problems
Stressful Life Events
Stressful Life Events
C C
C T
T T
0
5
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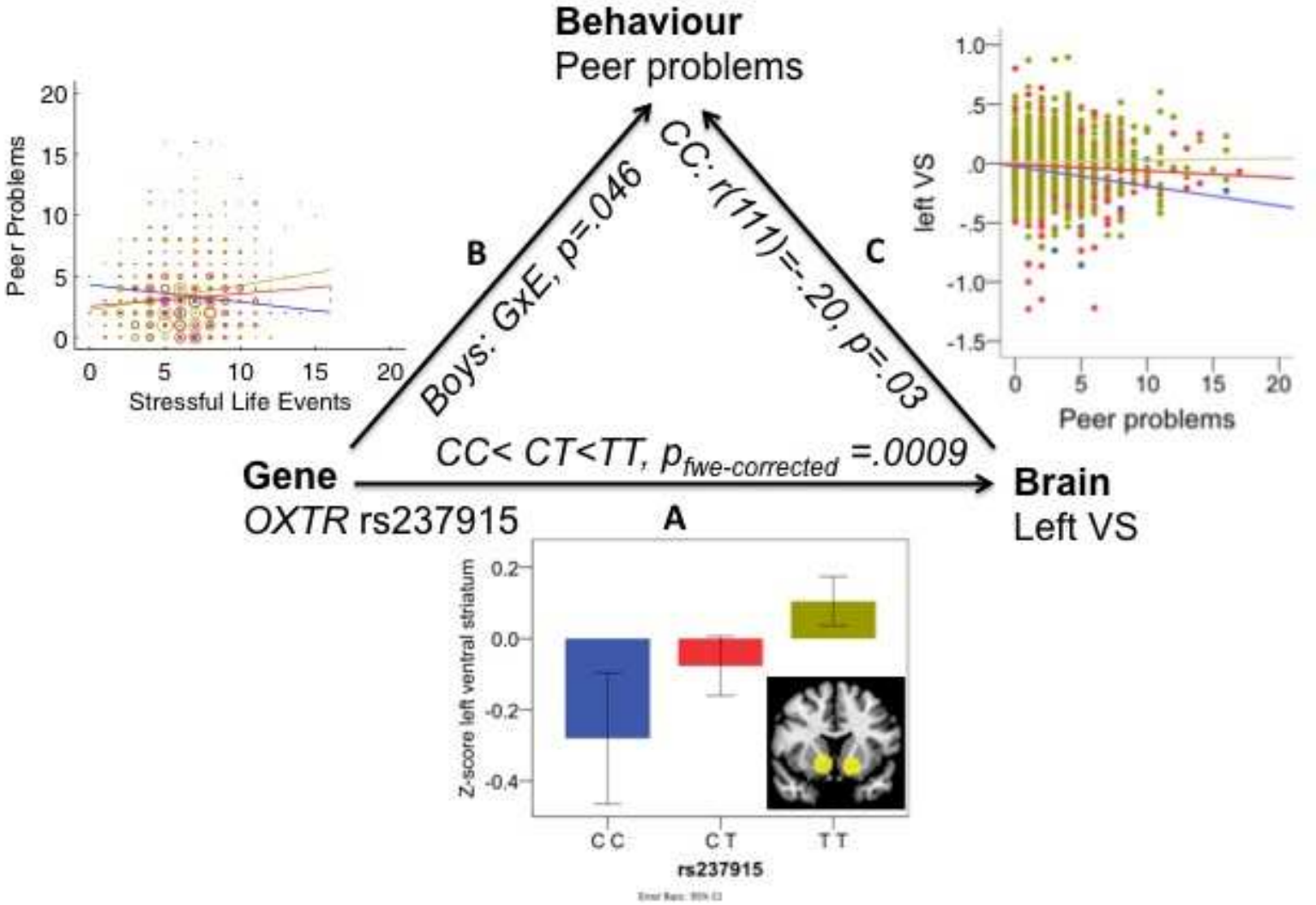
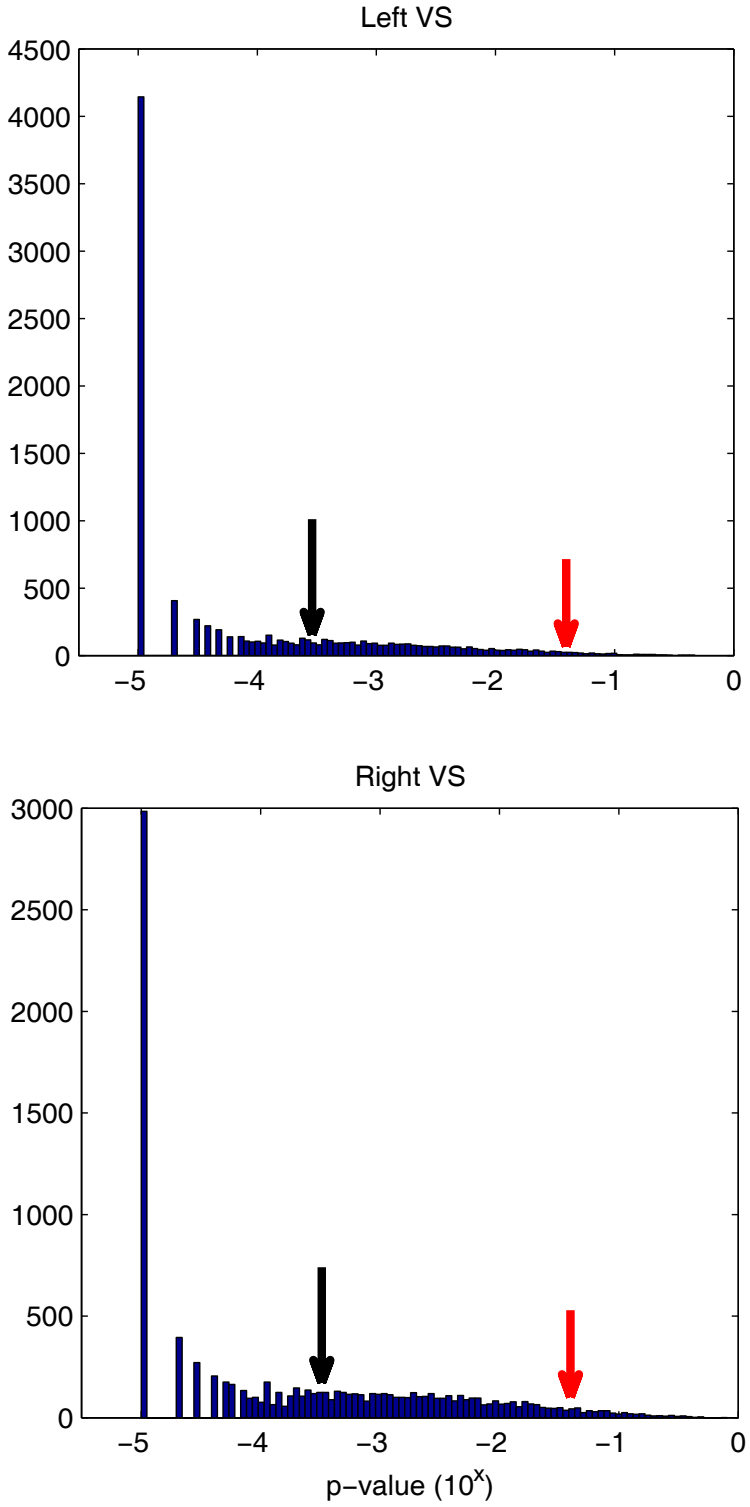
20

Figure

Click here to download high resolution image



Figure


Left VS
4500
4000
3500
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500
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−4
−3
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Right VS
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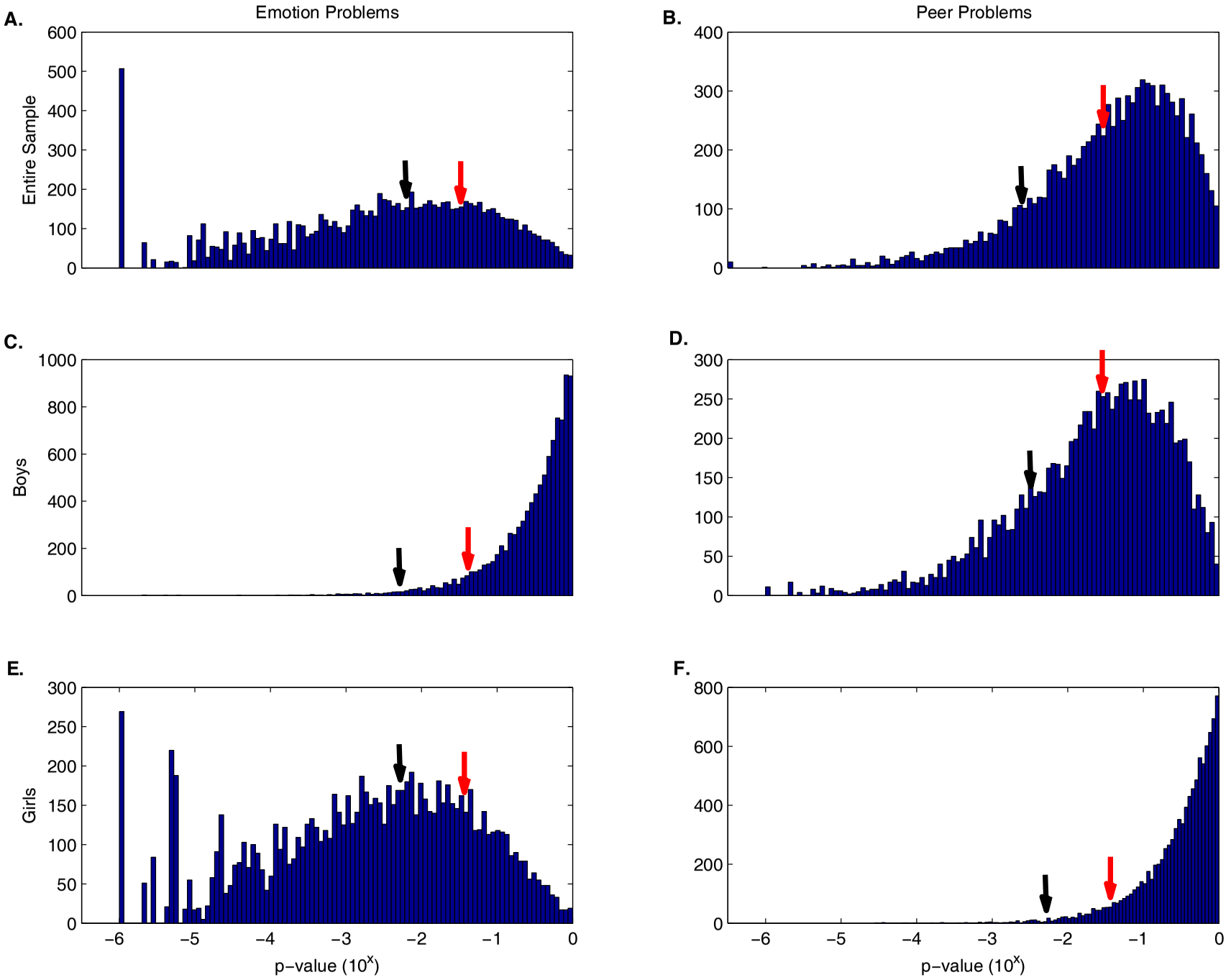
−2
−1
0
p−value (10^x)

Figure


A.
Emotion Problems
Entire Sample
600
500
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200
100
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B.
Peer Problems
400
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C.
Boys
1000
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D.
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E.
Girls
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Supplementary Table 1. Breakdown of excluded data sets

Exclusion criteria	**N excluded**
Available contrast maps Angry Faces vs. control	1,884
Suspected anatomical abnormalities, problems during scanning (e.g., goggles did not work; volunteer fell asleep)	23
Bad volumes, non-correctable	19
Failure to pass criterion on multi-variate outlier procedure, including outliers due to excessive head motion*	102
Missing genotype information (e.g., failure to obtain blood sample)	164
Non-Caucasian ancestry (based on population stratification)	121
Missing LEQ data, or failure to meet LEQ QC (e.g., inconsistent/ repetitive responses)	10
Total gene-neuroimaging	1,445
Gene-behaviour analyses: missing SDQ data, or failure to meet SDQ QC	13
Total gene-behaviour	1,432

*****We used the multivariate outlier procedure described by (Fritsch et al., Med Image Comput Comput Assist Interv. 2011) to detect and exclude contrast maps that exceeded criterion values in cortical regions, subcortical regions, and empirically determined 'ROIs'.

Supplementary Table 2. Position of tagged SNPs site and minor allele frequencies (MAF)

Name	Alleles	MAF	HWpval	Position
rs7632287	G:A	0.246	1	8766446
rs1042778	G:T	0.403	0.7345	8769545
rs11706648	A:C	0.341	0.4055	8771547
rs237887	A:G	0.409	0.2992	8772042
rs2268490	C:T	0.138	0.4488	8772085
rs237888	T:C	0.061	0.1711	8772095
rs2268491	C:T	0.112	0.3452	8775398
rs2268492	C:T	0.295	0.4023	8775672
rs2268494	T:A	0.089	1	8777046
rs2254298	G:A	0.115	0.2481	8777228
rs237889	C:T	0.372	0.7891	8777483
rs11131149	G:A	0.393	0.1366	8777851
rs53576	G:A	0.314	0.2726	8779371
rs35498753	T:G	0.103	0.9283	8780366
rs237893	A:G	0.448	0.3711	8780950
rs11711703	A:G	0.181	0.2515	8781314
rs4686302	C:T	0.123	0.1893	8784222
rs237915	T:C	0.292	0.9064	8785311
rs35413809	G:A	0.083	1	8785520
rs2301261	C:T	0.081	1	8785896
rs3806675	G:A	0.389	0.5729	8786646
rs1465386	G:T	0.068	1	8786653
rs6777726	G:A	0.062	0.6666	8788494

Supplementary Table 3: Definition and items of Stressful Life Events

The life events questionnaire is a self-report measure that includes 39 items (Newcombe et al., 1981). In the IMAGEN version, participants were asked to indicate whether the event had ever happened, and whether it had happened over the past 12 months. For the current purposes, only 'ever' ratings were used.
Stressful life events were defined based on the quality ratings of N=1,239 adolescents who had experienced the event, and had rated the event as 'unhappy' or 'very unhappy'. The experienced raters consistently rated 20 events as distressing and 16 events as positive. Three events were consistently considered neutral [LEQ 06, 12 & 11] and therefore omitted. Agreement between ratings of experienced and inexperienced raters (i.e., youth who had not experienced the event) was high.

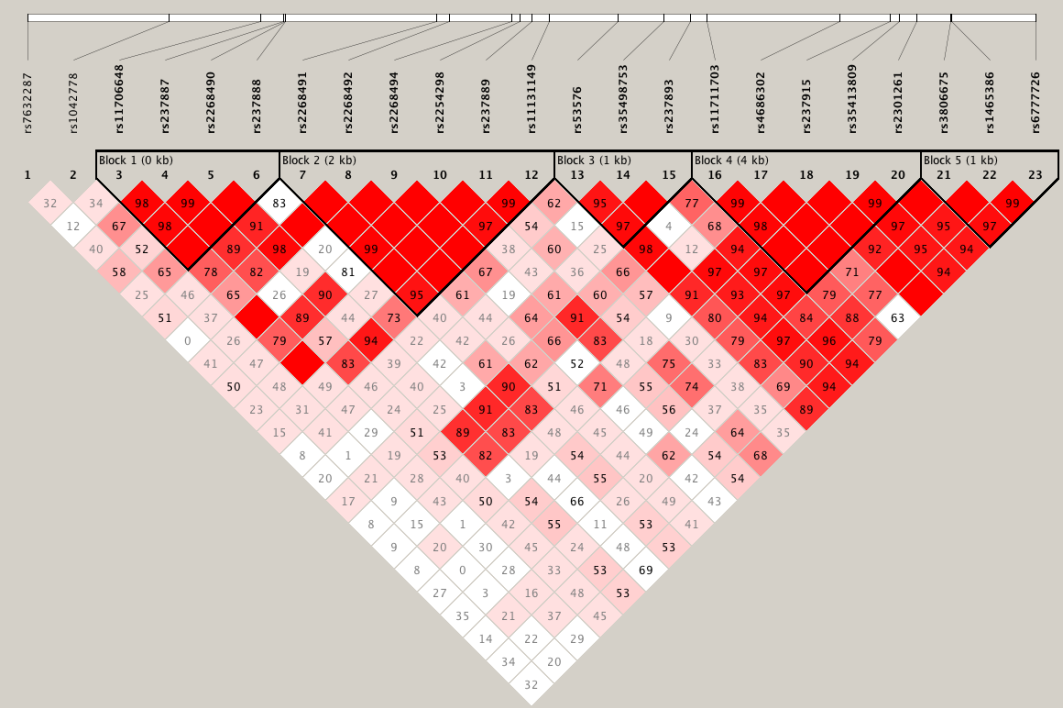
Stressful life events	**Positive life events**
LEQ_08 Death in the family	LEQ_26 Started going out with a boyfriend/girlfriend
LEQ_02 Family Accident/Illness	LEQ_23 Got own TV or Computer
LEQ_37 Serious accident/illness	LEQ_32 Started making own money
LEQ_22 Family had money problems	LEQ_07 Fell in love
LEQ_36 Gained a lot of weight	LEQ_29 Started driving a motor vehicle
LEQ_24 Parents argued or fought	LEQ_03 Found a new group of friends
LEQ_27 Got poor grades in school	LEQ_18 Joined a club or group
LEQ_16 Thought about suicide	LEQ_21 Met a teacher I liked a lot
LEQ_30 Broke up with a boyfriend/girlfriend	LEQ_15 Decided about college/university
LEQ_09 Face broke out with pimples	LEQ_28 Went on holiday without parents
LEQ_39 Parent abused alcohol	LEQ_13: Began a time consuming hobby
LEQ_01 Parents Divorced	LEQ_38 Lost virginity
LEQ_05 Stole something valuable	LEQ_35 Had a gay experience
LEQ_19 Got in trouble at school	LEQ_33 Found religion
LEQ_25 Ran away from home	LEQ_14 Got or made someone pregnant
LEQ_20 Got or gave an STD	LEQ_34 Parent remarried
LEQ_04 In trouble with the law	
LEQ_17 Changed Schools	
LEQ_10 Brother or Sister moved out	
LEQ_31 Family moved	

Supplementary Table 4. Results whole-brain random effects analyses

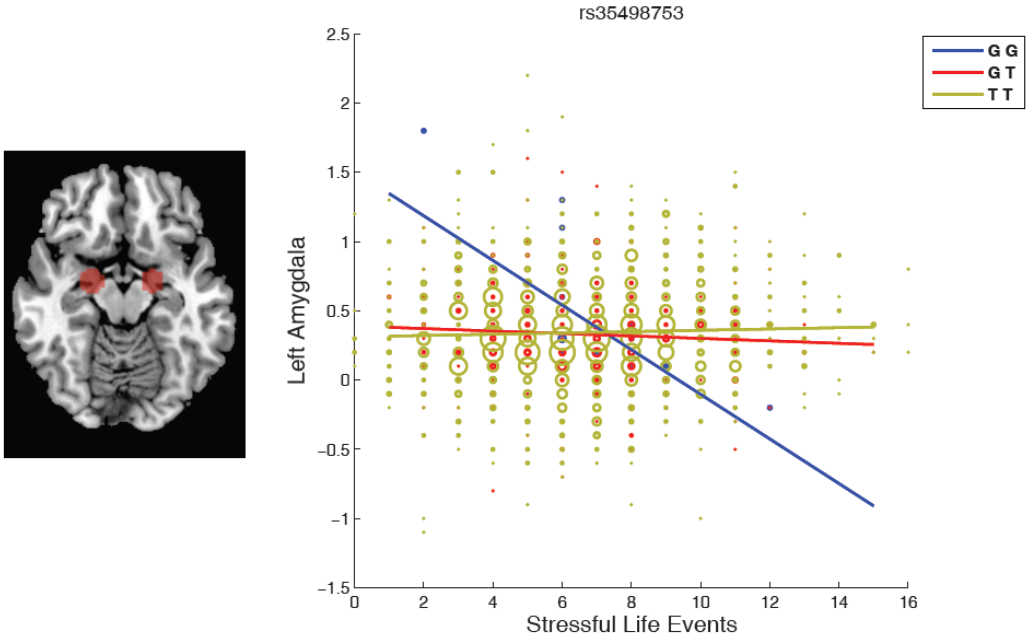
a. Brain regions activated by the Angry Faces vs. Control contrast. (Height threshold, T=4.9, p<.05 fwe-corrected, p=0.05 fwe-corrected, extent threshold, *k*=10 voxels)

Brain area	Talairach Coordinates	T-score	Cluster-size *k*	p-value
R posterior fusiform gyrus	42, -46, -23	33.51	12,001	<0.0001
R amygdala	21, -7, -14	32.70		
L amygdala	-18, -7, 14	31.09		
R superior frontal gyrus	6, 14, 67	19.77	800	<0.0001
R inferior frontal gyrus	3, 56, -14	12.79	126	<0.0001
R caudate	12, -22, 28	10.62	44	<0.0001
L cerebellum	-27, 38, -26	8.66	10	<0.0001
L cerebellum	-18, -40, -44	10.62	17	<0.0001
R superior parietal lobule	36, -61, 43	8.56	47	<0.0001

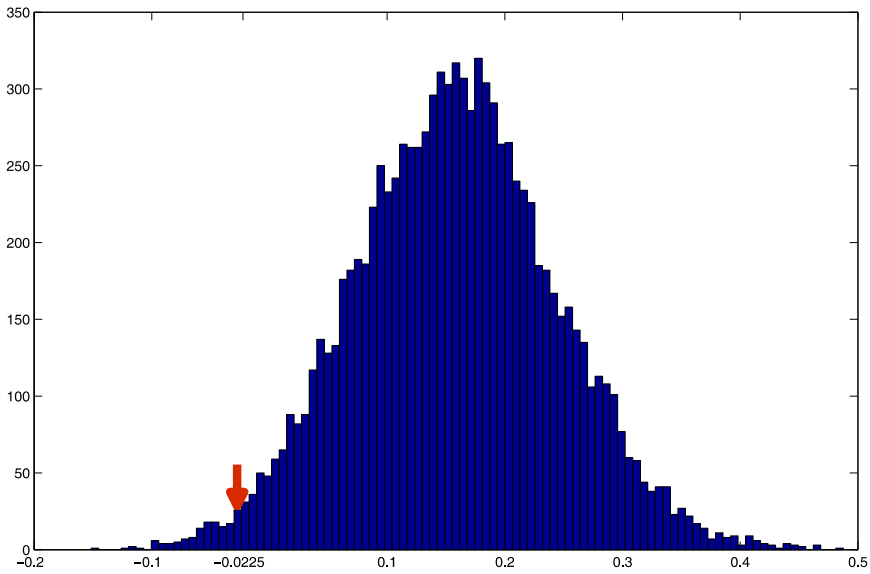
Supplementary Figure 1. Linkage Disequilibrium plot



Supplementary Figure 2: Interaction effect of rs35498753-genotype and SLEs on left amygdala activity. Minor GG-homozygotes had lower left amygdala activity as a function of SLEs (r(15)=-.48, p=.08), whereas in GT and TT-carriers the number of SLEs was not correlated with left amygdala activity (GT: r(262)=-.07, p=.26; TT(1160)=.043, p=.14).



Supplementary Figure 3: Histogram showing 10,000 surrogates of Ns=115 with a similar distribution of left VS activity and covariates as rs237915-CC homozygotes. The p-value of the negative slope of the CC-carriers is situated at the lower 95% tail of the distribution (p=.015).



Supplementary Methods 1

Internal validation using bootstrapping

In statistics, bootstrapping is a widely-used resampling method to estimate statistical accuracy and robustness. Bootstrapping works by assuming that if the original data's empirical distribution D' is analogous to the true distribution D, then the properties of inference of D could be obtained from the knowledge of D'.

Here we used bootstrapping to investigate the robustness of the results. The large sample (N=1,445) from which the subjects was drawn justified the assumption that the empirical distribution is a reasonable approximation of the real distribution.

Bootstrap for Linear Regression Models

We conducted the bootstrap for the Linear Regression Models (gene-fMRI analyses) as follows. First, subjects were resampled with replacement from the original 1,445 subjects, here referred to as the bootstrap sample. Second, the F-statistics F_0 for the variables of interest of the bootstrap sample was calculated. We shuffled the variables of interest of the bootstrap sample 100,000 times and recalculated the F, generating a NULL distribution of F for the bootstrap sample. Third, the empirical p-value of the bootstrap sample was determined as the portion of F statistics in the NULL greater than F_0. We repeated this bootstrap procedure 10,000 times to obtain an empirical distribution of the p-values for the variable of interest.

Bootstrap for Poisson Models (Generalized Linear Models)

For the Poisson Model (gene-behaviour analyses), the estimation procedure was more computationally intensive. Hence, as the procedure used for the Linear Regression Models was not computationally viable we adopted another less intensive procedure. More specifically, the NULL distribution for the test-statistics was determined once for all the bootstrap samples. Simulations on smaller samples

suggested that the result of this alternative did not deviate from the more computation demanding procedure. We resampled the bootstrap sample from the original sample, then permuted the variables of interest and calculated the Chi-square statistics. This procedure was repeated 1,000,000 times and generated a NULL distribution for the Chi-square statistics.

After that, we resampled one bootstrap sample and calculated the Chi-square statistics without shuffling, denoting C_0. Then the p-value of this bootstrap sample was determined as the portion of F statistics in the NULL greater than C_0. We repeated this 10,000 times and obtained an empirical distribution of the p-values for the variables of interest.

As the estimates of the coefficients of variables of interest were stable across the bootstrap samples, we used p-values throughout to demonstrate robustness of the results. The number of bootstrap-p-values <.05 were visualized in histograms, and the rank of the original p-values among the bootstrap p-values were computed. Original p-values lying within a 95% confidence interval indicate no evidence for rejecting the original p-value.

Simulations to indicate the likelihood of the original p-value

The p-value distribution obtained by bootstrap can give an indication of how likely the original p-value obtained from the full dataset is significant. The more significant p-values (less than 5%) in this distribution and the smaller they are, the more likely is the original effect significant. To get an indication of this, we constructed a score that sums the log of the p-values below the 5% threshold and compared this to the sum of the log of p-values above 5% (normalised by the total sum of the log of the p-values). We then compare this score to the ones obtained by simulating an effect with a p-value identical to the one originally obtained in a distribution of 1,445 normally distributed

values, generated the bootstraped p-values, and repeated this simulation 500 times. The position of the actual score within these 500 scores reflects how likely is the original p-value. A score of 50% indicates p-values of .05, a score of more than 80% indicates strong support of the original p-value or a more significant one, and a score of less than 20% indicates that the original p-value may in fact have been obtained in a distribution with a less significant effect.

Supplementary Methods 2

Additional tests to examine whether in CC-homozygotes reduced left VS activity modulates greater resilience against the effect of SLEs on peer problems.

A. To test whether reduced left VS activity *per se* mediates greater resilience against the effect of SLEs on peer problems we first calculated the quantiles of left VS activity across rs237915 genotype-groups (using Z-scores to account for site-differences). Then we used linear regressions to calculate separately per left VS quantile–group for each rs237915-genotype group the slope (B) for the association between SLEs and peer problems. Finally, for each quantile group the slopes between genotype-groups were compared.

	CC	CT	TT
Q1_lVS	0.059	0.17	0.2
Q2_lVS	-0.013	0.048	0.25
Q3_lVS	0.006	0.112	0.25
Q4_lVS	-0.24	0.17	0.11
Q5_lVS	0.008	0.14	0.079

B. To test the alternative hypothesis that the modulatory effect of left VS activity on greater robustness against the effect of SLEs on peer problems is rs237915 CC-genotype specific, we resampled the entire sample for 10,000 surrogate samples with N=115 subjects and calculated the slope for the surrogate sample. Study centre and sex were included as covariates of no interest in the design matrix. Of 10,000 simulations, the slope of the CC-homozygotes was at the lower 5% tail of the surrogate samples (0.0151 level). Then we further constrained the surrogate sample using the following criteria: (1) no significant deviation for the VS activation from the original CC sub-set (KS test, $p>0.5$) (2) similar mean and standard deviation (0.05 relative difference), (3) similar sex ratio (male between 56~78), (4) similar distribution of subjects from each study-center (no more than 50%

difference). The covariates were also used in the design matrix. After controlling for the distribution, the difference remained significant ($p = 0.0159$).

***IN THIS ISSUE Statement**

Oxytocin is a neuropeptide that influences social and affective behaviours. This study investigated in 1,445 adolescents whether common variants in the oxytocin receptor gene interact with stressful experiences in influencing brain function to angry face expressions and risk for social-affective problems. We show that minor-allele carriers of one common variant had lower ventral striatal activity to angry faces than major-allele carriers and were more resilient against the effect of stressful experience on social-affective problems.