

Stress-Related Negative Affectivity and Genetically Altered Serotonin Transporter Function

Evidence of Synergism in Shaping Risk of Depression

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Context: Genetic moderation of the depression-inducing effects of stressful life events (SLEs) has been reported, but findings suggest that genes may not moderate the effects of SLEs per se but instead may moderate the risk of depression associated with the stable tendency to develop negative emotions in response to minor environmental experiences.

Objective: To examine whether a functional polymorphism of the serotonin transporter gene (*5-HTTLPR*) moderates the association between negative affectivity (neuroticism) and depression and to what degree this can explain previous findings involving SLEs.

Design: A prospective cohort study involving 1 baseline and 4 follow-up measurements in 15 months analyzing change in self-reported depressive symptoms across time as a function of negatively attributed SLEs, neuroticism, *5-HTTLPR*, and their interactions.

Setting: General community.

Participants: A population-based sample of 374 ethnically homogeneous young adult female twins.

Main Outcome Measure: A continuous score of self-reported depressive symptoms.

Results: The depressogenic effect of SLEs in the 3 months before interview was significantly greater in women with 2 short (S) alleles compared with women with 1 or none. However, this effect disappeared after accounting for the effect of SLEs conditional on neuroticism. Similarly, the depressogenic effect of neuroticism was progressively greater with number of S alleles, and this was unchanged after accounting for the effect of neuroticism conditional on SLEs.

Conclusions: Genotype \times environment interactions in depression may be more productively interpreted by involving mechanisms more proximal to psychological experience itself. The probability that stress-related cognitive vulnerabilities for depression result in symptom formation may be moderated by a neurobiologic phenotype characterized by altered processing of negative emotions associated with variation in *5-HTTLPR*.

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IT IS WELL KNOWN THAT STRESSFUL life events (SLEs) increase risk of depression.¹ Because only a small proportion of the exposed individuals² develop a depressive disorder as a consequence, some individuals may be more sensitive to the depression-inducing effects of SLEs than others.^{3,4} Factors that have been shown to increase sensitivity to SLEs are the personality trait neuroticism,⁵⁻¹⁰ childhood adversity,^{9,11} and indirect measures of genetic risk for depression and anxiety, derived from twin or family studies.¹²⁻¹⁵

A variety of recent studies have reported that the moderating effect of genetic risk on the relationship between life events and depression can be traced to a length polymorphism in the gene encoding the serotonin transporter (*5-HTTLPR*).

Caspi et al¹⁶ reported that individuals with 1 or 2 short (S) alleles at this polymorphism (SL and SS) were more likely to develop depression after exposure to SLEs than individuals with 2 long (L) genotypes (LL). However, the temporal resolution of SLEs in relation to onset of depression was low, suggesting that what was moderated by the *5-HTTLPR* genotype may not be the direct relationship between life stress and depression.¹⁷ Kendler et al¹⁷ recently replicated the moderating effect of the *5-HTTLPR* genotype in a sample of adult twins, analyzing life events that were proximal to depression onset. Although the results resembled those reported by Caspi et al,¹⁶ the moderating effect of the *5-HTTLPR* genotype concerned only the impact of common, mild stressors rather than the impact of rarer and severe life events.¹⁷ A third study¹⁸ found that

5-HTTLPR moderated the association between a composite measure of environmental risk (that by itself did not increase risk for the depression outcome) and self-reported depressive symptoms in female (but not male) adolescents, and a fourth study¹⁹ did not find a moderating effect of 5-HTTLPR.

One way to explain a moderating effect of the 5-HTTLPR genotype on the association between depression and life events that may be (1) remote in relation to the onset of depression^{16,18} and (2) mild rather than severe¹⁷ is to invoke some third factor associated with both life events and depression. An attractive hypothesis is that rather than the occurrence of SLEs, per se, this factor is the personality trait that moderates their psychological impact, that is, determines how individuals appraise and cope with environmental adversity.^{20,21} The personality trait neuroticism, associated with stress sensitivity and negative affectivity,²²⁻²⁶ is a plausible candidate for several reasons. First, although an interaction between the 5-HTTLPR genotype and SLEs can be readily analyzed statistically, any causal effect of SLEs can only be meaningfully envisaged more “downstream” in terms of the psychological experience they engender. One way of taking this into account is to assess SLEs in terms of their contextual threat. Within this framework, not the SLE but its sequelae for the individual are crucial in the development of a possible depressive episode. It is well known that there are stable differences between individuals in the way they experience the environment as a source of negative emotions²⁷ and the way they respond with mood changes after stressors in their daily lives.^{15,28-30} Therefore, the origin of any interaction between a genetic polymorphism and a highly prevalent exposure, such as SLEs,² may alternatively represent a reflection of an interaction between a genetic polymorphism and a stable psychological trait to experience the environment as stressful. Second, neuroticism not only predicts the onset of depressive symptoms and depressive disorder³¹⁻³⁵ but also increases the risk of exposure to SLEs,^{8,34,36-40} in particular SLEs in the realm of getting along with other people, marital difficulties, financial difficulties, and work problems and, to a lesser extent, rarer SLEs, such as being robbed or assaulted or death and illness in the extended network.⁴⁰ Third, previous studies have suggested that 5-HTTLPR and neuroticism may interact in their effect on smoking behavior,^{41,42} a phenotype associated with depression.⁴³ Thus, one hypothesis is that rather than the association between SLEs and depression, the 5-HTTLPR genotype moderates the association between depressive disorder and the tendency to experience the environment as stressful. Individuals with easily provoked negative affectivity may be more likely to develop depression in the context of a predisposing genotype. In the analyses reported by Caspi et al,¹⁶ this would explain the moderating effect of the 5-HTTLPR genotype, although SLE exposure and depression outcome were arguably too remote from each other to represent a direct effect of stress on onset of mood disorder; in the replication study,¹⁷ this would explain why the moderating effect of the 5-HTTLPR genotype concerned only minor stressors.

In the present study, the hypothesis was tested that the apparent moderating effect of the 5-HTTLPR genotype on the association between negatively attributed SLEs

and depression in fact represents a moderating effect on the association between neuroticism and depression. This hypothesis can be tested by taking into account the fact that neuroticism, in addition to increasing the risk of exposure to SLEs, also moderates the effects of SLEs on depressive symptoms and depressive disorder.⁵⁻¹⁰ We thus hypothesized that the moderating effect of the 5-HTTLPR genotype on the depressogenic effect of SLEs would disappear if the genotype \times SLE interaction did not include the effect of SLEs conditional on neuroticism. Conversely, we hypothesized that the depressogenic effect of neuroticism would also be moderated by the 5-HTTLPR genotype and that this would be unchanged even if the genotype \times neuroticism interaction did not include the effect of neuroticism conditional on SLEs.

Testing these hypotheses assumes that there is no direct association between (1) the 5-HTTLPR genotype and SLEs, as demonstrated previously,^{16,17} and (2) the 5-HTTLPR genotype and neuroticism. Although reports of such an association exist,⁴⁴ this has not been replicated consistently.^{45,46} In addition, recent genomewide linkage studies of neuroticism have not found the region around *SLC6A4* (the human serotonin gene) on chromosome 17 to contain loci contributing to neuroticism,⁴⁷⁻⁵⁰ and a recent study⁴⁶ with 100% power to detect a genetic effect of 5% of the phenotypic variance similarly did not support the hypothesis that the 5-HTTLPR variant contributes significantly to neuroticism.

A variety of issues were also addressed in the study design. First, because context-sensitive constructs such as depression, neuroticism, and life events are difficult to assess precisely in a single cross-sectional measurement and risk is better assessed in a longitudinal rather than a cross-sectional design,²¹ a prospective design was used with 1 baseline and 4 follow-up measurements in 15 months. This allowed for added measurement precision and the assessment of change in depression across time in relation to neuroticism, SLEs, the 5-HTTLPR genotype, and their interactions.

METHODS

SAMPLE

Participants were taking part in an ongoing, longitudinal, general population twin study on gene-environment interaction in affective disorders, which has been described in detail elsewhere, showing a very high degree of compliance with research procedures.⁵¹ The sample was female only, given evidence of sex-specific genetic factors for neuroticism and depression⁵² and evidence of qualitative differences in the type of environmental stressors associated with depression in men and women.⁵³ All the participants were white, and all 4 grandparents were of Belgian origin. The study was approved by the ethics committee of Maastricht University, and participants provided written informed consent. Of 623 participants, 131 refused genotyping, and of the 492 consenting individuals, 384 sent back samples suitable for DNA analysis. Of the 384 individuals, 374 had complete baseline measures on depression, life events, and neuroticism at baseline. At the 4 follow-up measurements, these numbers were 332, 304, 271, and 241, respectively. The sample of 374 at baseline included 356 female twin pairs (231 individuals were members of monozygotic twin pairs, 124 were members of dizygotic twin pairs, and 1 was of

unknown zygosity) and 18 of their sisters. At baseline, there were no differences between the 384 genotyped and the 239 nongenotyped women in neuroticism ($F=0.42$; $P=.52$), life events ($F=0.58$; $P=.45$), or depression ($F=0.02$; $P=.90$).

Zygosity was determined through sequential analysis based on sex, fetal membranes, blood groups, and DNA fingerprints. In 81 pairs, determination of zygosity was based on self-report and mother's report of standard questions about physical similarity and the degree to which the twins are confused⁵⁴⁻⁵⁶ and, if necessary, on examination of DNA fingerprints. Participants were interviewed 5 times at approximately 3- to 4-month intervals. The mean number of days between baseline and the first measurement was 132, between the first and second measurements was 91, between the second and third measurements was 116, and between the third and fourth measurements was 91. The first interview was at the home of the individual, and follow-up data were collected by questionnaire.

MEASURES

Depression

Because Caspi et al¹⁶ showed that results for *5-HTTLPR* moderation were similar for self-reported and interview-based measurements of depression, a validated self-report measure was used. At baseline and at each of the 4 follow-up measurements, participants completed the 90-item Symptom Check List, a validated self-report clinical rating scale developed to measure 9 primary symptom dimensions observed in psychiatric outpatients.⁵⁷ The dimension of depressive symptoms consists of 16 items, such as "feeling low in energy or slowed down," "feeling no interest in things," and "experiencing feelings of worthlessness." Participants were instructed to rate the degree of discomfort associated with each depressive symptom during the past week on a 5-point scale ranging from "not at all" to "extremely." A continuous weighted depression score (sum of scores on the depression items divided by the number of items completed) was calculated at each measurement occasion. This continuous measure was used in the analyses.

Recent Life Events

An inventory of recent life events was made based on the event list of the Interview for Recent Life Events.⁵⁸ Participants reported whether 1 of the 61 events happened in the past 6 months (at baseline) and since the last measurement occasion (at follow-up) and rated their impact on a 5-point scale (from 1=very pleasant to 5=very unpleasant). These recent life events were divided into 10 categories: work, education, finance, health, bereavement, migration, courtship and cohabitation, legal, family and social relationships, and marital relationships, all representing datable occurrences involving changes in the external social environment. Internal occurrences, such as changes in perceptions or satisfactions, were excluded except for onset of physical illness because the implications of this event are much the same as those of an event that is purely external in origin.⁵⁸ Events rated as unpleasant (ie, a score of 4, "unpleasant," or a score of 5, "very unpleasant") were included in the analysis, and a variable was made representing the number of such unpleasant events that happened in the past 6 months. In the analyses, an SLE score was used and was coded as follows: 0 SLEs=0, 1 SLE=1, 2 SLEs=2, 3 SLEs=3, 4 SLEs=4, and 5 or more SLEs=5.

Neuroticism

At baseline and at each of the 4 follow-up measurements, participants completed the neuroticism-extraversion subscale of

the Eysenck Personality Scale.⁵⁹ The neuroticism-extraversion subscale consists of 12 yes-or-no items measuring neuroticism and 12 yes-or-no items measuring extraversion. A weighted neuroticism score (sum of "yes" divided by the number of items completed) was calculated at each follow-up measurement.

Genotyping

Placental tissue for DNA analysis was available for 156 participants, blood samples for 14, and buccal cell samples for 208 using a sterile swab specifically designed for the collection of buccal cell samples for DNA testing (Omni Swabs; Whatman plc, Brentford, England).

Genomic DNA was isolated using the QIAamp DNA Mini Kit (Qiagen, Westburg, Leusden, the Netherlands) according to the appropriate protocol for each sample type (placenta, blood, or buccal swabs). Determination of the *5-HTTLPR* genotype was performed as previously described,⁶⁰ with some modifications. The forward primer was labeled with carboxyfluorescein and had the sequence (5'-GGCGTTGCCGCTCTGAATGC-3') and the reverse (5'-GAGGGACTGAGCTGGACAACCCAC-3'). Polymerase chain reactions were performed in 96-well microtiter plates on a T1 Thermocycler (Biometra GmbH, Goettingen, Germany). We used approximately 10 to 100 ng of genomic DNA in a 25- μ L reaction mixture containing 1 \times polymerase chain reaction buffer (Invitrogen, Breda, the Netherlands), 0.2mM deoxynucleoside triphosphates, 0.4 μ M of each primer, 0.75mM magnesium chloride, and 1 U of Taq DNA polymerase (Invitrogen). Cycling conditions were as follows: initial 3-minute denaturation at 95°C; 5 cycles of denaturation at 94°C for 30 seconds, annealing at 65°C (touchdown 0.3°C) for 1 minute, and extension at 72°C for 1 minute; 30 cycles of denaturation at 94°C for 30 seconds, annealing at 63°C for 1 minute and extension at 72°C for 1 minute; and a final extension for 10 minutes at 72°C. Polymerase chain reaction products were analyzed using an ABI 3100 Genetic Analyzer and GeneScan analysis software (Applied Biosystems, Nieuwerkerk aan den IJssel, the Netherlands).

For every monozygotic twin in the sample with genotypic data, the same genotypic data were included for the co-twin, assuming that both twins had identical genotypes. In the analyses, the *5-HTTLPR* genotype was expressed as follows: 1 = 2 long alleles (*LL*); 2 = 1 short and 1 long allele (*SL*); and 3 = 2 short alleles (*SS*). In a model of SLE score at baseline, the *5-HTTLPR* genotype was not associated with SLE score ($\chi^2=1.82$; $P=.40$). Similarly, in a model of neuroticism at baseline, the *5-HTTLPR* genotype was not associated with neuroticism ($\chi^2=2.78$; $P=.25$).

STATISTICAL METHODS

To take the 3-level grouping structure of the data (measurement occasion, twin pair, and participant) into account, multilevel random regression analysis was applied in Stata version 9 using the *xtmixed* command (StataCorp, College Station, Tex) to fit linear mixed models of the continuous depression outcome. Mixed models are characterized as containing fixed and random effects. The fixed effects are analogous to standard regression coefficients and are estimated directly. The grouping structure of the data in *xtmixed* may consist of multiple levels of nested groups.

To assess associations with change in the depression outcome across time, the depression outcome measured at follow-up was modeled in the multilevel random regression equation, corrected for baseline depression.

The first basic model included the main effects of SLE score, neuroticism, and the interaction between SLE and neuroticism. Subsequently, we examined to what degree possible interactions between genotype and life events on the one hand and genotype

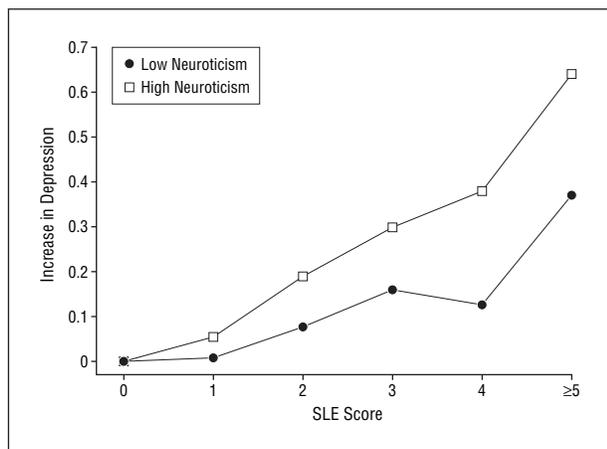


Figure 1. Regression coefficients indicating baseline-adjusted increase in the depression outcome as a function of stressful life event (SLE) exposure (score 0 to 5 or more SLEs; score 0 was the reference category), stratified by level of neuroticism (median-split groups).

and neuroticism on the other would remain in this basic model. If an interaction between the *5-HTTLPR* genotype and SLE was apparent but disappeared after adding neuroticism and the interaction between neuroticism and SLE to the model (ie, after modeling the genotype \times SLE interaction with only the effect of SLE that is not conditional on neuroticism), the genotype \times SLE interaction is unlikely to reflect a direct effect independent of neuroticism. If, in addition, an interaction between the *5-HTTLPR* genotype and neuroticism was apparent that did not disappear after adding SLE and the interaction between neuroticism and SLE to the model (ie, after modeling the genotype \times neuroticism interaction with only the effect of neuroticism that is not conditional on SLE), the genotype \times neuroticism interaction is more likely to reflect the true moderating mechanism.

Given that linear¹⁶ and nonlinear¹⁷ moderating effects of the *5-HTTLPR* genotype have been reported, interactions were tested first with the continuous variable indicating the degree of *S* loading (0=0 *S* alleles, 1=1 *S* allele, and 2=2 *S* alleles), followed by an interaction with both the *5-HTTLPR* genotype dummy variables, with *LL* as the reference category. From this latter model, effect sizes were calculated for SLE and neuroticism for each genotype separately by applying and testing the appropriate linear combinations using the Stata *lincom* command. Main effects and interactions were assessed by means of the Wald test.⁶¹

RESULTS

SAMPLE

The mean (SD) participant age was 27 (8) years (range, 18-46 years); 2% completed only primary school, 34% finished secondary school, and 64% had a college degree. Most participants were currently employed (65% employed, 30% students, 2% homemakers, and 2% unemployed). Aggregated over participants' means, the mean (SD) neuroticism score was 0.45 (0.27) (range, 0-1), and the mean (SD) Symptom Check List depression score was 1.47 (0.48) (range, 1-4.54). The proportions of individuals with at least 1 life event at baseline and the 4 follow-up measurements were 74%, 67%, 73%, 61%, and 64%, respectively. Across the 5 measurement occasions, the proportion of individuals with 1 SLE was 26%; 2 SLEs, 20%; 3 SLEs, 10%; 4 SLEs, 7%; and 5 or more SLEs, 7%.

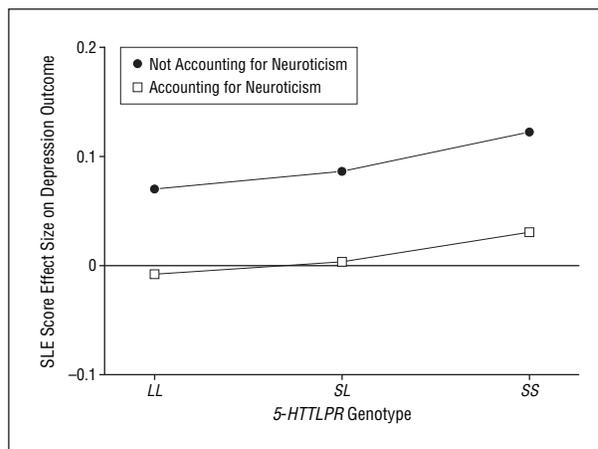


Figure 2. Regression coefficients indicating baseline-adjusted increase in depression outcome as a function of stressful life event (SLE) score (coefficient indicates change in depression per unit increase in SLE score coded 0-5), stratified by genotype at the serotonin transporter length polymorphism (*5-HTTLPR*) (*LL*, *SL*, and *SS*; *L* indicates long allele and *S*, short allele). Effects of SLE not taking into account the part conditional on neuroticism: *LL*: $\beta=0.071$; $\chi^2=18.1$; $P<.001$; *SL*: $\beta=0.087$; $\chi^2=33.8$; $P<.001$; and *SS*: $\beta=0.122$; $\chi^2=43.4$; $P<.001$. Effects of SLE taking into account neuroticism: *LL*: $\beta=-0.007$; $\chi^2=0.12$; $P=.73$; *SL*: $\beta=0.003$; $\chi^2=0.02$; $P=.88$; and *SS*: $\beta=0.03$; $\chi^2=1.30$; $P=.26$.

The frequencies of the 3 *5-HTTLPR* genotypes were as follows: *LL*, 31%; *SL*, 47%; and *SS*, 22%, comparable with previous reported frequencies in similar samples^{16,17} and in Hardy-Weinberg equilibrium ($\chi^2=1.4$; $P=.24$).

MAIN EFFECTS OF SLE AND NEUROTICISM

In the model including SLE score and neuroticism without their interaction, a significant positive association was apparent for both, showing progressively greater increases in the depression outcome with higher levels of SLE (SLE: $\beta=0.07$; $\chi^2=67.7$; $P<.001$; and neuroticism: $\beta=0.82$; $\chi^2=202.6$; $P<.001$). In the model including the interaction between SLE and neuroticism, strong effect modification was apparent ($\beta=0.14$; $\chi^2=23.5$; $P<.001$). This interaction is depicted in **Figure 1**, showing the effect of SLE on the depression outcome stratified by 2 levels of neuroticism around the median split. Adding the *5-HTTLPR* genotype did not further improve this model, entered either as 2 dummy variables with *LL* as the reference category ($\chi^2=1.49$; $P=.47$) or as *S* loading ($\chi^2=1.19$; $P=.28$).

GENOTYPE \times SLE INTERACTION

First, a limited model was tested that included genotype, Symptom Check List score, and the genotype \times SLE interaction. There was a significant interaction between SLE score and *S* loading ($\beta=0.026$; $\chi^2=4.2$; $P=.04$), whereas the interaction with genotype dummy variables revealed a positive, but nonsignificant, contrast in SLE effect size between *SL* and *LL*, respectively ($\beta=0.016$; $\chi^2=0.51$; $P=.48$) and a larger and significant contrast between *SS* and *LL* ($\beta=0.051$; $\chi^2=4.3$; $P=.04$). The SLE effect sizes by genotype calculated from this latter model are depicted in **Figure 2**.

When neuroticism and the interaction between neuroticism and SLE were added to the equation, the interac-

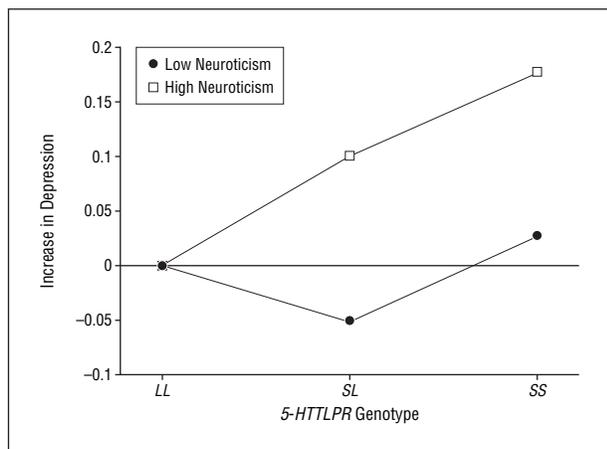


Figure 3. Regression coefficients indicating baseline-adjusted increase in the depression outcome as a function of a polymorphism of the serotonin transporter gene (*5-HTTLPR*) genotype (*LL*, *SL*, and *SS*; *L* indicates long allele and *S*, short allele), stratified by level of neuroticism (median-split groups).

tion between genotype and SLE was reduced and no longer significant ($SLE \times S$ loading: $\beta=0.017$; $\chi^2_1=2.2$; $P=.14$; $SLE \times$ genotype dummies: SL vs LL : $\beta=0.010$; $\chi^2_1=0.26$; $P=.61$; and SS vs LL : $\beta=0.034$; $\chi^2_1=2.17$; $P=.14$). The SLE effect sizes by genotype calculated from this latter model are depicted in Figure 2 and were all but nullified.

GENOTYPE \times NEUROTICISM INTERACTION

In the simple model including genotype, neuroticism, and the genotype \times neuroticism interaction, a significant interaction was apparent between neuroticism and *S* loading ($\beta=0.18$; $\chi^2_1=5.7$; $P=.02$) (**Figure 3**), whereas the interaction with genotype dummy variables revealed an increasing contrast in neuroticism effect size in the comparison, first, between *SL* and *LL* ($\beta=0.24$; $\chi^2_1=3.8$; $P=.05$) and, second, between *SS* and *LL* ($\beta=0.34$; $\chi^2_1=5.1$; $P=.03$). Neuroticism effect sizes by genotype calculated from this latter model are depicted in **Figure 4**.

When SLE and the interaction between neuroticism and SLE were added to the equation, the interaction between genotype and neuroticism was only marginally reduced (neuroticism \times *S* loading: $\beta=0.15$; $\chi^2_1=4.7$; $P=.03$; neuroticism \times genotype dummies: SL vs LL : $\beta=0.24$; $\chi^2_1=4.1$; $P=.04$; and SS vs LL : $\beta=0.29$; $\chi^2_1=4.0$; $P=.05$). Neuroticism effect sizes by genotype calculated from this latter model are depicted in Figure 4.

In a final analysis, the full model, including all 3 interactions, revealed strong neuroticism \times *S* loading ($\beta=0.22$; $\chi^2_1=15.3$; $P<.001$) and neuroticism \times SLE ($\beta=0.14$; $\chi^2_1=24.1$; $P<.001$) interactions but not an SLE \times *S* loading interaction ($\beta=0.009$; $\chi^2_1=0.64$; $P=.43$).

COMMENT

FINDINGS

Replication

This study was a replication of a specific genotype interacting with a specific environmental measure⁶²⁻⁶⁵ and con-

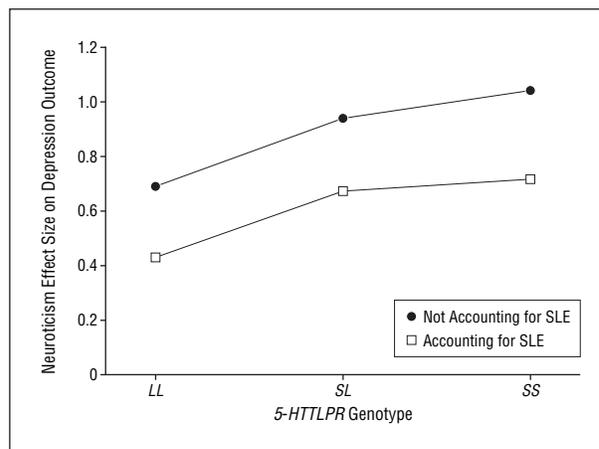


Figure 4. Regression coefficients indicating baseline-adjusted increase in depression outcome as a function of neuroticism (coefficient indicates change in depression per unit increase in neuroticism), stratified by genotype at the serotonin transporter length polymorphism (*LL*, *SL*, and *SS*; *L* indicates long allele and *S*, short allele). Effects of neuroticism not taking into account the part conditional on stressful life event (SLE): *LL*: $\beta=0.70$; $\chi^2_1=48.0$; $P<.001$; *SL*: $\beta=0.94$; $\chi^2_1=130.9$; $P<.001$; and *SS*: $\beta=1.04$; $\chi^2_1=79.5$; $P<.001$. Effects of neuroticism taking into account SLE: *LL*: $\beta=0.44$; $\chi^2_1=18.1$; $P<.001$; *SL*: $\beta=0.68$; $\chi^2_1=58.9$; $P<.001$; and *SS*: $\beta=0.72$; $\chi^2_1=36.2$; $P<.001$).

curred with earlier reports that *5-HTTLPR* moderates the association between SLEs or related environmental risks and depressive symptoms and disorder.¹⁶⁻¹⁸ One study,¹⁹ however, did not find a moderating effect of *5-HTTLPR* on the association between SLEs and dimensional and categorical self-report measures of depression, which may have been related to the fact that the sample was much older than that of most other studies in this area. It has been shown that older samples predominantly experience “independent” SLEs, which are outside personal control and less related to high levels of neuroticism.⁶

Beyond Replication

Beyond replication, however, these findings suggest that of 2 possible models of interaction—(1) moderation of the association between SLEs perceived as negative or threatening on the one hand and depression on the other or (2) moderation of the association between the stable tendency to perceive the environment as negative or threatening on the one hand and depression on the other—the latter is more likely than the former. This finding may explain the discrepancy in the moderating effect between mild and more severe SLEs¹⁷; the effects of the former on psychological well-being may be more associated with the personality-related experience of the environment than the latter. In addition, the finding that *5-HTTLPR* moderated an association between SLEs and depression of which the temporal resolution was possibly too limited to reflect a direct effect¹⁶ is suggestive of an indirect relationship moderated by neuroticism. The findings, therefore, suggest that what is moderated by the *5-HTTLPR* genotype may not just be the relationship between a threatening event and the onset of depression; instead, it may moderate the way individuals continually respond to, and cope with, mild SLEs and minor environmental experiences in daily life. This model is com-

patible with transactional models of stress that conceive of stress as person-environment interactions in the flow of daily life,^{15,66,67} and the likely complicated relationships between genotype and phenotype in psychiatry.¹⁷ Although plausible and in line with 2 previous studies showing 5-HTTLPR \times neuroticism interactions in the area of smoking behavior,^{41,42} the findings should nevertheless be considered hypothesis generating until replicated by future studies.

NEUROTICISM AND THE 5-HTTLPR GENOTYPE

Neuroticism is generally conceived of as a stable and highly general trait dimension that reflects the extent to which an individual perceives the world as threatening and distressing.²⁷ Conceptualized as a higher-order trait in a hierarchical model, neuroticism incorporates a variety of lower-order personality traits, such as low self-esteem,⁶⁸ perfectionism,¹³ rumination,⁶⁹ self-criticism,⁷⁰ and sociotropy,⁷¹ that have been associated with increased risk of depression. The hypothesis that the association between these personality traits and depression is moderated by the 5-HTTLPR genotype is biologically plausible given that the 5-HTTLPR *S* allele is associated with lower serotonin transporter transcription and reduced serotonin transporter function.⁴⁴ This has been confirmed in human in vivo imaging studies^{72,73} using radioligand serotonin transporter binding. Recent studies^{74,75} show increased amygdala reactivity in response to fearful and angry human facial expressions in healthy *S* allele carriers. In addition, it has been shown that functional connectivity of the amygdala with corticolimbic regions is altered in *S* allele carriers. The degree of this *S* allele-moderated disconnectivity was inversely related to a measure of temperamental anxiety associated with heightened risk of depression (harm avoidance subscale of the Tridimensional Personality Questionnaire).⁷⁶ These findings indicate that the 5-HTTLPR *S* allele may alter neurobiologic circuits implicated in the processing of negative emotions. It has been proposed that the underlying mechanism of this neurodevelopmental disturbance is related to the lifelong increased availability of serotonin in *S* allele carriers and its possible sequences, such as alterations in receptor sensitivity.^{76,77} Concerning the latter, recent evidence indicates that individuals with at least 1 *S* allele have reduced serotonin 1A receptor density,⁷⁸ as is seen in patients with major depression.⁷⁹

LIMITATIONS

A major strength of this study is that rather than relying on a single interview, participants were assessed 5 times in 15 months, allowing us to model prospectively collected measures of change. Nevertheless, the findings should be interpreted in light of 5 limitations. First, the continuous outcome measure of depressive symptoms is different from clinical diagnosis according to *DSM* criteria. However, 4 arguments can be brought to bear to demonstrate that this would not have biased or otherwise negatively affected the results. (1) Caspi et al¹⁶

showed that results were similar for continuous measures of self-reported symptoms and major depressive episode according to *DSM-IV* criteria. (2) It would be difficult to conceive of how any error associated with self-report measures would be nonrandom regarding the 5-HTTLPR genotype, a requirement that must be met to be able to produce spurious results. (3) There is good evidence that constructs such as major depressive episode defined by *DSM* or *ICD* criteria may be arbitrary diagnostic conventions imposed on a continuum of depressive symptoms.⁸⁰⁻⁸⁴ (4) Using a continuous depression outcome allowed us to model interactions on the additive scale, which recent studies^{15,85} suggest is more likely biologically meaningful.

A second limitation is that the modeling approach relied on analyzing the interaction between SLEs and neuroticism, as this person-environment interaction was central to the hypothesis. This approach, however, does not allow for simultaneously taking into account the fact that the relationship between SLEs and neuroticism is also characterized by person-environment correlation; that is, neuroticism also affects the likelihood of exposure to SLEs per se.^{86,87} The degree to which this person-environment correlation may have contributed to explaining the apparent interaction between SLEs and the 5-HTTLPR genotype, therefore, cannot be estimated. This would not change the central argument, however, that the apparent interaction between SLEs and the 5-HTTLPR genotype reflects the underlying effect of neuroticism.

A third limitation is the possibility of systematic measurement errors leading to inflated correlations between the neuroticism and depression outcomes. However, the focus of this study was not on the relationship between these measures but on the interaction between neuroticism and the 5-HTTLPR genotype on the depression outcome. An inflated correlation between neuroticism and the depression outcome would not generalize to 5-HTTLPR, which is the main exposure of this study.

The fourth limitation is that the assessment of SLEs and depression did not make it possible to examine which SLEs were the result rather than the possible cause of the depression outcome. However, the Interview for Recent Life Events was designed specifically to collect datable occurrences involving changes in the external social environment rather than internal occurrences, such as changes in perceptions or satisfactions. In addition, the fact that measures were collected on 5 separate occasions will have brought many truly dynamic changes in mental states occasioned by changes in the external social environment. Finally, measurements of SLEs were based on self-reports as opposed to structured interviews such as the Life Events and Difficulties Schedule, which increases the possibility of retrospective bias and error in the timing of event occurrence. Although SLEs were not rated specifically regarding level of contextual threat, the rating of negative emotional attribution in the Interview for Recent Life Events arguably constitutes a valid way of identifying occurrences most likely to shape the risk of future depression.

A fifth limitation relates to the female-only constitution of the sample. The main rationale for this was the evidence showing that men and women differ not only

in experiencing various SLEs but also in their (depressogenic) response to them.^{53,71} In addition, sex-specific genes likely affect the neuroticism and the depression phenotype.^{13,82,88} Therefore, although our approach for a restricted sample was justified, the findings cannot be extrapolated directly to the male population.

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