Serotonin Transporter Polymorphism Moderates Effects of Prenatal Maternal Anxiety on Infant Negative Emotionality

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Background: Consistent with the fetal programming hypothesis, effects of maternal prenatal anxiety have been found to predict various measures of infant temperament in the early postnatal period. In recent years, a polymorphism in the serotonin transporter gene (5-HTTLPR) emerged as a moderator of diverse environmental influences on different outcomes, with individuals carrying the short allele being generally more vulnerable to adversity.

Methods: We tested whether the association between self-reported maternal anxiety at 20 weeks gestation (Brief Symptom Inventory) and mother-rated infant negative emotionality at 6 months after birth (Infant Behavior Questionnaire-Revised) would be moderated by the 5-HTTLPR in a large Dutch cohort sample ($n = 1513$). We hypothesized that infants carrying the 5-HTTLPR short allele would be more susceptible and therefore more affected by both low and high prenatal maternal anxiety vis-à-vis negative emotionality than other genotypes.

Results: Findings of a significant gene × environment interaction ($B = .65$, $p = .01$) were supportive of a vulnerability model, with infants carrying the short allele being more negatively emotional when mothers reported anxiety during pregnancy, whereas there was no difference between genotypes on negative emotionality when maternal anxiety was low.

Conclusions: The association between maternal anxiety during pregnancy and negative emotionality in early infancy was significant in infants carrying one or more copies of the short allele but not in those homozygous for the long allele. The 5-HTTLPR short allele might increase vulnerability to adverse environmental influences as early as the fetal period.

Key Words: 5-HTTLPR, differential susceptibility, gene × environment (GXE) interaction, infant temperament, prenatal programming, serotonin transporter

Early experiences and environmental influences have been found to shape human development as early as the fetal period. This observation has been interpreted in terms of the fetal programming hypothesis (1,2), which stipulates that the fetus adjusts its phenotype (e.g., metabolism and stress reactivity) in utero—on the basis of placental transferred maternal nutritional and hormonal cues about the "outside" world—as a means of optimally adapting to the (anticipated) conditions of the postnatal environment.

Findings consistent with the fetal programming hypothesis have been reported repeatedly (3), along with perhaps related evidence linking prenatal maternal anxiety and mother-reported infant temperament. For example, higher levels of maternal anxiety during pregnancy has been found to predict: 1) greater infant temperament reactivity at 8 weeks after birth (4); 2) greater infant negative behavioral reactivity at 4 months after birth (5); 3) greater infant difficult temperament at 4 and 6 months after birth (6); and 4) decreased infant attention regulation at 3 and 8 months after birth (7). The fact that all the just-cited investigations controlled for postnatal maternal anxiety clearly suggests that the predicted differences in infant temperament are a function of prenatal maternal anxiety rather than just of postnatal maternal psychological state.

What the available research has yet to address is whether the putative effects of maternal anxiety on infant temperament vary across fetuses due to their genetic make-up. That this might be the case is certainly suggested by recent studies of gene × environment (GXE) interaction. Most prominently, perhaps, a polymorphism in the serotonin transporter promoter gene area (SLC6A4), the 5-HTTLPR, has been found to moderate the apparent effect of adverse early environmental influences on a variety of phenotypic outcomes. For example, severe childhood maltreatment has been associated with more depression symptoms in adulthood in individuals that carried one or two copies of the short 5-HTTLPR allele but not in individuals homozygous for the long allele (8). Similarly, low maternal sensitivity at 7 months predicted insecure attachment at 15 months exclusively for infants carrying 5-HTTLPR short alleles, whereas attachment quality of infants homozygous for the long allele was independent of observed levels of maternal sensitivity (9).

Most such GXE results have been interpreted in terms of diathesis-stress thinking (10), with the 5-HTTLPR short allele regarded as a vulnerability factor (or diathesis) predisposing individuals toward problematic functioning (e.g., depression) in the face of contextual adversity (e.g., child maltreatment). But as noted by Taylor *et al.* (11) in their study of a GXE interaction involving 5-HTTLPR and quality of the early family environment in the prediction of adult depression as well as by Belsky *et al.* (12,13) in their analysis of many other GXE findings, the short allele might perhaps be better conceptualized as a "plasticity gene" rather than a "vulnerability gene." This is because individuals with the short allele seem in some research to be not only more likely than others to succumb to the negative effects of adverse environments but also more likely than others to benefit from positive supportive ones (14). This proves true even in work in which environmental support is operationalized as merely the ab-

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sence of negative contextual conditions (e.g., no childhood maltreatment).

Evidence of this kind is consistent with Belsky's (13,15–17) "differential-susceptibility hypothesis," which posits that some individuals—including those with the short allele of the 5-HTTLPR—are more affected by both negative and positive environmental conditions than others (i.e., for better and for worse) rather than just disproportionately and negatively affected by contextual adversity than others (11,18). A recent reanalysis of data from a GXE study by Neuman *et al.* (19) of effects of maternal smoking during pregnancy on ADHD in childhood (20) provided first evidence of genetically related differential susceptibility to effects of prenatal experiences. The study, however, investigated the moderating effect of DRD4 and not 5-HTTLPR. Children carrying the dopamine DRD4 7-repeat allele—an allele repeatedly associated with differential susceptibility (12,13,21)—tended to be most and least likely to develop ADHD, depending, respectively, on whether their mothers did or did not smoke during pregnancy. It remains to be determined whether the effect of stressful prenatal experiences is moderated in a manner consistent with differential susceptibility when the moderator is 5-HTTLPR.

In light of evidence that prenatal maternal anxiety predicts infant temperament and that the short allele of 5-HTTLPR might function as a plasticity gene, moderating environmental influences in a for-better-and-for-worse manner (17), the current study tested whether the temperaments of infants with one or two short alleles would be more affected by maternal prenatal anxiety than those homozygous for long alleles and whether this moderation would be more consistent with a differential susceptibility than diathesis-stress model (i.e., whether they would prove to be less negatively emotional than others under conditions of low maternal prenatal anxiety, yet more negatively emotional than others under conditions of high maternal prenatal anxiety).

Methods and Materials

Design

This research was embedded in the Generation R Study, a population-based cohort study investigating growth, development, and health from fetal life into young adulthood in Rotterdam, the Netherlands. The Generation R Study has previously been described in detail (22). Briefly, all pregnant women living in the study area with a delivery date between April 2002 and January 2006 were informed about the research project by community midwives and obstetricians. Inclusion criteria were: 1) residency in study area at delivery date; 2) delivery date between April 2002 and January 2006; and 3) informed consent. Importantly, mothers with psychiatric disorders were not identified or excluded from study participation. Written informed consent and genetic data were available for 4345 study families.

The Generation R study has been approved by the Medical Ethics Committee (MEC) of the Erasmus Medical Center, Rotterdam (numbers: prenatal, MEC 198.782/2001/31, and postnatal, MEC 217.595/2002/202).

Participants

Only infants with at least one parent of self-reported Dutch ethnicity were included in the present study to avoid confounding effects of ethnic differences in gene frequency. Of the 3639 qualifying Dutch families 1513 had data on 5-HTTLPR, infant negative emotionality, and prenatal maternal anxiety and were consequently included in the study; see Table 1 for sample characteristics (when analyses were restricted to the 1136 infants with two parents of Dutch ethnicity, results remained the same). Comparisons between included and excluded families revealed no significant differences with regard to 5-HTTLPR, infant negative emotionality, infant gender, and postnatal maternal anxiety or depression. Significant differences emerged, however, for prenatal maternal anxiety, which was significantly greater for excluded than included mothers (mean = .22, SD = .38 vs. mean = .18, SD = .31, $p < .01$, $D = .14$), and for some variables which are not reported here because of restricted space (and are available on request).

Table 1. Demographic Characteristics of the Sample

Variables	*n* (%)
Age at First Contact (yrs)	mean = 31.81, SD = 4.03 (range: 17–43)
Educational Level	
No education	20 (1.3%)
Low (12 yrs or less)	129 (8.5%)
Mid-low (13–15 yrs)	369 (24.4%)
Mid-high (16–17 yrs)	399 (26.4%)
High (18 yrs or more)	596 (39.4%)
Living Situation	
Living with partner	1443 (95.3%)
Living without partner	70 (4.6%)
Income	
< €1200	69 (4.6%)
€1200–2200	261 (17.3%)
> €2200	1183 (78.2%)
Smoking During Pregnancy	174 (11.5%)
Alcohol During Pregnancy	861 (56.9%)
Anxiety During Pregnancy	mean = .18, SD = .31
Anxiety at 6 Months Postnatal	mean = .22, SD = .36
Depression at 6 Months Postnatal	mean = .16, SD = .35
Child Gender	
Boy	761 (50.3%)
Girl	752 (49.7%)
Child Gestational Age at Birth (weeks)	mean = 40.16, SD = 1.44
Child Birth Weight (g)	mean = 3552.40, SD = 508.28
Child 5-HTTLPR	
l/l	497 (32.8%)
s/l	738 (48.8%)
s/s	278 (18.4%)
Child Negative Emotionality at 6 Months	
Fear	mean = .33, SD = .27
Distress to limitations	mean = .62, SD = .30
Recovery of distress	mean = 1.56, SD = .28
Negative emotionality composite (standardized)	mean = .00, SD = 2.18

N = 1513.
5-HTTLPR, serotonin transporter polymorphism.

Measures

Maternal Prenatal and Postnatal Psychopathology Maternal psychopathology was assessed at 20 weeks of pregnancy and at 6 months after birth with the Brief Symptom Inventory, a validated self-report questionnaire with 53 items answered on a 5-point scale ranging from 0 = "not at all" to 4 = "extremely" (23–25). The Brief Symptom Inventory is a short version of the Symptom Checklist 90 (26) and defines a broad spectrum of psychiatric symptoms over the preceding 7 days. For this study, the prenatal and postnatal anxiety and the postnatal depression subscales were used.

Infant Negative Emotionality Infant temperament was assessed at 6 months after birth with an abbreviated version of the Infant Behavior Questionnaire—Revised (IBQ-R) (27). This measure

is based on maternal reports of frequencies of specific infant behaviors observed over the past week. Only 6 of the original 14 IBQ-R subscales were administered, and the original 7-point response scale was truncated to a 3-point scale (0 = never present; 1 = sometimes present; 2 = often present) after a pilot study revealed that respondents seldom used the extreme positions of scales (28). Total scores for each subscale were calculated by dividing the sum of the items by the number of endorsed items. Internal consistencies for the adapted IBQ-R ranged from .71 to .85—similar to the internal consistencies of the original IBQ-R (27). A composite measure for infant negative emotionality was derived by standardizing and averaging three of the subscales (distress to limitations, fear, and recovery of distress [reflected]). Higher scores on this composite measure represent greater negativity.

Covariates Birth weight and infant gender were obtained from midwife and hospital registries shortly after birth. Gestational age was established by fetal ultrasound examinations. Information about income, maternal educational level, maternal smoking, and maternal alcohol consumption during pregnancy was obtained by questionnaires. The highest completed education determined the educational level of the mothers. Following the definition of Statistics Netherlands (29), educational level was categorized as "no education," "low" (12 years of education or less), "mid-low" (13–15 years), "mid-high" (16–17 years), and "high" (18 years or more). Maternal smoking and maternal alcohol consumption were assessed in the first, second, and third trimester and summarized as either "yes, at least sometime during pregnancy" or "never during pregnancy."

Genotyping

The DNA was derived from cord blood samples at birth. The 43-base pair insertion/deletion in the promoter region of the serotonin gene was genotyped with TaqMan allelic discrimination. Primer sequences were taken from Hu *et al.* (30). Reactions were performed in a 384-well format in a total volume of 5 μL containing 2 ng DNA, 120 nmol/L FAM-probe, 80 nmol/L VIC-probe, polymerase chain reaction primers (100 nmol/L each), dimethyl sulfoxide (4% by volume), and 1 × genotyping master mix (Applied Biosystems, Foster City, California). Polymerase chain reaction cycling consisted of initial denaturation for 10 min at 95°C, and 40 cycles with denaturation of 15 sec at 96°C and annealing and extension for 90 sec at 62.5°C. Signals were read with the TaqMan 7900HT (Applied Biosystems) and analyzed with the sequence detection system 2.3 software (Applied Biosystems). To evaluate genotyping accuracy, 225 random samples were genotyped a second time. No discrepancies were found. To check for potential contamination with maternal blood, gender was determined in male participants. Contamination occurred in < 1% of cases, which were excluded. Genotype distribution (l/l: 32.8%; l/s: 48.8%; s/s: 18.4%) conformed to the Hardy–Weinberg Equilibrium ($p > .99$).

Statistical Analysis

Unadjusted associations between the different measures were evaluated with bivariate correlations (Pearson, two-tailed). The moderating effect of 5-HTTLPR was tested with a hierarchical regression model. All variables included in the regression analysis were centered. Missing data occurred in this longitudinal project due to attrition and failure to complete all assessments, as follows: maternal education (1.0%), living with partner (2.7%), income (5.5%), 6-month maternal depression (.1%), drinking during pregnancy (4.1%), IBQ-R fear (.6%), IBQ-R distress to limitations (2.0%), and IBQ-R recovery from distress (3.6%). Missing data were imputed with multiple imputation (31). Test statistics and regression coefficients were averaged across five imputed datasets. When analyses were run with only cases with complete data, results did not differ from those derived from the imputed data sets. The level of significance for all analyses was set at $\alpha = .05$.

Given the negative findings of a recent meta-analysis of GXE studies involving 5-HTTLPR and life event stress in the prediction of adult depression (32), the robustness of any GXE interaction discerned in the research reported herein is rigorously evaluated by randomly dividing the sample into two subsamples and determining whether the results that emerge from each can be cross-validated on the other, following procedures pioneered by Bakermans-Kranenburg *et al.* (33). All statistical analyses were carried out with PASW Statistics, version 18.0 for Windows (SPSS, Chicago, Illinois) (34).

Results

The serotonin transporter polymorphism, 5-HTTLPR, was not associated with infant negative emotionality and, critically, maternal prenatal anxiety, according to bivariate correlations. The latter fact rules out the possibility of gene–environment correlation being misinterpreted as GXE interaction (17). See Table 2 for the bivariate correlations between variables.

For the hierarchical regression analysis, variables were entered in three steps to predict infant negative emotionality: Step 1 included all the covariates; Step 2 included infant 5-HTTLPR (0, 1, 2 for, respectively, l/l, s/l, and s/s) and maternal anxiety during pregnancy; and Step 3 included the 2-way interaction between 5-HTTLPR and maternal prenatal anxiety. Income, depression at 6 months after birth, and anxiety during pregnancy significantly predicted infant negative emotionality. Most importantly, although there was no main effect of 5-HTTLPR, the interaction between 5-HTTLPR and maternal prenatal anxiety was significant ($B = .65$, $p = .01$, Effect Size [f^2] = .004) in the prediction of infant negative emotionality 6 months after birth (Table 3). Running the regression model separately for male ($n = 761$) and female subjects ($n = 752$) did not reveal any gender differences, although the interaction term for male subjects was only marginally significant ($B = .55$, $p = .09$), whereas it remained significant for female subjects ($B = .87$, $p = .03$). To investigate the small effect size of the interaction term for the full sample ($f2 = .004$), we ran additional hierarchical regression models—stratified by genotype—with Step 1 including the same covariates as in the preceding text and Step 2 maternal anxiety during pregnancy. Although there was no significant effect of maternal prenatal anxiety on negative emotionality for infants homozygous for the long allele ($B = .27$, $p = .43$, $f2 = .001$), significant effects emerged for heterozygous infants ($B = .63$, $p = .05$, $f2 = .005$) and for infants homozygous for the short allele ($B = 1.39$, $p < .01$, $f2 = .033$). Thus, for infants carrying one or more short alleles, greater prenatal anxiety predicted more negative emotionality.

The sample was randomly split into two subsamples of 767 and 747 cases, and the robustness of the regression model was retested by cross-validation of the regression equation in each subsample. The regression equation for subsample 1 ($R = .28$) showed a cross-validation correlation for subsample 2 of .25, and the equation for subsample 2 ($R = .28$) showed a cross-validation correlation for subsample 1 of .25. To investigate the sensitivity of the predicted scores with respect to the exact form of the regression equation, the estimated scores for infant negative emotionality from both regression equations were also correlated within each subsample. The correlation between the two estimates within subsample 1 was $r = .93$, and the correlation within subsample 2 was $r = .94$. Thus, the predicted scores from both regression models seemed to be largely similar within the two subsamples, thereby indicating that the equation coefficients of the regression model in this study were highly robust.

Table 2. Unadjusted Associations Between Variables

	Variables	1	2	3	4	5	6	7	8	9	10	11	12	13
1	Maternal Age	–												
2	Maternal Education	.32[a]	–											
3	Living with Partner (1 = yes; 2 = no)	−.15[a]	−.14[a]	–										
4	Income	.28[a]	.39[a]	−.39[a]	–									
5	Smoking During Pregnancy (1 = no; 2 = yes)	−.06[b]	−.27[a]	.10[a]	−.21[a]	–								
6	Alcohol During Pregnancy (1 = no; 2 = yes)	.22[a]	.26[a]	<.01	.14[a]	.07[b]	–							
7	Anxiety During Pregnancy	−.12[a]	−.13[a]	.15[a]	−.21[a]	.16[a]	−.02	–						
8	Anxiety at 6 Months Postnatal	−.06[b]	−.06[b]	.11[a]	−.11[a]	.10[a]	−.01	.47[a]	–					
9	Depression at 6 Months Postnatal	−.10[a]	−.09[a]	.11[a]	−.17[a]	.15[a]	<.01	.42[a]	.70[a]	–				
10	Child Gender (1 = male; 2 = female)	.02	.04	−.01	.08[a]	−.04	−.01	−.03	−.04	−.06[b]	–			
11	Child Gestational Age at Birth	.01	.08[a]	−.01	.01	.01	.07[a]	.01	.04	.03	−.05[b]	–		
12	Child Birth Weight	.09[a]	.10[a]	−.07[b]	.07[a]	−.12[a]	.05	−.03	.05	.03	−.12[a]	.51[a]	–	
13	Child 5-HTTLPR (0 = l/l; 1 = s/l; 2 = s/s)	−.03	<.01	<.01	<.01	<.01	.01	−.01	.02	.05[b]	.01	<.01	−.02	–
14	Child Negative Emotionality at 6 Months	−.08[a]	−.06[b]	.12[a]	−.16[a]	.01	−.06[b]	.16[a]	.16[a]	.17[a]	<.01	.04	.01	<.01

$N = 1513$.
5-HTTLPR, serotonin transporter polymorphism.
[a] $p < .01$.
[b] $p < .05$.

To illuminate the nature of the interaction, we plotted regression slopes of maternal anxiety during pregnancy vis-à-vis infant negative emotionality separately for each of the three genotypes (Figure 1). These simple slopes revealed what Belsky and Pluess (13) labeled a "plasticity gradient": the positive relation between prenatal maternal anxiety and postnatal infant negative emotionality being strongest for infants homozygous for the short allele (β = .28, $p < .01$), intermediate for heterozygotes (β = .18, $p < .01$), and weakest (and only marginally significant) for those homozygous for the long allele (β = .08, $p = .06$). After z-transformation of the standardized regression coefficients (35), the slopes of infants with s/s and s/l genotype were significantly larger than that of l/l genotypes ($p < .01$ and $p < .05$, respectively), whereas the difference of slopes between s/s and s/l was only marginally significant ($p = .08$).

Table 3. Summary of Hierarchical Regression Analysis

Predictor Variables	Infant Negative Emotionality at 6 Months B
Step 1	
Maternal age	−.01
Maternal education	.04
Living with partner (1 = yes; 2 = no)	.50
Income	−.47[a]
Smoking during pregnancy (1 = no; 2 = yes)	−.28
Alcohol during pregnancy (1 = no; 2 = yes)	−.19
Anxiety at 6 months postnatal	.28
Depression at 6 months postnatal	.53[b]
Child gestational age at birth	.06
Child gender (1 = male; 2 = female)	.08
Child birth weight	<.01
Step 2	
5-HTTLPR	−.01
Anxiety during pregnancy	.62[a]
Step 3	
5-HTTLPR × anxiety during pregnancy	.65[a]

The displayed coefficients of the variables at steps 1 and 2 represent the values after inclusion of interaction terms at Step 3; $N = 1513$, after Step 3: adjusted $R^2 = .06$[a] [$F(14,1498) = 7.74$, $p < .01$].
5-HTTLPR, serotonin transporter polymorphism.
[a] $p < .01$.
[b] $p < .05$.

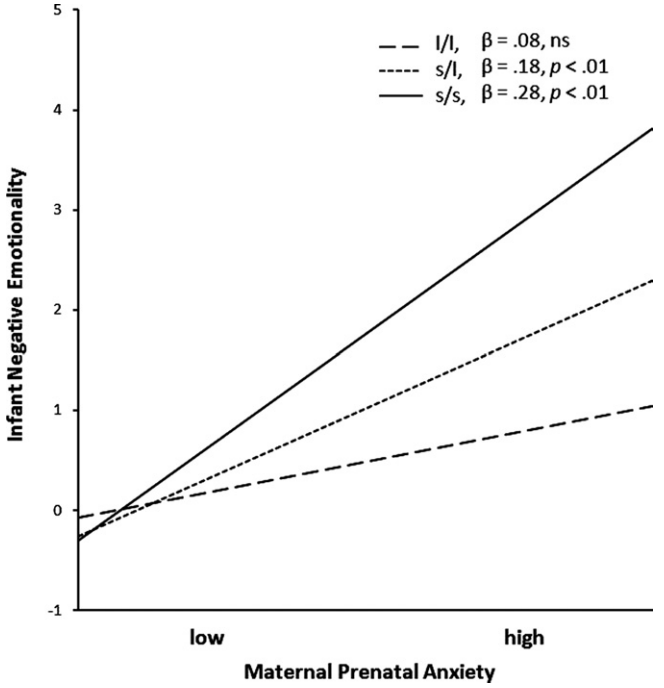

Figure 1. Linear relations between maternal reports of anxiety during pregnancy and infant emotional negativity at 6 months after birth as a function of the serotonin transporter polymorphism, 5-HTTLPR.

Consideration of Figure 1 indicates that the results are more consistent with a diathesis-stress/dual-risk than a differential-susceptibility model of environmental action, because infants with at least one short allele had the highest negative emotionality scores when mothers reported prenatal anxiety but did not differ from those homozygous for the long allele when maternal prenatal anxiety was low. At the same time, however, the simple slopes in Figure 1 do suggest a slight trend for a crossover interaction consistent with differential susceptibility. Follow-up analyses revealed that infants with short alleles had lower scores in negative emotionality (mean = -.31, SD = 2.05) when mothers did not report anxiety during pregnancy compared with infants homozygous for the long alleles (mean = −.21, SD = 2.28), but that this difference was not significant [$t(843) = 1.29$, $p = .26$].

Discussion

The serotonin transporter polymorphism, 5-HTTLPR, moderated the effects of maternal anxiety during pregnancy on infant negative emotionality at 6 months after birth, as hypothesized. The hypothesized association between higher levels of maternal anxiety during pregnancy and higher levels of infant negative emotionality proved strongest for individuals with two short alleles, weakest for individuals with two long alleles, and intermediate for heterozygotes. To investigate whether the significant GXE interaction was more consistent with a diathesis-stress or differential-susceptibility framework, we applied the criteria for the testing of differential susceptibility stipulated by Belsky *et al.* (17). The criteria that the susceptibility factor (i.e., 5-HTTLPR) be unrelated to predictor and outcome variables was met in that there were no significant associations between, respectively, 5-HTTLPR and maternal anxiety or between 5-HTTLPR and infant negative emotionality. At the same time, however, the graphical display of simple slopes linking prenatal anxiety and infant negative emotionality failed to document a clear crossover pattern, a further criterion for differential susceptibility; in other words, the interaction under consideration chronicled more a diathesis-stress than differential-susceptibility process of environmental action. This could be the result of reliance on a contextual predictor, prenatal maternal anxiety, whose positive pole merely reflected the absence of an adverse condition and not, say, the presence of a development facilitating positive one. A contextual variable capturing the range of prenatal environmental influences from negative to positive and not just the presence and absence of adversity as in the current analysis might have revealed a crossover interaction of the kind anticipated by the differential susceptibility framework.

According to the fetal programming hypothesis, the fetus adapts its phenotype to the anticipated postnatal environment on the basis of maternal cues regarding the quality of the outside world (1,2,36) to function optimally in that specific environment. Gluckman and Hanson (1) make reference to "predictive adaptive responses" that, if the actual environment ends up being different from the one anticipated, generates a mismatch between the programmed phenotype and environment and consequently proves dysfunctional rather than adaptive (36). This raises the question of whether prenatal-stress effects on sequelae like infant negative emotionality should be regarded as adaptive or maladaptive. Here we entertain the former possibility.

Infant difficult temperament is generally considered a risk factor for and precursor of a range of problematic outcomes (37,38). However, Belsky's (15,16) reconceptualization of difficult temperament as a marker for developmental plasticity stipulates that highly negatively emotional infants and children have an especially sensitive nervous system and thus are not simply more vulnerable to adversity but also more likely to benefit from enriching and supportive environmental influences. Bradley and Corwyn (39) and Pluess and Belsky (40,41) provide evidence to this effect with respect to the quality of both parenting and child care. Recently, numerous related findings have been reviewed by Belsky and Pluess (13).

Therefore, prenatal programming of negative emotionality as chronicled in the current study, especially in the case of infants carrying at least one short allele of the 5-HTTLPR, might itself represent an adaptive response: stressful environmental experiences during pregnancy contribute to the fetal programming of developmental plasticity—demarcated by negative emotionality/difficult temperament—as a means of enhancing the organism's adaptation to the postnatal environment. For example, negative emotionality might sensitize children to carefully observe a potentially threatening postnatal environment and/or help them to regain attention from caregivers that might be distracted by other concerns. It is thus proposed that the association between prenatal maternal anxiety and infant temperament reflects adaptive prenatal programming of postnatal plasticity (42). The current study provides new evidence for such fetal programming of infant temperament but further suggests that such fetal programming effects differ as a function of genotype with the effect of prenatal anxiety on negative emotionality—and therefore hypothetically developmental plasticity—being strongest in individuals carrying the 5-HTTLPR short allele. However, whether negative emotionality moderates postnatal environmental influences in the current sample, as the differential susceptibility hypothesis would predict, remains to be tested.

This research is not without limits. Consider first that the interaction effect detected was statistically significant but very small, which was mostly because maternal prenatal anxiety exerted no apparent effect on negative emotionality for infants homozygous for the long allele. In contrast, for infants carrying two copies of the short allele, maternal prenatal anxiety explained up to 3% of the variance in infant negative emotionality, thereby implying clinical significance. The second limitation of this inquiry is that infant negative emotionality was based exclusively on maternal report; additionally, ethnicity of the sample was restricted to those of Dutch ancestry. Whether the discerned association between maternal anxiety during pregnancy and infant negative emotionality, especially as moderated by 5-HTTLPR, can be replicated with behavioral measures of negativity and/or in samples comprising other ethnic groups remains to be determined. Attention needs also to be drawn to the fact that mothers with psychiatric disorders were not identified or excluded from the study, a limitation that would seem to be mitigated by the statistical controls instituted for postnatal depression and anxiety. Finally, it must be appreciated that the study design was correlational, thereby limiting the confidence that can be placed in any causal inferences drawn. Conceivably, for example, the association between maternal anxiety and infant temperament could be an artifact of shared genes and thus heritability, with mothers more easily distressed during pregnancy bearing children who inherit the same propensity to experience stress more readily than others (43,44).

In conclusion, the work presented herein provides first empiric evidence for the hypothesis that effects of fetal programming are moderated by the 5-HTTLPR. The association between maternal anxiety during pregnancy and negative emotionality in early infancy was significant in infants carrying one or more copies of the short allele but not in those homozygous for the long allele. Consequently, the 5-HTTLPR short allele might increase vulnerability to adverse environmental influences as early as the fetal period.

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