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Maternal childhood adversity and child temperament: An association moderated by child 5-HTTLPR genotype

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We examined transgenerational effects of maternal childhood adversity on child temperament and a functional promoter polymorphism, 5-HTTLPR, in the serotonin-transporter gene (*SLC6A4*) as potential moderators of such maternal influences in 154 mother–child dyads, recruited into a longitudinal birth cohort study. We examined the interactive effects of maternal childhood experience using an integrated measure derived from Childhood Trauma Questionnaire (CTQ) and Parental Bonding Index (PBI). Triallelic genotyping of 5-HTTLPR was performed. A measure of 'negative emotionality/behavioural dysregulation' was derived from the Early Childhood Behaviour Questionnaire at 18 and 36 months. Negative emotionality/behavioural dysregulation was highly stable between 18 and 36 months and predicted psychosocial problems at 60 months. After controlling multiple demographics as well as both previous and concurrent maternal depression there was a significant interaction effect of maternal childhood adversity and offspring 5-HTTLPR genotype on child negative emotionality/behavioural dysregulation ($\beta = 1.03$, $t_{11,115} = 2.71$, $P < .01$). The results suggest a transgenerational effect of maternal developmental history on emotional function in the offspring, describing a pathway that likely contributes to the familial transmission of vulnerability for psychopathology.

Keywords: Differential susceptibility, maternal adversity, negative emotionality, 5-HTTLPR genotype, transgenerational transmission

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Child abuse or neglect increases the risk and chronicity of depression and anxiety disorders (Heim & Nemeroff 2001; Kendler *et al.* 2000; Molnar *et al.* 2001; Stein *et al.* 1996; Widom *et al.* 2007) as well as treatment outcomes (Nanni *et al.* 2012). Likewise, persistent emotional neglect, family conflict, and conditions of harsh, inconsistent discipline increase the risk for depression and anxiety disorders (e.g. Hill *et al.* 2001; Shanahan *et al.* 2008). Thus, cold, distant parent–child relationships as well as more overt forms of trauma associate with an increased risk of affective disorders as well as childhood endophenotypes, such as behavioural inhibition (Hane & Fox 2006). These findings suggest that the influence of parental care on child development extends across a wide parent–child interactions and is not unique to more extreme forms of maltreatment (e.g. Hane & Fox 2006).

The risk stemming from a maternal history of early adverse experiences may be transmitted to the next generation (Collishaw *et al.* 2007). While studies of the transgenerational risk transmission of maternal history of early adversity are scarce, longitudinal analyses confirm the familial transmission of depression and related disorders (Weissman *et al.* 2006). The offspring of depressed mothers are at a significantly increased risk for depression than are those of parents with no history of depression. While heritable, sequence-based genomic variations are inevitably an influence on future mental health, the results of treatment studies suggest non-genomic effects (Weissman *et al.* 2006). Successful treatment of maternal depression reduces the risk for psychopathology in the offspring (Wickramaratne *et al.* 2011).

Children of depressed mothers show an increase in forms of temperament, such as negative emotionality, that predict a greater risk for depression in later life (Caspi *et al.* 2003; Weissman *et al.* 2006). A recent meta-analysis confirmed the relation between maternal depression and negative emotionality (Goodman *et al.* 2011), however, the effect size of the associations was small suggesting the importance of moderating variables. This conclusion is consistent with studies showing that the impact of early environmental influences

is moderated by the genotype of the child (e.g. Belsky & Pluess 2009a,b; Caspi *et al.* 2003). Indeed, it is increasingly apparent that vulnerability for depression emerges from the interaction of environmental influences, including genotype (Meaney 2010; Rutter *et al.* 2006).

Depression involves alterations in serotoninergic synaptic transmission and there is evidence that variation in genes encoding for proteins that regulate serotonin metabolism and transmission, such as the serotonin-transporter polymorphism (5-HTTLPR) that codes for the serotonin-transporter gene, moderate the effects of environmental factors on both the risk for depression as well as on childhood expression of endophenotypes that associate with depression (Fox *et al.* 2005; Pluess *et al.* 2011). The most extensively characterized 5-HTTLPR genotype is that of 43 bp insertion/deletion in the promoter region that produces long (L) and short (S) variants in the serotonin-transporter-linked promoter region. The L and S functional alleles alter 5-HTTLPR transcription such that the S variant results in significantly reduced *in vitro* basal transcription of 5-HTT mRNA (Hu *et al.* 2006). The S allele is associated with increased negative emotion, such as heightened anxiety, elevated neuroticism, harm avoidance and fear conditioning (see Homberg & Lesch 2011, for a review). The S allele of 5-HTTLPR also associates with greater vulnerability for depression in children, adolescents and young adults exposed to early-life stress, whereas in the same context the L allele appears to be protective (Caspi *et al.* 2003; Pluess *et al.* 2011; Taylor 2010). While these results have not been uniformly replicated, thorough analyses suggest that the failure to replicate is associated with differences in methodologies as opposed to the fidelity of the interaction effect on depression (Uher & McGuffin 2008). It is important to note that a variant of the L allele, the L_G allele (Hu *et al.* 2006, and see below) also confers vulnerability to depression, which might explain some of the discordant results.

These findings suggest that maternal childhood adversity might compromise the emotional development of the child, an effect that may be moderated by the child genotype. We examined the relation between maternal childhood adversity and negative emotionality/behavioural dysregulation (NE/BR) in the offspring using data from a longitudinal birth cohort. We first derived a factor of NE/BR from the Early Childhood Behaviour Questionnaire (Putnam *et al.* 2006). We hypothesized (1) a significant, positive relation between maternal adversity and NE/BR in the child, and (2) that the effect of maternal childhood adversity would be moderated by child 5-HTTLPR genotype.

Method

Participants

Our community sample consisted of 154 mothers recruited in Montreal (Quebec) and Hamilton (Ontario) at 13–20 weeks gestation from antenatal care clinics at the time of routine ultrasound or through advertisements at hospitals. Participants were part of the Maternal Adversity, Vulnerability and Neurodevelopment (MAVAN) study, a longitudinal descriptive study, which examines the development of individual differences in phenotypes associated with multiple forms of psychopathology. This group of mothers and children (Table 1) constitute a portion of the larger population of mothers that were part of MAVAN and whose children had reached the age of 36 months at the time of this analysis. Eligibility criteria included age 18 or over, singleton gestation, and fluency in French or English and excluded women with severe chronic illness (other than hypertension, asthma or diabetes) and other serious medical conditions (e.g. placenta previa). Only babies born at a gestational age of 37 weeks or later, above 2000 g and with APGAR scores >7 were included in the cohort. Mothers were first assessed during their pregnancy (~26 weeks) and

Table 1: Means (standard deviation) of demographic variables, predictor variables and outcome variables in offspring by 5-HTTLPR genotype (36 months postpartum)

	5-HTTLPR	
	L_A/L_A	S or L_G carriers
Sample size	46	108
Maternal age*	31.7 (5.0)	33.0 (5.3)
Family income†	12.4 (4.1) ($40–50 000)	14.0 (3.2) ($50–60 000)
Dual parenting‡	38	105
Gender§	27	48
Birth weight (grams)	3339.4 (555.1)	3354.9 (551.0)
Maternal early adversity (log)	−0.2 (.6)	−0.3 (.6)
Negative emotionality/behavioural dysregulation	0.2(.8)	0.7 (1.2)
SDQ (mother) total difficulties	9.38 (4.63)	9.90 (4.58)
SDQ (mother) emotional symptoms	1.75 (1.83)	2.43 (1.93)
SDQ (mother) conduct problems	2.25 (1.75)	1.93 (1.44)
SDQ (mother) hyperactivity	4.38 (2.00)	3.81 (2.24)
SDQ (mother) peer problems	1.00 (1.41)	1.74 (1.74)
SDQ (mother) prosocial	9.25 (1.17)	8.10 (1.91)
SDQ (father) total difficulties	8.75 (6.11)	8.91 (4.28)
SDQ (father) emotional symptoms	2.38 (2.33)	2.45 (1.94)
SDQ (father) conduct problems	1.75 (1.49)	1.97 (1.53)
SDQ (father) hyperactivity	3.38 (2.33)	3.15 (1.96)
SDQ (father) peer problems	1.25 (1.04)	1.33 (.92)
SDQ (father) prosocial	8.25 (1.83)	8.15 (1.42)

Differences between both genotype groups were not significant for all listed variables (all *P*'s > .05).

*Postpartum (36 months).

†Combined family income at 36 months, where 0 = no revenue, 1 = less than $5000, 2 = at least $5000, 3 = less than $10 000, 4 = at least $10 000, 5 = less than $15 000, 6 = at least $15 000, 7 = less than $20 000, 8 = at least $20 000, 9 = less than $30 000, 10 = at least $30 000, 11 = less than $40 000, 12 = at least $40 000, 13 = between $40 000 and $50 000, 14 = between $50 000 and $60 000, 15 = between $60 000 and $80 000, 16 = between $80 000 and $100 000, and 17 = at least $100 000.

‡Information was available for only 147 of the 154 participants.

§Number of females.

then followed at multiple time points that included both home visits and laboratory sessions. Written, informed consent was obtained from all participants. Ethics approval was obtained from the Douglas Mental Health University Institute (McGill University, Montreal) and St-Joseph Healthcare/McMaster University, Hamilton.

In terms of ethnicity, 88.7% of the sample was European/Caucasian, 8.1% was African descent/African American, and 3.2% was Hispanic/Latino.

Measures

Maternal adversity

Maternal history of early adversity was assessed with a combination of the Childhood Trauma Questionnaire (CTQ; Bernstein *et al.* 1994) and the Parental Bonding Instrument (PBI; Parker *et al.* 1979). Whereas the CTQ assesses more severe instances of adversity, the PBI captures the subjects' perception of variation in parental experience across the normal range. The CTQ was administered to mothers during a home visit both prenatally and when children were aged 24 months. All five subscales (emotional neglect, emotional abuse, physical neglect, physical abuse and sexual abuse) were used in our analyses. The PBI, which is highly stable over time (Wilhelm *et al.* 2005), was administered during a home visit when the infants were aged 6 months. Only the maternal care scale of the PBI was entered into the analytical models since it was the scale that related to the construct under study and focused on maternal transmission.

We used a previously validated principal component analysis to derive one factor and reduce our measures of maternal childhood adversity (CTQ and PBI) (Mileva-Seitz *et al.* 2011). This factor explained 52% of the total variance (eigenvalue = 3.134; see Table 2a).

Maternal mood

The depressive state of the mothers was assessed at 6 and 36 months postpartum with the Center for Epidemiologic Studies Depression Scale (CES-D), a self-report, 20-item measure (Radloff 1997). CES-D scores were centered and standardized.

Infant genotype

Buccal swabs were collected at 36 months. DNA extraction and 5-HTTLPR genotyping was performed at the Center for Addiction and Mental Health, in Toronto (Canada). For the 5-HTTLPR, 4 μl total genomic DNA was combined with 1× MBI Fermentas polymerase chain reaction (PCR) buffer containing $(NH_4)2SO_4$, 1.5 mM $MgCl_2$ (MBI Fermentas, Burlington, Canada), 0.0325 μg each primer (Cook *et al.* 1997; forward primer labeled with 5′ HEX fluorescent tag), 0.16 mM each dNTP (MBI Fermentas) and 1 U Taq polymerase (MBI Fermentas) to a total volume of 25 μl. The PCR reactions were subjected to an initial denaturation for 3 min at 95°C, followed by 40 cycles of amplification in an AB 2720 (Thermofisher Scientific, Burlington, Canada) thermal cycler: denaturing for 30 seconds at 95°C, annealing for 30 seconds at 61°C and extension for 1 min at 71°C, and a final extension at 72°C for 10 min. Five microlitres of the PCR product was combined with 1× New England Biolabs Buffer 2, 10 U MspI restriction enzyme (New England Biolabs, Whitby, Canada) in a total volume of 30 μl was digested overnight at 37°C. Digested products were electrophoresed on an AB 3130-Avant Genetic Analyzer as per manufacturer's directions, and product sizes determined by comparison to GeneScan 500 ROX size standard using GeneMapper (version 4.0; Thermofisher Scientific). Ten percent of samples were genotyped in duplicate. Error rate was below 1%. Any discrepant genotypes were repeated with a new aliquot of stock DNA. Whichever genotype result the third result matched was retained as correct. If a new aliquot was not available, the genotype was removed.

When children were aged 36 months, buccal swabs were also collected for mothers. Maternal genotype was used in our analyses as a covariate.

There is evidence for two functional variants of the L allele (L_A and L_G) result from a single nucleotide polymorphism (A → G, rs25531) in the 5-HTTLPR region (Hu *et al.* 2006; Uher & Mcguffin 2008). The L_A/L_A genotype is associated with a greater 5-HTT binding potential in human putamen (Praschak-Rieder *et al.* 2005) and midbrain (Reimold *et al.* 2007) as well as with higher mRNA expression *in vitro* (Hu *et al.* 2006). We grouped the L_G and S alleles since these variants are functionally similar with respect to 5-HTT expression (*Hu et al*. 2006). We compared L_A/L_A homozygote infants to S/L_G allele carriers.

Negative emotionality/behavioural dysregulation

Infant NE/BR was measured using the Early Childhood Behaviour Questionnaire (ECBQ; Putnam *et al.* 2006) at 18 and 36 months. The ECBQ is a maternal-report questionnaire comprised of 201 items grouped in 18 subscales (see Table 2b) and is based on a 7-point Likert scale. A principal component analysis was performed to derive a NE/BR factor at both 18 and 36 months (see Results).

Behavioural problems

The Strengths and Difficulties Questionnaire (SDQ; Goodman 1997) was administered at 60 months to validate ECBQ-derived measures. The SDQ is a parental report comprised of 25 items divided into 5 scales: emotional symptoms, conduct problems, hyperactivity/inattention, peer relationship problems and prosocial behaviour. A total difficulties score is also obtained by summing all scores from all subscales except for the prosocial subscale. Respondents are asked to rate each item on a 3-point scale. The validity of the SDQ as a screening measure for child psychopathology is well established (e.g. Goodman 1997). The SDQ was administered to both mothers and fathers during a home visit, when children were aged 60 months.

Results

Negative emotionality/behavioural dysregulation

We first attempted to replicate a negative affectivity factor using the ECBQ (Putnam *et al.* 2006) performing a principal axis factor analysis with an oblimin rotation. We were required to force a three-factor solution to obtain the same factor loadings as Putnam *et al.* (2006). We decided to derive our own construct without a predetermined number of factors. We entered the 18 ECBQ subscales into a principal component analysis. After excluding all items that loaded with a coefficient absolute value below .40, we obtained one factor termed 'negative emotionality/behavioural dysregulation' that included discomfort, fear, frustration and sadness, which are core negative emotionality/behavioural dysregulation components. The NE/BR factor explained 21% of the total variance in the ten remaining input variables (eigenvalue = 3.8). This factor was comprised of positive ratings of discomfort, fear, frustration, activity level, motor activation, and sadness, and of negative ratings of attentional focusing, cuddliness, inhibitory control and soothability. The scores were normalized and centered for all further analyses. Rotating the matrix solution did not affect the solution. Since this factor included items that reflect cognitive and motor aspects not included in the Putnam *et al.* (2006) negative affectivity factor, notably inhibitory control, attentional focusing, activity level, and because such measures are not typically related to negative emotionality per se, we labeled our factor as 'negative emotionality/behavioural dysregulation'. Our factor nevertheless correlated strongly with that of *Putnam et al*. (2006) ($r = .73$, $P < .01$) (see Table 2b for factor loadings).

We derived a similar factor with the same variables obtained when infants were aged 18 months to assess the stability of our NE/BR measure. NE/BR scores at 18 and 36 months were strongly associated ($r = .65$, $P < .01$;

Table 2: Factor loadings for (a) ECBQ negative emotionality/behavioural dysregulation related scales at 18 and 36 months and (b) maternal history of early adversity factor

(a) Scales	Loadings
Activity level	.58
Attentional focusing	−.41
Cuddliness	−.42
Discomfort	.57
Fear	.42
Frustration	.74
Inhibitory control	−.68
Motor activation	.55
Sadness	.58
Soothability	−.63

(b) Instruments; scales	Loadings
CTQ; Physical neglect	.76
CTQ; Physical abuse	.75
CTQ; Emotional neglect	.73
CTQ; Emotional abuse	.85
CTQ; Sexual abuse	.72
PBI; Maternal care	−.48

Cronbach's $\alpha = .79$). This finding is consistent with a previous study demonstrating the longitudinal stability of the ECBQ (Putnam *et al.* 2006).

We then examined the predictive validity of our NE/BR scores by examining the relation with SDQ ratings obtained at 60 months of age for the sub-sample for which such scores were available ($n = 70$ mothers; $n = 55$ fathers). The NE/BR scores at 36 months were positively associated with maternal reports of total difficulties ($r = .47$, $P < .01$), emotional symptoms ($r = .30$, $P < .01$), conduct problems ($r = .21$, $P < .05$), hyperactivity ($r = .32$, $P < .01$) and peer problems ($r = .46$, $P < .01$), and negatively associated with prosocial scores ($r = -.30$, $P < .01$). The NE/BR scores at 36 months were also positively associated with paternal reports of total difficulties ($r = .26$, $P < .05$), conduct problems ($r = .26$, $P < .05$), hyperactivity ($r = .25$, $P < .05$) and negatively associated with prosocial scores ($r = -.42$, $P < .01$). However, paternal ratings were not associated with emotional symptoms and peer problems (P's $> .05$). These findings are consistent with those linking negative emotionality to phenotypes associated with an increased risk for depression (Anthony *et al.* 2002; Caspi *et al.* 1996). Finally, we found that children did not differ in NE/BR as a function of their 5-HTTLPR genotype ($t_{184} = .62$, *ns*) nor that of their mother ($t_{206} = .32$, *ns*).

5-HTTLPR genotype frequencies and demographics

Genotype was coded for the presence of the S allele: 0 = no copies of S or L_G; 1 = one or two copies of S or L_G. The frequency of mothers and children with the L_A/L_A genotype (30%; Table 3) is consistent with the literature with Caucasian populations (Hu *et al.* 2006). Tests of Hardy Weinberg Equilibrium (HWE) were performed for each locus. Three of four comparisons did not deviate from expected values. For L_A and L_G alleles, offspring values were not in HWE ($\chi^2 = 6.2$; $n = 54$; $P < .05$) whereas mothers' values were ($\chi^2 = .31$; $n = 59$; *ns*). The fact that offspring values were not in HWE might be explained by a very small sample size in the L_G category. For L and S alleles, both offspring and mothers values were in HWE ($\chi^2 = .07$; $n = 154$; *ns and* $\chi^2 = 1.83$; $n = 154$; *ns*, respectively). Comparisons using *t*-tests, assuming equal variances, showed that the 5-HTTLPR genotype of the child was unrelated to maternal age, combined family income at intake into the study, gender of the child, infant birth weight, maternal early adversity or child NE/BR scores.

Maternal childhood adversity and postpartum depression

We log-transformed values to normalize the maternal adversity measure, which tended to show a negative skew. Mothers did not differ in their history of childhood adversity as a function of their 5-HTTLPR genotype ($t_{142} = .72$, *ns*) or that of their offspring ($t_{152} = 1.37$, *ns*). Maternal childhood adversity was positively related to maternal depression at 6 months postpartum ($r = .38$, $P < .01$) and 36 ($r = .33$, $P < .01$) and, as expected, the depression scores at the two time points were inter-correlated (Table 4). Hence, we controlled for maternal depression at the 6- and 36-month time points in our analyses by using CES-D scores as covariates to assess the effects of maternal depression on ratings of NE/BR. The 36-month time point corresponds to a time of maternal report and permits an analysis of the influence of the potential of maternal mood, while the 6-month time point predates the time of the

Table 3: Offspring 5-HTTLPR genotype frequencies (a) and maternal 5-HTTLPR genotype frequencies (b)

Genotype	L_A/L_A	L_A/L_G	L_G/L_G	S/L_A	S/L_G	S/S	Total
(a)							
n	46	6	2	63	10	27	154
Frequencies	.30	.04	.01	.40	.07	.18	1.00
Frequencies for S, L_A and L_G alleles were as follows: .41, .52 and .07.							
(b)							
n	48	10	1	60	6	29	154
Frequencies	.31	.07	.01	.38	.04	.19	1.00
Frequencies for S, L_A and L_G alleles were as follows : .40, .54 and .06.							

Table 4: Bivariate correlations between all study variables

	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.
1. Maternal age	.32**	.11*	−.15**	−.15*	−.03	−.24**	−.21	−.15	−.12	−.24*−	−.01	−.18	−.28*	−.24	−.12	−.19	−.20	−.10
2. Family income	—	.00	−.38**	−.35**	−.27**	−.27**	−.26*	−.13	−.20	−.19	−.20	−.02	−.16	.00	−.21	−.15	−.05	.00
3. Birth weight		—	.03	.13*	.02	.06	−.12	−.19	−.16	−.05	.08	.21	.08	.11	.10	−.02	.06	.01
4. Maternal depression (6 m)			—	.57**	.40**	.36**	.50**	.20	.49**	.43**	.19	−.01	.11	−.07	.15	.14	.12	.05
5. Maternal depression (36 m)				—	.35**	.42**	.37**	.28*	.29*	.22	.24*	−.21	.11	.01	.23	.00	.11	−.13
6. Maternal adversity					—	.20**	.20	.20	.09	.16	.07	−.04	.01	−.17	.10	.02	.15	.12
7. Child NE/BR						—	.47**	.30*	.21	.32**	.46**	−.30*	.26	.04	.26	.25	.17	−.42**
8. SDQ (M) total difficulties							—	.68**	.69**	.74**	.64**	−.38**	.52**	.34**	.40**	.36**	.32*	−.50*
9. SDQ (M) emotional difficulties								—	.32**	.14	.37**	−.06	.32*	.50**	.17	.01	.20	−.09
10. SDQ (M) conduct problems									—	.48**	.17	−.32**	.51**	.30*	.51**	.31*	.26*	−.29*
11. SDQ (M) hyperactivity										—	.30**	−.37**	.34**	−.09	.26*	.53**	.15	−.49**
12. SDQ (M) peer problems											—	−.33**	.19	.19	.13	.02	.22	−.41**
13. SDQ (M) prosocial												—	−.13	.09	−.30*	−.11	−.06	.50**
14. SDQ (F)total difficulties													—	.69**	.72**	.70**	.55**	−.33**
15. SDQ (F) emotional difficulties														—	.38**	.15	.33**	−.12
16. SDQ (F) conduct problems															—	.34**	.24	−.20
17. SDQ (F) hyperactivity																—	.19	−.29*
18. SDQ (F)peer problems																	—	−.33**
19. SDQ (F) prosocial																		—

*$P < .05$; **$P < .01$

first maternal report (i.e. 18 months) and informs on maternal mood in the early postpartum period. Including these depression measures as covariates prior to the main adversity, genotype and G × E values, allowed us to determine whether the main predictors remained significant after accounting for previous and current maternal depression.

Maternal childhood adversity and offspring 5-HTTLPR genotype

Multiple linear hierarchical regression analyses were performed to assess the influence of maternal childhood adversity and offspring 5-HTTLPR genotype on infant NE/BR at 36-months of age. We controlled for sample of origin recruitment differences as well as the effects of selected demographics, maternal depression and maternal 5-HTTLPR genotype (see Table 4). We inserted variables in the following order: Step 1, *origin of subject*, *gender*, *birth weight corrected for gestational age*, *maternal* 5-HTTLPR *genotype, maternal age and family income;* Step 2, *maternal depression at 6, and 36 months postpartum*; Step 3, *maternal adversity*, *offspring 5-HTTLPR genotype*; Step 4, the multiplicative interaction term of *maternal adversity* and *offspring 5-HTTLPR genotype.* Both the first full model and the reduced model are included in Table 4 and show the same basic outcome. None of the demographic variables with the exception of birth weight (corrected for gestational age) contributed to the variation in NE/BR ($\beta = .01$, $t_{11,115} = 1.83$, $P < .10$; Table 5). Maternal depression at 6 months did not associate with NE/BR. In contrast depression at 36 months was associated with NE/BR ($\beta = .36$, $t_{11,115} = 3.53$, $P < .01$). Nevertheless, accounting for the effects of demographics as well as both early (6 month) and concurrent (36 month) maternal depression, there remained a significant main effect of maternal childhood adversity on child NE/BR ($\beta = -.62$, $t_{11,115} = -2.04$, $P < .05$) such that higher maternal adversity scores were significantly associated with higher NE/BR ratings. Furthermore, there was a significant interaction effect of maternal childhood adversity and offspring 5-HTTLPR genotype on child NE/BR ($\beta = 1.03$, $t_{11,115} = 2.71$, $P < .01$) such that NE/BR scores increased as a function of maternal adversity in S/L_G allele carriers (Table 5).

This interaction was explored both statistically and graphically (Fig. 1). We followed procedures for post-hoc probing of moderation effects described by Holmbeck (2002). First, the effects of adversity on child NE/BR were tested as a function of child 5-HTTLPR genotype. The results revealed a significant and positive slope for S/L_G allele carriers ($\beta = .26$, SE = .09, $P < .01$). The slope for L_A/L_A homozygotes was not significant ($\beta = -.25$, SE = .14, *ns*).

We then explored the effects of child genotype on NE/BR at different levels of maternal adversity, which is the inverse of the previous post hoc probe. At one standard deviation above the mean of maternal adversity, there were no statistically significant differences between the NE/BR scores between subgroups ($\beta = .32$, SE = .09, *ns*). However, at 1.5 standard deviations above the mean, NE/BR scores were significantly higher in S/L_G allele carriers than the L_A/L_A homozygotes group ($\beta = .71$, SE = .32, $P < .05$). Interestingly, at one standard deviation below the mean in maternal adversity, S/L_G allele carriers showed a significantly lower NE/BR scores ($\beta = -.55$, SE = .24, $P < .05$). These findings reflect a cross-over interaction where children with the less functional 5-HTTLPR (S/L_G allele carriers) alleles are significantly higher in NE/BR scores than L_A/L_A homozygotes at high levels of maternal adversity, but significantly lower in NE/BR scores than at low levels of maternal adversity. Indeed L_A/L_A homozygote children do not vary significantly in NE/BR as a function of maternal adversity. These interactive effects of maternal childhood adversity and child genotype were retained in the reduced regression analysis after removal of all non-predictive variables.

Table 5: Beta regression coefficients (*t*-statistics in brackets) for analyses predicting offspring negative emotionality/behavioural dysregulation at 36 months

	5-HTTLPR	
	Full model	Reduced model
Site of origin	−.34 (−1.65)	–
Gender	−.21 (−1.12)	–
Birth weight (percentile)	.01 (1.83)∼	.00 (.76)
Maternal 5-HTTLPR genotype	.07 (.29)	—
Maternal age*	−.01 (−.53)	—
Family income†	−.03 (−.97)	—
Maternal depression at 6 months	.00 (.02)	—
Maternal depression at 36 months	.36 (3.53)**	.40 (5.18)**
Maternal adversity (E)	−.62 (−2.04)*	−.34 (−1.36)
Child 5-HTTLPR genotype (G)	.21 (.88)	.06 (.33)
G × E	1.03 (2.71)**	.62 (2.04)*
R^2 (adj.)	0.30	0.19
df	(11, 115)	(5, 149)

The 'full model' contains several covariates whereas reduced model contains only variables that contributed significantly to the model. Since an overall multiple imputation for missing data was not undertaken, differences in sample sizes reflect missing data on some of the demographic variables.

*Postpartum (36 months).

†Combined family income at 36 months, where 0 = no revenue, 1 = less than $5000, 2 = at least $5000, 3 = less than $10 000, 4 = at least $10 000, 5 = less than $15 000, 6 = at least $15 000, 7 = less than $20 000, 8 = at least $20 000, 9 = less than $30 000, 10 = at least $30 000, 11 = less than $40 000, 12 = at least $40 000, 13 = between $40 000 and $50 000, 14 = between $50 000 and $60 000, 15 = between $60 000 and $80 000, 16 = between $80 000 and $100 000, and 17 = at least $100 000.

*$P < .05$, **$P < .01$; ∼$P < .10$.

Discussion

We examined the transgenerational influence of maternal childhood adversity on offspring NE/BR focusing on the

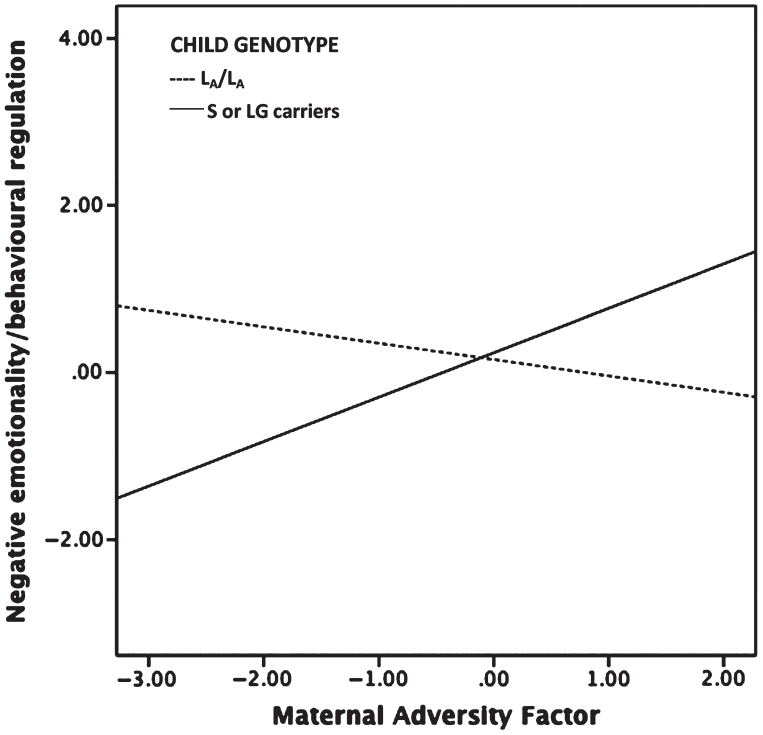

Figure 1: Interaction effect of maternal history of early adversity and offspring 5-HTTLPR genotype on offspring negative emotionality/behavioural regulation at 36 months, controlling for child birth weight and maternal depression at 36 months postpartum.

potential moderation of such effects by offspring 5-HTTLPR genotype, independent of maternal depression. We found that maternal childhood adversity associated with increased NE/BR in 36 months-old children, suggesting a transgenerational effect. As hypothesized, this transgenerational effect was moderated by the offspring 5-HTTLPR genotype and independent of previous and concurrent maternal depression.

The findings are consistent with previous studies showing that 5-HTTLPR moderates the influence of prenatal maternal anxiety (Pluess *et al.* 2011), social support (Fox *et al.* 2005), and attachment security (Kochanska *et al.* 2009) on the childhood expression of phenotypes linked to depression, including negative emotionality. The influence of the 5-HTTLPR polymorphism in this study conforms to the criteria established for a 'susceptibility factor' (Belsky & Pluess 2009a), since 5-HTTLPR status was unrelated to either the predictors (maternal adversity or maternal depression) or the outcome (negative emotionality/behavioural dysregulation). More compelling evidence for differential susceptibility is the finding of the cross-over interaction effect such that S/L_G allele carrier whose mothers presented with a history of adversity showed significantly increased NE/BR compared to L_A/L_A homozygotes. In contrast, among the offspring of mothers with a more favourable developmental history, children carrying the same 5-HTTLPR genotype showed significantly reduced NE/BR compared to L_A/L_A homozygotes. These findings suggest that 5-HTTLPR genotype is a differential susceptibility factor for early emotional development (Belsky & Pluess 2009a; Chen *et al.*, 2015).

Our analyses produced a derived measure of NE/BR in children using the ECBQ that was stable between 18 and 36 months. The inclusion of subscales associated with behavioural dysregulation is consistent with a definition formulated by Rothbart and Posner (2006) who viewed temperament as '… individual differences in reactivity and self-regulation, as observed in the domains of emotionality, motor activity and attention.' This assessment of child NE/BR is statistically more homogenous and thus potentially more focused than the ECBQ, which yields 18 different scale scores. The predictive validity of this measure is reflected in the strong correlations across multiple subscales of the SDQ (Goodman 1997) administered at 60 months of age and evident in both maternal and paternal ratings. Temperament at this age predicts internalizing and externalizing problems, vulnerability for depression (Bruder-Costello *et al.* 2007; Caspi *et al.* 1996; Degnan *et al.* 2010) and anxiety (Degnan *et al.* 2010). We found that the influence of maternal adversity was moderated by 5-HTTLPR genotype. It is important to note that the interaction between maternal adversity and 5-HTTLPR genotype in the offspring may be specific to certain developmental outcomes, and should not suggest that L_A/L_A homozygote children are necessarily immune from the influence of maternal adversity across all developmental domains although differential susceptibility to specific environmental conditions may be both tissue- and function-specific (Chen *et al.*, 2015).

Our results suggest that the mental health of the offspring may reflect maternal childhood adversity. Although maternal depression was associated with child temperament, both the main effect of maternal childhood adversity and the significant interaction between maternal adversity and child genotype on NE/BR were significant even when controlling for maternal depression. The distinguishable effects

of maternal adversity and maternal depression on child temperament suggest independent pathways that influence emotional development (also see Hill *et al.* 2001). Parenting is a candidate mediator of the relation between maternal developmental adversity and offspring temperament. Individual differences in parenting are transmitted from mother to daughter across a wide range of species (Belsky *et al.* 2005; Francis *et al.* 1999; Gonzalez *et al.* 2001; Maestripieri & Mateo 2009; Miller *et al.* 1997). Mothers sexually abused in childhood are more likely to exhibit child neglect, diminished confidence in their parenting skills, heightened negative self-appraisal as a parent, greater use of physical punishment, and lack of emotional control in parenting situations (Roberts *et al.* 2004). Childhood maltreatment associates with impaired attention and emotional regulation, and with less sensitive parenting (Belsky & Pluess 2009b; Gonzalez *et al.* 2012). Moreover, infant negative emotionality also affects the quality of the interactions that adults direct towards infants (Tronick & Reck 2009). These findings suggest a cascade of influences that reinforce NE/BR along a vulnerability pathway.

One limitation to this study is the use of retrospective reports of maternal childhood adversity and maternal reports of NE/BR. However, both the CTQ and the PBI are well-established measures with good psychometric properties (Bernstein *et al.* 1994; Wilhelm *et al.* 2005). The inclusion of maternal depression at multiple time points, including a time that corresponds to the completion of the ECBQ (i.e. 36 months), also accounts for the potential confound of maternal affect as a reporting bias. Moreover, the fact that NE/BR was predictive of both maternal and parental ratings of psychosocial function at 60 months argue against shared method variance as does the fact that NE/BR ratings remained consistent and stable over a 3.5-year period. Finally, we showed that the effects reported here were independent of maternal 5-HTTLPR genotype. Thus, these findings appear to represent a transgenerational effect of maternal childhood adversity on cognitive – emotional function in the offspring. The sample size of this study is consistent with multiple recent reports of G × E interactions. Nevertheless, the findings should be considered as preliminary pending replication and extension.

References

Anthony, J.L., Lonigan, C.J., Hooe, E.S. & Phillips, B.M. (2002) An affect-based, hierarchical model of temperament and its relations with internalizing symptomatology. *J Clin Child Adolesc* **31**, 480–490.

Belsky, J. & Pluess, M. (2009a) Beyond diathesis stress: differential susceptibility to environmental influences. *Psychol Bull* **135**, 885–908.

Belsky, J. & Pluess, M. (2009b) The nature (and nurture?) of plasticity in early human development. *Perspect Psychol Sci* **4**, 345–351.

Belsky, J., Jaffee, S.R., Sligo, J., Woodward, L. & Silva, P.A. (2005) Intergenerational transmission of warm-sensitive-stimulating parenting: a prospective study of mothers and fathers of 3-year-olds. *Child Dev* **76**, 384–396.

Bernstein, D.P., Fink, L., Handelsman, L., Foote, J., Lovejoy, M., Wenzel, K., Sapareto, E. & Ruggiero, J. (1994) Initial reliability and validity of a new retrospective measure of child abuse and neglect. *Am J Psychiatry* **151**, 1132–1136.

Bruder-Costello, B., Warner, V., Talati, A., Nomura, Y., Bruder, G. & Weissman, M. (2007) Temperament among offspring at high and low risk for depression. *Psychiatry Res* **153**, 145–151.

Caspi, A., Moffitt, T.E., Newman, D.L. & Silva, P.A. (1996) Behavioral observations at age 3 years predict adult psychiatric disorders – longitudinal evidence from a birth cohort. *Arch Gen Psychiatry* **53**, 1033–1039.

Caspi, A., Sugden, K., Moffitt, T.E., Taylor, A., Craig, I.W., Harrington, H., McClay, J., Mill, J., Martin, J.L., Braithwaite, A. & Poulton, R. (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* **301**, 386–389.

Chen, L., Pan, H., Tuan, T.A., Teh, A.L., MacIsaac, J.L., Mah, S.M., McEwen, L.M., Li, Y., Chen, H., Broekman, B.F.P., Buschdorf, J.P., Chong, Y.S., Kwek, K., Saw, S.M., Gluckman, P.D., Fortier, M.V., Rifkin-Graboi, A., Kobor, M.S., Qiu, A., Meaney, M.J. & Holbrook, J.D. (2015) Brain-derived neurotrophic factor (BDNF) Val66Met polymorphism influences the association of the methylome with maternal anxiety and neonatal brain volumes. *Dev Psychopathol* **27**, 137–150.

Collishaw, S., Dunn, J., O'Connor, T.G. & Golding, J. (2007) Maternal childhood abuse and offspring adjustment over time. *Dev Psychopathol* **19**, 367–383.

Cook, E.H., Courchesne, R., Lord, C., Cox, N.J., Shuya, Y., Lincoln, A., Haas, R., Courchesne, E. & Leventhal, B.L. (1997) Evidence of linkage between the serotonin transporter and autistic disorder. *Mol Psychiatry* **2**, 247–250.

Degnan, K.A., Almas, A.N. & Fox, N.A. (2010) Temperament and the environment in the etiology of childhood anxiety. *J Child Psychol Psychiatry* **51**, 497–517.

Fox, N.A., Nichols, K.E., Henderson, H.A., Rubin, K., Schmidt, L., Hamer, D., Ernst, M. & Pine, D.S. (2005) Evidence for a gene-environment interaction in predicting behavioral inhibition in middle childhood. *Psychol Sci* **16**, 921–926.

Francis, D., Diorio, J., Liu, D. & Meaney, M.J. (1999) Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science* **286**, 1155–1158.

Gonzalez, A., Lovic, V., Ward, G.R., Wainwright, P.E. & Fleming, A.S. (2001) Intergenerational effects of complete maternal deprivation and replacement stimulation on maternal behavior and emotionality in female rats. *Dev Psychobiol* **38**, 11–32.

Gonzalez, A., Jenkins, J.M., Steiner, M. & Fleming, A.S. (2012) Maternal early life experiences and parenting: the mediating role of cortisol and executive function. *J Am Acad Child Psychiatry* **51**, 673–682.

Goodman, R. (1997) The Strengths and Difficulties Questionnaire: a research note. *J Child Psychol Psychiatry* **38**, 581–586.

Goodman, S.H., Rouse, M.H., Connell, A.M., Broth, M.R., Hall, C.M. & Heyward, D. (2011) Maternal depression and child psychopathology: a meta-analytic review. *Clin Child Fam Psychol* **14**, 1–27.

Hane, A.A. & Fox, N.A. (2006) Ordinary variations in maternal caregiving influence human infants' stress reactivity. *Psychol Sci* **17**, 550–556.

Heim, C. & Nemeroff, C.B. (2001) The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry* **49**, 1023–1039.

Hill, J., Pickles, A., Burnside, E., Byatt, M., Rollinson, L., Davis, R. & Harvey, K. (2001) Child sexual abuse, poor parental care and adult depression: evidence for different mechanisms. *Brit J Psychiatry* **179**, 104–109.

Holmbeck, G.N. (2002) Post-hoc probing of significant moderational and mediational effects in studies of pediatric populations. *J Pediatr Psychol* **27**, 87–96.

Homberg, J.R. & Lesch, K.-P. (2011) Looking on the bright side of serotonin transporter gene variation. *Biol Psychiatry* **69**, 513–519.

Hu, X.Z., Lipsky, R.H., Zhu, G., Akhtar, L.A., Taubman, J., Greenberg, B.D., Xu, K., Arnold, P.D., Richter, M.A., Kennedy, J.L., Murphy, D.L. & Goldman, D. (2006) Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. *Am J Hum Genet* **78**, 815–826.

Kendler, K.S., Bulik, C.M., Silberg, J., Hettema, J.M., Myers, J. & Prescott, C.A. (2000) Childhood sexual abuse and adult psychiatric and substance use disorders in women: an epidemiological and cotwin control analysis. *Arch Gen Psychiatry* **57**, 953–959.

Kochanska, G., Philibert, R.A. & Barry, R.A. (2009) Interplay of genes and early mother-child relationship in the development of self-regulation from toddler to preschool age. *J Child Psychol Psychiatry* **50**, 1331–1338.

Maestripieri, D. & Mateo, J.M. (2009) *Maternal Effects in Mammals*. U Chicago Press.

Meaney, M.J. (2010) Epigenetics and the biological definition of gene x environment interactions. *Child Dev* **81**, 41–79.

Mileva-Seitz, V., Kennedy, J., Atkinson, L., Steiner, M., Levitan, R., Matthews, S.G., Meaney, M.J., Sokolowski, M.B. & Fleming, A.S. (2011) Serotonin transporter allelic variation in mothers predicts maternal sensitivity, behavior and attitudes toward 6-month-old infants. *Genes Brain Behav* **10**, 325–333.

Miller, L., Kramer, R., Warner, V., Wickramaratne, P. & Weissman, M. (1997) Intergenerational transmission of parental bonding among women. *J Am Acad Child Psychiatry* **36**, 1134–1139.

Molnar, B.E., Buka, S.L. & Kessler, R.C. (2001) Child sexual abuse and subsequent psychopathology: results from the National Comorbidity Survey. *Am J Public Health* **91**, 753–760.

Nanni, V., Uher, R. & Danese, A. (2012) Childhood maltreatment predicts unfavorable course of illness and treatment outcome in depression: a meta-analysis. *Am J Psychiatry* **169**, 141–151.

Parker, G., Tupling, H. & Brown, L.B. (1979) A parental bonding instrument. *Brit J Med Psychol* **52**, 1–10.

Pluess, M., Velders, F.P., Belsky, J., van IJzendoorn, M.H., Bakermans-Kranenburg, M.J., Jaddoe, V.W., Hofman, A., Arp, P.P., Verhulst, F.C. & Tiemeier, H. (2011) Serotonin transporter polymorphism moderates effects of prenatal maternal anxiety on infant negative emotionality. *Biol Psychiatry* **69**, 520–525.

Praschak-Rieder, N., Wilson, A.A., Hussey, D., Carella, A., Wei, C., Ginovart, N., Schwarz, M.J., Zach, J., Houle, S. & Meyer, J.H. (2005) Effects of tryptophan depletion on the serotonin transporter in healthy humans. *Biol Psychiatry* **58**, 825–830.

Putnam, S.P., Gartstein, M.A. & Rothbart, M.K. (2006) Measurement of fine-grained aspects of toddler temperament: the early childhood behavior questionnaire. *Infant Behav Dev* **29**, 386–401.

Radloff, L.S. (1997) The CES-D Scale: a self-report depression scale for research in the general population. *Appl Psychol Meas* **1**, 385–401.

Reimold, M., Smolka, M.N., Schumann, G., Zimmer, A., Wrase, J., Mann, K., Hu, X.Z., Goldman, D., Reischl, G., Solbach, C., Machulla, H.J., Bares, R. & Heinz, A. (2007) Midbrain serotonin transporter binding potential measured with [11C]DASB is affected by serotonin transporter genotype. *J Neural Transm* **114**, 635–639.

Roberts, R., O'Connor, T., Dunn, J., Golding, J. & the ALSPAC Study team (2004) The effects of child sexual abuse in later family life; mental health, parenting and adjustment of offspring. *Child Abuse Neglect* **28**, 525–545.

Rothbart, M.K. & Posner, M.I. (2006) Temperament, attention, and developmental psychopathology. In Cicchetti, D. & Cohen, D.J. (eds), *Developmental Psychopathology*. Developmental Neuroscience, 2nd edn. Vol. **2**. John Wiley & Sons, Hoboken, NJ, pp. 465–501.

Rutter, M., Moffitt, T.E. & Caspi, A. (2006) Gene-environment interplay and psychopathology: multiple varieties but real effects. *J Child Psychol Psychiatry* **47**, 226–261.

Shanahan, L., Copeland, W., Costello, E.J. & Angold, A. (2008) Specificity of putative psychosocial risk factors for psychiatric disorders in children and adolescents. *J Child Psychol Psychiatry* **49**, 34–42.

Stein, M.B., Walker, J.R., Anderson, G., Hazen, A.L., Ross, C.A., Eldridge, G. & Forde, D.R. (1996) Childhood physical and sexual abuse in patients with anxiety disorders and in a community sample. *Am J Psychiatry* **153**, 275–277.

Taylor, S.E. (2010) Mechanisms linking early life stress to adult health outcomes. *Proc Natl Acad Sci USA* **107**, 8507–8512.

Tronick, E. & Reck, C. (2009) Infants of depressed mothers. *Harvard Rev Psychiatry* **17**, 147–156.

Uher, R. & McGuffin, P. (2008) The moderation by the serotonin transporter gene of environmental adversity in the aetiology of mental illness: review and methodological analysis. *Mol Psychiatry* **13**, 131–146.

Weissman, M.M., Wickramaratne, P., Nomura, Y., Warner, V., Pilowsky, D. & Verdeli, H. (2006) Offspring of depressed parents: 20 years later. *Am J Psychiatry* **163**, 1001–1008.

Wickramaratne, P., Gameroff, M.J., Pilowsky, D.J., Hughes, C.W., Garber, J., Malloy, E., King, C., Cerda, G., Sood, A.B., Alpert, J.E., Trivedi, M.H., Fava, M., Rush, A.J., Wisniewski, S. & Weissman, M.M. (2011) Children of depressed mothers 1 year after remission of maternal depression: findings from the STAR*D-Child study. *Am J Psychiatry* **168**, 593–602.

Widom, C.S., DuMont, K. & Czaja, S.J. (2007) A prospective investigation of major depressive disorder and comorbidity in abused and neglected children grown up. *Arch Gen Psychiatry* **64**, 49–56.

Wilhelm, K., Niven, H., Parker, G. & Hadzi-Pavlovic, D. (2005) The stability of the Parental Bonding Instrument over a 20-year period. *Psychol Med* **35**, 387–393.

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