

Regulatory variant of the TPH2 gene and early life stress are associated with heightened attention to social signals of fear in infants

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Background: Cross-species evidence suggests that genetic and experiential factors act early in development to establish individual emotional traits, but little is known about the mechanisms that emerge during this period to mediate long-term outcomes. Here, we tested the hypothesis that known genetic and environmental risk conditions may heighten infants' natural tendency to attend to threat-alerting stimuli, resulting in a cognitive bias that may contribute to emotional vulnerability. **Methods:** Data from two samples of 5–7-month-old infants ($N = 139$) were used to examine whether established candidate variations in the serotonin-system genes, i.e., TPH2 SNP rs4570625 (-703 G/T) and HTR1A SNP rs6295 (-1019 G/C), and early rearing condition (maternal stress and depressive symptoms) are associated with alterations in infants' attention to facial expressions. Infants were tested with a paradigm that assesses the ability to disengage attention from a centrally presented stimulus (a nonface control stimulus or a neutral, happy, or fearful facial expression) toward the location of a new stimulus in the visual periphery (a geometric shape). **Results:** TPH2 -703 T-carrier genotype (i.e., TT homozygotes and heterozygotes), presence of maternal stress and depressive symptoms, and a combination of the T-carrier genotype and maternal depressive symptoms were associated with a relatively greater difficulty disengaging attention from fearful facial expressions. No associations were found with infants' temperamental traits. **Conclusions:** Alterations in infants' natural attentional bias toward fearful facial expressions may emerge prior to the manifestation of emotional and social behaviors and provide a sensitive marker of early emotional development. **Keywords:** Attention, facial expression, fear, tryptophan hydroxylase 2 gene, infancy, maternal stress.

Introduction

The first years of life appear to constitute a period of heightened plasticity when an organism's biological systems are particularly sensitive to genetic and experiential influences, and may undergo functional alterations that are foundational for individual emotional traits (Hertzman & Boyce, 2010). Studies examining how developmental processes during this period are influenced by genetic and environmental adversity (e.g., Feldman et al., 2009; Holmboe et al., 2010) may hence provide important new markers for identifying individuals at risk and for understanding the ontogenetic origins of emotional vulnerability.

One tractable, but as yet little examined, aspect of early development relates to functional emergence of mechanisms that mediate attentional vigilance to biologically relevant stimuli (LeDoux, 2012). In human infants, the first signs of such vigilance are observed during the second half of the first year of life when infants begin to discriminate between facial expressions and exhibit an attentional bias toward

faces that express fear (Peltola, Hietanen, Forssman, & Leppänen, 2013). Similar vigilance to danger-alerting cues is observed in several species (Olsson & Phelps, 2007), suggesting a conserved mechanism that may serve as an adaptive function in detecting harmful stimuli. Yet, there are also indications that genetic and experiential factors may exacerbate such species-typical perceptual predispositions (Shackman, Shackman, & Pollak, 2007), and that a difficulty disengaging attention from threat-related facial expressions is correlated with trait anxiety (Georgiou et al., 2005) and predicts vulnerability to anxiety in children and adults (Bar-Haim, Morag, & Glickman, 2011; Fox, Hane, & Pine, 2007; Pine, 2007).

In the present study, we examined whether the development of attention to emotional cues is modulated by established genetic and environmental risk factors. Although attentional biases cannot be directly linked with particular genetic or environmental factors, we reasoned that the rapid development of these processes during the first year of life may make them susceptible to modulatory effects by factors that are more nonspecific in nature (cf. Leonardo & Hen, 2007).

Conflict of interest statement: No conflicts declared.

The primary focus of our genetic analyses was a common G to T base substitution (rs4570625) in the promoter region of a gene that encodes tryptophan hydroxylase-2 (TPH2), a brain-specific enzyme of serotonin synthesis. The T-allele of this single nucleotide polymorphism (SNP) has been associated with altered TPH2 mRNA expression (Chen, Vallender, & Miller, 2008), enhanced amygdala and cortical responsiveness to emotional cues (Brown et al., 2005; Herrmann et al., 2007), reduced attention control (Strobel et al., 2007), and increased susceptibility to depressive disorders (Gao et al., 2012). The possibility that these genetic effects are established through altered neurodevelopment is supported by evidence showing that TPH2 expression commences very early in the developing brain (embryonic day 11 in mice, Waider, Araragi, Gutknecht, & Lesch, 2011) and the results of our previous study showing that the TPH2 -703 T-carrier genotype (i.e., T-carriers, including both TT homozygotes and heterozygotes) are associated with increased difficulty disengaging attention from happy and fearful expressions in 7-month-old infants (Leppänen et al., 2011). Our goal in the present study was to examine the replicability of these results in a new sample of infants.

A second genetic variation examined in the present study was a polymorphism in the serotonin autoreceptor 1A gene (HTR1A SNP rs6295 -1019 G/C). The C/C genotype of this SNP has been associated with increased serotonergic tone and heightened amygdala reactivity to facial expressions (Fakra et al., 2009). In our previous study (Leppänen et al., 2011), no significant association of this SNP on attention disengagement in infants was found, but the results showed a trend-level association between the C/C genotype and increased difficulty disengaging.

To examine whether the predicted effects of the TPH2 SNP rs4570625 (-703 G/T) genotype and infants' attentional bias to fearful facial expressions are dependent on early rearing conditions, we examined maternal stress and depressive symptoms. Maternal stress and depression have been associated with adverse changes in several aspects of child development (Bornstein, Arterberry, Mash, & Manian, 2011; Feldman et al., 2009), with some studies (Bagner, Pettit, Lewinsohn, & Seeley, 2010), although not all (Naicker, Wickham, & Colman, 2012), suggesting that depressive symptoms during pregnancy and first postnatal months are especially strongly associated with adverse outcomes. It is noteworthy that stress and depressive symptoms, as assessed by typical self-report questionnaires, are likely to be linked with several changes in the child's genetic predispositions as well as pre- and postnatal environment (e.g., Feldman et al., 2009). Our study was not aimed at teasing apart these factors but rather to examine whether maternal stress as an established risk condition is linked with an early-emerging and heightened attention bias toward signals of negative emotion.

Methods

Participants

The present analyses included data from two ongoing longitudinal studies. Most of the participants in the two studies came from urban, middle-class families of Finnish origin. The first sample (Cohort 1) consisted of 7-month-old infants ($n = 66$) from a study that was started in October 2007 and consisted of laboratory assessments at 7, 24, and 48 months of age. The methodology and results of the 7-month assessment were reported in a previous publication (Leppänen et al., 2011) and were used here in pooled analyses of TPH2 SNP rs4570625 effects across two samples. The second sample (Cohort 2) consisted of 5–7-month-old infants from a study that began in April 2012 and include laboratory assessments at 5, 7, 12, 24, and 48 months of age. DNA samples were available from 73 full-term (≥ 37 weeks) infants (35 females) with scorable attention assessments at 5 ($M = 151.58$ days, $SD = 3.28$ days) and 7 ($M = 213.44$ days, $SD = 4.61$ days) months of age. Maternal stress and depression data were available for 87 infants with scorable attention data at 5 ($M = 151.84$ days, $SD = 3.26$ days) and 7 ($M = 213.13$ days, $SD = 3.23$ days) months of age. Additional infants enrolled in the study were excluded from one of the analyses because of missing DNA ($n = 20$) or questionnaire data ($n = 5$), and both analyses because of premature birth ($n = 1$), fussiness ($n = 6$) or technical problems during testing ($n = 6$), experimenter's error ($n = 1$), dropping out ($n = 1$), or providing insufficient gaze data ($< 50\%$ trials in one or several conditions at one or both time points; $n = 12$). Genetic and stress-related data from Cohort 2 have not been reported previously, but the attention data of subgroup of infants in this sample were used in a previous report by Peltola et al. (2013).

Ethical permission for the study was obtained from the Ethical Committee of Tampere University Hospital and a written informed consent was given by the parents of the participants before the start of the study.

DNA extraction and genotyping

A volume of 3.0 ml EDTA-whole blood was taken from the participants when they were 7 months by an experienced laboratory nurse and stored at -20°C . DNA was extracted by using QIAamp*DNA Blood Minikit and BioRobots* M48 (Qiagen, Hilden, Germany). Consistent with the analyses conducted for Cohort 1 (Leppänen et al., 2011), the first DNA analyses of Cohort 2 focused on single nucleotide polymorphisms (SNPs) in the TPH2 (rs4570625) and HTR1A -1019 (rs6295) genes. Genotyping was performed by using Taqman*SNP Genotyping Assays and ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) with the following assays: C_226207_10 (rs4570625) and C_11904666_10 (rs6295). No discrepancies were

detected in the genotyping results of duplicate samples.

Maternal stress and depressive symptoms

Assessment of stressful life events and maternal depressive symptoms was performed through questionnaires. The child's biological mother or both parents were reported as the primary caregiver of the child for all except two dyads with father as the primary caregiver. The mothers were given the questionnaires after the 7-month-laboratory visit, were asked to fill them out within 2 weeks from the visit, and return them to the laboratory in a prepaid envelope. The recent life events questionnaire (based on Brugha, Berington, Tennant, & Hurry, 1985) consisted of 18 stressful life events and one open-response item (a full description of the events is provided in Table S3). The mothers were asked to tick a 'yes' box if the event had occurred during the past 12 months, and tick a 'still affects me' box if the event was still having an effect on their life. The sum of all 'yes' responses and 'still affects me' responses (except responses to the item related to child birth) were calculated separately and were used as measures of maternal stress. To assess maternal postnatal depressive symptoms, mothers filled out the 10-item Edinburgh Postnatal Depression Scale (EPDS, Ref. Cox, Holden, & Sagovsky, 1987). The sum scores from the EPDS were used to index depressive symptoms, with higher values indicating higher levels of depressive symptoms.

Assessment of attention to facial expressions

A detailed description of the paradigm that was used to assess attention disengagement can be found in Peltola et al. (2013). During the laboratory assessment at 5 and 7 months of age, the infants sat on their parents lap at a ~60-cm viewing distance in front of a 23-inch monitor that was part of a corneal-reflection eye-tracker (Tobii TX300; Tobii Technology, Stockholm, Sweden). Every experimental trial was preceded by a presentation of an attention-grabbing stimulus (a red circle that expanded from 0.4° to 4.3° in a continuous fashion) to attract the infant's attention to the center of the screen. The experimenter monitored the infant's gaze direction through a hidden video camera, and when the child was attending to the red circle, pressed a button to start the trial. On each trial, the infants were presented with a control stimulus (i.e., a scrambled face) or one of three different facial expressions (i.e., neutral, happy, or fearful facial expression) on the center of the screen. After 1000 ms, the central stimulus was flanked by a lateral stimulus (a black-and-white picture of vertically arranged circles or a checkerboard pattern) 13.6° equiprobably on the left or right for 3000 ms, as shown in Figure 1. The present analyses are

based on the infants' attention disengagement from the central stimuli during the first part of the testing session (24 trials; 6 trials/condition). The second part of stimulus presentation was added for the purposes of EEG measurement and will be reported in a separate publication.

Identical to Cohort 1, video recordings of each infant's behavior in Cohort 2 were coded frame-by-frame by an observer who was blind to the stimulus condition. In cases where the video recording was missing or invalid (e.g., video camera did not record due to technical errors, video recording showed a deviation in frame rate, or the child was out of view), data points were replaced with gaze data from the eye-tracker recording. A trial was considered invalid if the infant did not look at the central stimulus for at least 75% during the initial 1000 ms presentation, if the infant made an anticipatory eye movement (i.e., eye movement commenced <160 ms after the onset of the lateral stimulus), or an eye movement toward an incorrect location (i.e., not toward the lateral stimulus). Of the scorable trials, the number of missing attention shifts (i.e., no eye movement toward the lateral stimulus during a time window from 160 to 1000 ms after the onset of the lateral stimulus) were calculated and served as the dependent variable in the study.

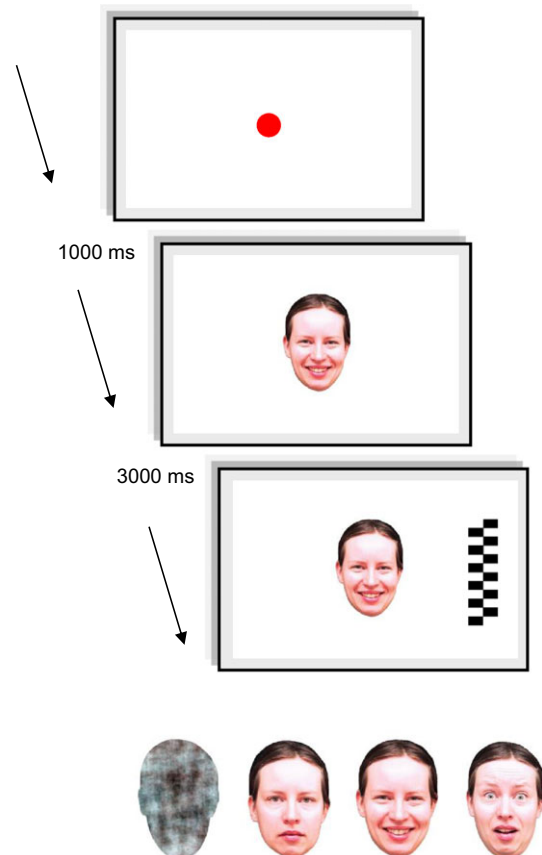


Figure 1 The sequence of events and stimuli in the paradigm used to assess infants' attention. The proportion of missing saccades from the central stimulus to the lateral stimulus was used as a measure of attention disengagement

Assessment of temperament

Parents rated their child's temperament using the Infant Behavior Questionnaire (Rothbart, 1981) when their child was 7 months.

Statistical analysis

Our first analysis was aimed at examining whether the previously reported association between the T-allele of the TPH2 SNP rs4570625 (-703 G/T) and attention disengagement (number of missing attention shifts) was replicated in Cohort 2. As in the previous study (Leppänen et al., 2011), a dominant effect of the T-allele was tested by dividing the participants into G/G homozygotes and T-carriers, and by conducting a Generalized Estimating Equations (a Poisson log linear model, SPSS 20) with Genotype, Age, and Facial Expression as factors, the number of missing saccades as a dependent variable (a Kolmogorov–Smirnov test showed that the number of missing saccades followed a Poisson distribution, $p > .06$), and a natural log of the number of trials as an offset variable. A corresponding analysis was conducted to examine the association between HTR1A SNP rs6295 (-1019 G/C genotypes (G-carriers vs. C/C homozygotes) and attention disengagement in Cohort 2. Following the statistical approach of the previous study (Leppänen et al., 2011), an unadjusted alpha level of .05 was used for both statistical analyses.

Second, we examined whether the number of maternal stressful life events were associated with the attentional bias toward facial expressions. Data on stressful life events were available for Cohort 2 only. In the primary analysis, variables describing the number of life events and the number of life events 'still affecting' the mother were divided into three groups (i.e., groups with 0, 1, and 2 or more life events), and their effects were examined by Generalized Estimating Equations with Life Events, Age, and Facial Expression as factors in a Poisson log linear model and the number of missing saccades as a dependent variable. In a secondary correlation analysis (Spearman rho), intended to supplement the original analysis by using the original ungrouped variables, we correlated the raw number of life events with a variable that described attentional bias toward fearful facial expressions (i.e., the difference in the proportion of missing attention shifts for all nonfearful stimuli and fearful facial expressions).

Third, we examined whether the effects of TPH2 SNP rs4570625 (-703 G/T) were dependent on rearing condition. For this analysis, data from Cohorts 1 and 2 were pooled to attain a maximal sample size, and mother's ratings of depressive symptoms that were available from both cohorts were used as a variable reflecting the early rearing conditions. A univariate analysis of variance (ANOVA) was performed with genotype (between factor; GG: $n = 88$

vs. T-carriers: $n = 51$) as an independent variable, EPDS scores as a continuous covariate, and the fear bias score as a dependent variable. Estimates of effect sizes for independent variables (i.e., proportion of variance explained by the variable) are given as partial η^2 as provided by SPSS. The fear bias scores met the standard criteria for univariate normality (skewness = .26; kurtosis = -.63).

Finally, although the primary purpose of this study was to examine whether the selected genetic and psychosocial factors were associated with the early development of attention disengagement, corresponding analyses were also conducted to examine whether parallel associations are observed in measures of infant temperament. For these analyses, individual *t*-tests were used to compare TPH2 SNP rs4570625 (-703 G/T) genotypes with respect to different temperamental scales (Rothbart, 1981), and Pearson correlations were calculated to examine the associations between maternal stressful life events and infant temperament dimensions in Cohort 2, as well as maternal depressive symptoms and infant temperamental traits using pooled data from Cohort 1 and 2.

Results

TPH2 SNP rs4570625 (-703 G/T) and attention disengagement

The allelic frequencies of the TPH2 SNP rs4570625 (-703 G/T) were in Hardy–Weinberg equilibrium in the replication sample, $\chi = .06$, $p = .79$, and infants in the G/G ($n = 45$) and T-carrier ($n = 28$; G/T: $n = 26$, T/T: $n = 2$) groups did not differ in gender ratio, age, birth-weight, maternal depressive scores, stressful life events scores, or number of scorable trials (Table S2). The minor allele frequency for rs4570625 in the present sample (.2055) was comparable to that in general Finnish population sampling (.1953, Raitakari et al., 2008). A Generalized Estimating Equations (Poisson) model with genotype, facial expression, and age as factors revealed no significant main effect of TPH2 SNP rs4570625 (-703 G/T) on number of missing attention shifts, $p < .10$, but there was a significant main effect of facial expression, $\chi^2 = 126.05$, $df = 3$, $p < .001$, and a significant interaction between genotype and facial expression, $\chi^2 = 10.89$, $df = 1$, $p = .012$. Inspection of the mean proportion of missing attention shifts in the two genotype groups shows that the two genotype groups did not differ in overall levels of missing attention shifts, but the T-carriers displayed a larger relative increase in number of missing saccades for fearful facial expressions compared to the other conditions ($M_{increase} = .14$, $SD = .15$ in the GG group vs. $M_{increase} = .23$, $SD = .16$ in the T-carrier group). Thus, both G/G homozygotes and T-carriers show the typical patterns of increased number of missing attention shifts in the context fearful facial

expressions (see Figure S1), but the typical pattern of increased number of missing shifts for fear is significantly larger in the T-carriers. There was no interaction between TPH2 -703 genotype, emotion, and age, $p > .13$, indicating that the effects of genotype group and facial expression were not age-related. Together, these results show that the relative increase in missing attention shifts for happy expressions in T-carriers (Leppänen et al., 2011) did not replicate in the new sample, but the increase in missing saccades for fearful facial expression was consistently larger in T-carriers across the two studies. Figure 2 illustrates the genotype effect on proportion of missing attention shifts from the neutral and affectively salient stimuli in the total sample (Cohorts 1 and 2 combined).

Corresponding analysis of the HTR1A SNP rs 6295 (-1019 G/C) showed no differences between the G-carriers ($n = 55$; C/G: $n = 34$, G/G: $n = 21$) and C/C ($n = 18$) genotypes on attention disengagement. This SNP was left out from all subsequent data analyses in the study.

Maternal stress and attention disengagement

The mean number of stressful life events reported by the mothers was 1.4 (range 0–5), with 23 mothers reporting no life events, 33 one event, and 31 two or more life events. The mean number of events still affecting the mother was 0.6 (range 0–3), with 54 mothers reporting no affecting events, 19 reporting one still affecting event, and 14 reporting two or more affecting events. Stressful life events were not associated with the number of scorable trials in attention task, all $ps > .10$, indicating that maternal stress was not related to the success or quality of the data collection per se.

A Generalized Estimating Equations (Poisson) model with the number of stressful life events, facial expression, and age as factors revealed no significant main effect of life events nor a significant interaction between the number of life events and facial expression on missing attention shifts, $p = .12$. A similar analysis using the number of life events 'still affecting' the mother revealed a significant interaction between the number of events and facial expression, $\chi^2 = 18.82$, $df = 6$, $p = .004$. As shown in Figure 3, increased number of life events 'still affecting' the mother was associated with a linear increase in missing attention in the fearful facial expression condition (i.e., relatively larger fear bias scores). The interaction between the number of events and facial expression was further qualified by an interaction with age, $\chi^2 = 12.9$, $df = 6$, $p = .045$. Separate analysis (univariate ANOVAs) showed that the association between life events and fear bias scores was weaker at 5 months ($p = .16$) than at 7 months of age ($p = .003$). The association between stressful life events and infants' fear bias scores was also seen in correlations between the raw (ungrouped) numbers of life events and fear bias scores. The reported number of stressful life events and the number of events 'still affecting' were both significantly associated with the strength of the fear bias in infants, Spearman's rho .32 and .37, $ps < .004$.

TPH2 SNP rs4570625 (-703 G/T), maternal depression, and infants' fear bias

To examine whether the observed effect of the TPH2 SNP rs4570625 (-703 G/T) genotype on missing attention shifts was dependent on exposure to

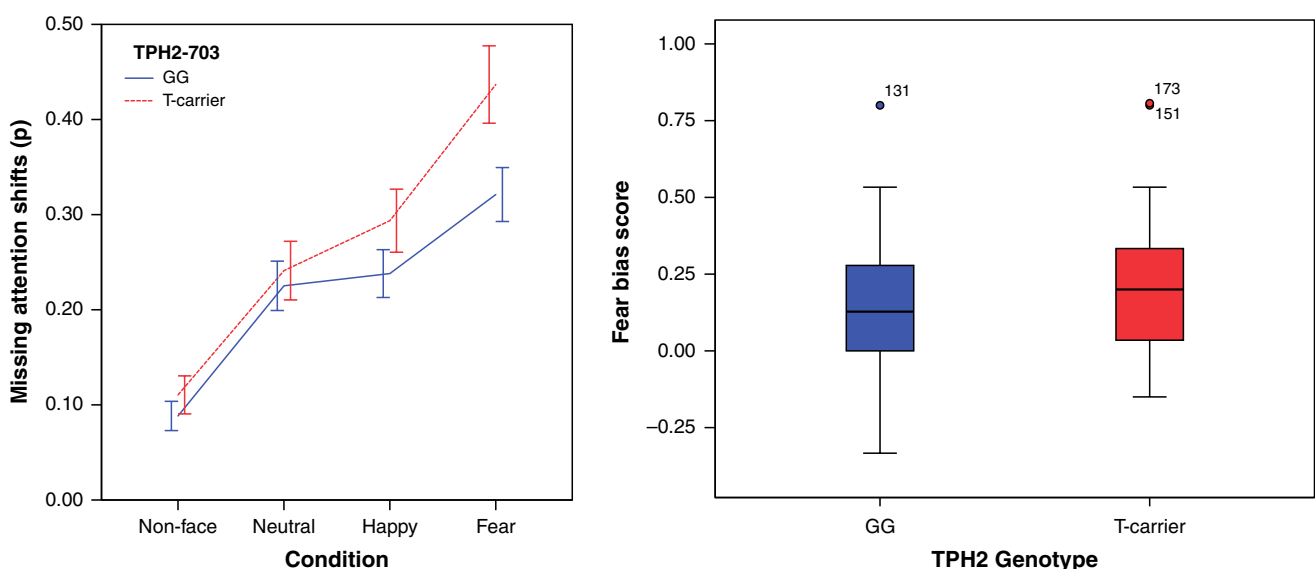


Figure 2 Left: Mean proportion of missing attention shifts in the total sample (Cohort 1 and 2) from the central to the lateral stimulus in the attention disengagement task grouped by facial expressions and TPH2 SNP rs4570625 (-703 G/T; G/G $n = 88$; T-carrier $n = 51$). Error bars represent ± 1 standard error. Right: A boxplot showing the effect of TPH2 SNP rs4570625 (-703 G/T) genotypes on attention bias toward fearful facial expressions, calculated as the difference in missing saccades for nonfearful (i.e., control, neutral, and happy stimuli) and fearful facial expressions (fear bias score)

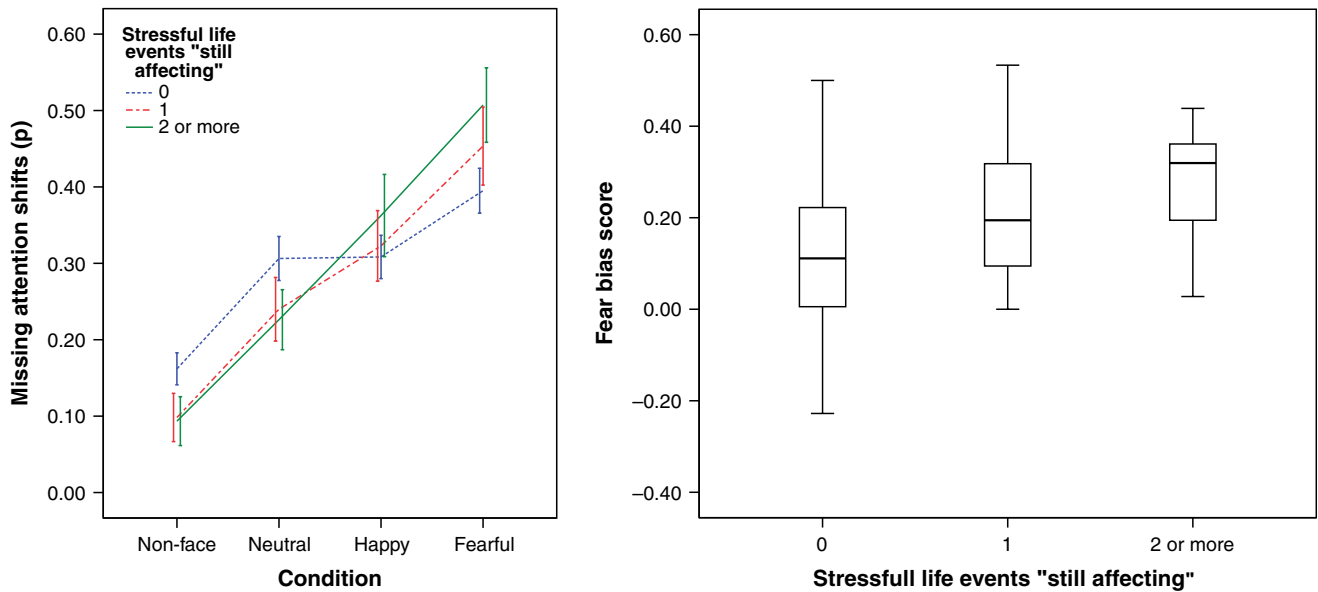


Figure 3 Left: Number of stressful life events ‘still affecting’ the mother and mean proportions of infants’ missing saccades in different stimulus conditions. Right: A boxplot showing the association between ‘still affecting’ stressful events and fear bias scores

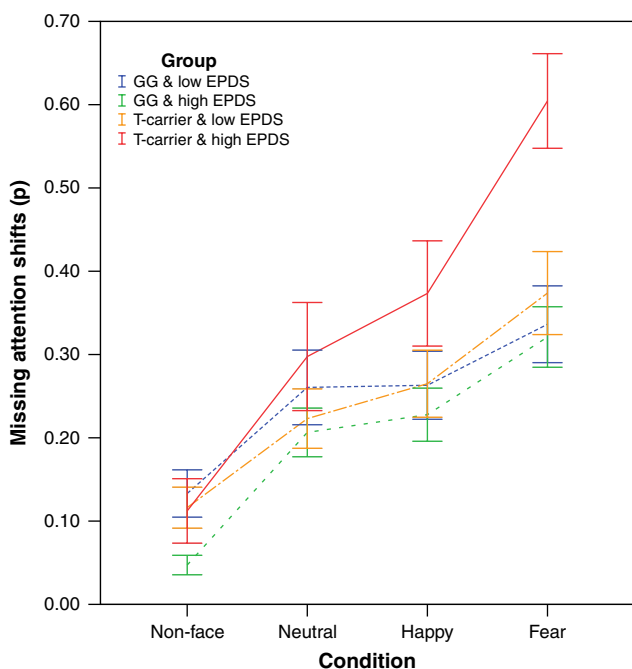


Figure 4 Mean proportion of missing attention shifts in the total sample (Cohort 1 and 2) from the central stimulus (Nonface, Neutral, Happy, or Fearful facial expression) to the lateral target grouped by maternal depression score (Edinburgh Postnatal Depression Scale; EPDS; Median-split: high vs. low) and TPH2 SNP rs4570625 (-703 G/T) genotypes (G/G and low EPDS $n = 42$; G/G and high EPDS $n = 42$; T-carrier and low EPDS $n = 34$; T-carrier and high EPDS $n = 15$). Error bars represent ± 1 standard error

maternal depressive symptoms, we carried out a univariate ANOVA with genotype (between factor; GG homozygotes vs. T-carriers) as an independent variable and maternal depressive score as a covariate using pooled data from Cohort 1 and 2. This analysis showed a significant main effect of TPH2 SNP rs4570625 (-703 G/T), $F(1, 130) = 7.0, p = .009$, partial $\eta^2 = .05$, and maternal depression, $F(1,$

130) = 8.3, $p = .005$, partial $\eta^2 = .06$, on the proportion of missing attention shifts for fear. These main effects were qualified by a significant interaction between genotype and maternal depression, $F(1, 129) = 8.5, p < .001$, partial $\eta^2 = .12$. As shown in Figure 4, T-carriers with mothers who scored relatively higher on postnatal depression exhibited highest levels of missing attention shifts for fearful facial expression. No significant main effect or interactions were found for Cohort (between factor; Cohort 1 vs. Cohort 2, p -values = .14–.92.) or any substantial change to the above described results, when the ANOVA was conducted with this factor as an independent variable.

TPH2 SNP rs4570625 (-703 G/T), maternal stress, and infant temperament

No association between genotype and any of the temperamental dimensions were found in pooled analysis of data from Cohort 1 and 2 ($n = 157$), or between maternal stress and infant temperamental orienting, distress to limitations, and negative affectivity, $r_s .18-.23, p_s < .05$ (uncorrected), in pooled analysis of data from Cohort 1 and 2.

Discussion

There has been increasing recent interest in the possibility that variations in the TPH2 gene may alter early neurodevelopment, partially contingent on exposure to adverse experiences. Consistent with this possibility, our results showed that the T-carrier genotype of the TPH2 SNP rs4570625 (-703 G/T) is associated with a similar trend for relatively greater

difficulty disengaging attention from fearful facial expressions in two independent samples of 5- to 7-month-old infants. We also found that this association was dependent on the infant's rearing conditions in a predicted way, being most pronounced in T-carriers whose mothers reported higher levels of depressive symptoms. If confirmed, the potential dominant association of the T-allele of the TPH2 SNP rs4570625 (-703 G/T) with increased serotonin synthesis and serotonergic tone (Chen & Miller, 2012), and the dependence of TPH2 expression on early experiences (Gardner et al., 2009) may provide a neurobiologically plausible mechanism for enhanced attention to fear. Indeed, a randomized controlled study in human adults has shown that increased tryptophan and serotonergic function heightens perceptual sensitivity to fearful facial expressions (Attenburrow et al., 2003). At present, this interpretation must be with caution, however, given the absence of direct evidence for the functional significance of the TPH2 SNP rs4570625 (-703 G/T). Furthermore, the present study focused on a single polymorphism in the TPH2 gene while it is likely that multiple SNPs, genes, and neurotransmitter systems are involved in early attentional development.

In future research, it will be important to examine whether the observed genetic associations were affected by genetic ancestry by analyzing ancestry informative genetic markers and by replicating the study in samples from different ancestries. It should also be noted that the current analyses do not rule out the possibility that the observed genetic associations in the infants may be partly attributable to indirect effects arising from the fact that the parents are likely to share the same genotype and associated behavioral traits (such as heightened depression).

Stressful life events (particularly when perceived as 'still affecting' the mother) were also associated with a selective difficulty disengaging attention from fearful facial expressions in infants. Besides shared genetic influences between the mother and the child, there are several plausible experience-related mechanisms that may mediate the association between maternal stress and heightened infant attention to fearful facial expressions. Maternal stress may pose a nonspecific risk condition that is associated with a number of pre- and postnatal adverse influences (e.g., reduced maternal sensitivity) and co-occurring conditions, each of which may lead to exacerbations in infants stress reactivity and related processes, possibly also increasing infants attentiveness to social signals of fear (Loman & Gunnar, 2010). A related possibility is that experienced stress increases caregivers' protective and precautionary behaviors (Hahn-Holbrook, Holbrook, & Haselton, 2011). Presumably, one of the primary means by which caregivers exert such behaviors is by facial signaling of fear. If continued over time, repeated exposure to such behaviors may increase both

adults' and infants' sensitivity to fear cues (see Shackman et al., 2007 for a similar explanation for presumably adaptive enhancement in attention to angry facial expressions in maltreated children).

Our results were limited by the reliance on self-reports as the only source of information regarding maternal stress and depressive symptoms, as well as maternal reports as the only source of information concerning infants' temperament. Nevertheless, questionnaires have been extensively used in developmental research and were considered to provide a sufficient proxy of rearing condition and infants' temperament for the purposes of the present study. It is clear, however, that further research with a broader approach to studying the role of infants' rearing environment for the development of attention to social signals, for example through direct home observations and the assessment of mother-child interaction, during the first months of life will be important for uncovering the mechanisms that underlie the association between stress and heightened fear bias. Also, it will be important to rule out the possibility that maternal depression biases the ratings of infant temperament by using more objective measures of temperament.

In summary, the present study suggests that infants' natural attentional bias toward fearful facial expressions may be exacerbated by genetic and psychosocial risk factors within a relatively short time period in early development. These alterations in attentional biases may provide a sensitive and accessible marker of early emotional development, as alterations in attention were observed without any concomitant changes in mother-reported temperamental traits. Although we did not examine the long-term functional significance of heightened fear bias in the current study (such analyses will be conducted when longitudinal assessments have been completed), it is intriguing to note that a wealth of recent evidence suggests that exaggerated attention to threat-alerting cues may represent one of the key ontogenetic mechanisms that catalyzes the development of fear-related temperamental traits and increases vulnerability to anxiety disorders (e.g., Bar-Haim et al., 2011). Naturally, the inference of such causal associations between attention and emotion requires not only longitudinal data but, ultimately, controlled experiments with techniques to attenuate maladaptive attention biases (Bar-Haim et al., 2011). Recent studies have shown that eye-tracking systems can be used to enhance attention disengagement in infants (Wass, Porayska-Pomsta, & Johnson, 2011), but it is not yet known whether such effects extend to infants attention biases toward threat-alerting cues.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Distribution of missing saccades for fearful facial expressions across the two samples.

Table S1 Mean proportion of missing saccades in the attention disengagement task.

Table S2 Demographic information, sum scores of maternal rating on the Edinburgh Postnatal Depression Scale.

Table S3 Life events questionnaire.

Appendix S1. Regulatory variant of the TPH2 gene and early life stress are associated with heightened attention to social signals of fear in infants.

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Key points

- The early stages of human development are highly sensitive to genetic and environmental influences, and functional adaptations during this period may be foundational for lifetime emotional traits.
- Little is known about the mechanisms that emerge during this period to mediate long-term outcomes.
- Our study shows that infants' natural attentional bias toward fearful facial expressions is heightened in association with established genetic and psychosocial risk factors.
- Heightened attentional bias toward fear may provide a sensitive and accessible marker for the assessment of early development and potentially also for understanding the mechanisms that mediate emotional vulnerability.

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