Genetic and neural dissociation of individual responses to emotional expressions in human infants

Tobias Grossmann a,b,*, Mark H. Johnson a, Amrisha Vaish c, David A. Hughes d, Dominique Quinque d, Mark Stoneking d, Angela D. Friederici b

a Centre for Brain and Cognitive Development, Birkbeck, University of London, Malet Street, London WC1E 7HX, UK
b Department of Neuropsychology, Max Planck Institute for Human Cognitive and Brain Sciences, Stephanstrasse 1a, 04103 Leipzig, Germany
c Department of Developmental and Comparative Psychology, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, 04103 Leipzig, Germany
d Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, 04103 Leipzig, Germany

Article history:
Received 29 April 2010
Received in revised form 5 July 2010
Accepted 6 July 2010

Keywords:
Emotion
Face
ERP
Individual differences
Infancy
Genetics

1. Introduction

Interacting with others by interpreting and responding to their facial expressions is an essential skill for humans (Darwin, 1872). Reading emotional expressions during social interactions permits us to detect another person’s emotional state or reactions, and can provide cues on how to respond appropriately in different situations (Frith, 2009). The ability to discriminate and recognize various emotional expressions from faces emerges during infancy (Grossmann, 2010). Given the well-studied developmental processes involved in the sensitive responding to facial expressions of emotion, an important further question is whether and how genetic variation might influence infants’ brain responses to facial expressions and thus contribute to individual differences in emotional sensitivity and temperament. Addressing this question of specific genetic pathways that contribute to social behavior is critical to our understanding of how such differences confer vulnerability to psychiatric diseases (Meyer-Lindenberg and Weinberger, 2006). In addition, studying emotion processing in infancy provides the opportunity to examine gene effects at a time in development when genetic association might be more robustly demonstrated because effects of postnatal experience are still relatively small (Ebstein, 2006).
In adults, variation in specific genes acting on neurotransmitter systems has been found to impact emotion processing. Specifically, a number of genetic neuroimaging studies have shown effects of catechol-O-methyltransferase (COMT) and serotonin transporter (SLC6A4/5-HTTLPR) genotypes on the processing of emotional stimuli in general and of facial expressions in particular (for reviews, see Canli and Lesch, 2007; Heinz and Smolka, 2006).

COMT is an important enzyme involved in the elimination of dopamine (DA) in the prefrontal cortex (Goldberg and Weinberger, 2004). A functional polymorphism in the COMT gene (val158met) accounts for a significant difference in enzyme activity: while the high-active val allele is presumed to be associated with lower concentration of synaptic DA, the low-active met allele is thought to result in higher concentrations of DA (Chen et al., 2004; Heinz and Smolka, 2006). At the cognitive level, the met allele is associated with improved working memory and executive functioning (Weinberger and Goldberg, 2004). This better performance in executive functions and working memory is reflected in a more focal response in prefrontal cortex as measured with functional magnetic resonance imaging (fMRI), indexing more efficient neural processing (Egan et al., 2001). The met allele, moreover, is associated with an increased sensitivity to emotionally unpleasant stimuli. More specifically, in an fMRI study with adults, the met allele was associated with increased activity in limbic and prefrontal brain regions in response to fearful and angry facial expressions (Drabant et al., 2006). This increased neural sensitivity associated with the met allele was not found in response to positive stimuli, suggesting that it is specific to negative stimuli (Herrmann et al., 2009; Smolka et al., 2005).

Serotonin (5-HT) plays a major role in emotion regulation and social behavior. A functional polymorphism (5-HTTLPR) in the regulatory regions of the serotonin transporter gene has a short (s) and a long (I) allele (14- and 16-repeat alleles, respectively) that alter promoter activity: the s variant produces significantly less serotonin transporter mRNA and protein than the l variant, resulting in higher concentrations of serotonin in the synaptic cleft (Canli and Lesch, 2007). Individuals carrying the s allele appear to have increased anxious temperament, resulting in an elevated risk to develop depression (Lesch et al., 1996). On the neural level, healthy non-depressed adults carrying the s allele showed an increased amygdala response to threatening stimuli such as fearful faces (Hariri et al., 2002). Furthermore, structural analyses revealed reduced gray matter in s allele carriers in anterior cingulate and amygdala, and during the processing of fearful faces, these regions showed less functional coupling in carriers of the s allele (Pezawas et al., 2005).

Taken together, in adults, both the met allele of the COMT gene and the s allele of the 5-HTTLPR gene appear to be associated with an increased sensitivity to negative, specifically fearful, expressions. Although both polymorphisms affect neural processes in the limbic system, the COMT variation is thought to be more specifically implicated in affecting prefrontal brain processes (Goldberg and Weinberger, 2004; Heinz and Smolka, 2006). Event-related brain potential (ERP) studies that allow for the precise investigation of the timing of neural processes have shown that, in adults, variation in COMT and 5-HTTLPR genotype affect the brain processing of emotional stimuli at early stages at occipital electrodes, starting approximately 200 ms after stimulus onset (Herrmann et al., 2006, 2009). Furthermore, in a recent study with adolescent twins, individual differences in ERP responses to emotional facial expressions have been found to be highly heritable (Anokhin et al., 2010).

In the present study, we thus assessed the effects of COMT and 5-HTTLPR genotypes on the brain processing of facial expressions (fearful and happy) in 7-month-old infants using ERPs, the method most readily used to study brain processes in human infants (de Haan, 2007). The analysis of genotype effects was focused on the Negative central (Nc) component in infants’ ERPs, and the preceding so-called Positivity before (Pb). Both components have been shown to be similarly modulated by facial expressions in infancy (Nelson and de Haan, 1996). The Nc, generated in the prefrontal cortex, occurs from approximately 300 to 600 ms, has its maximum at central electrodes, and is thought to reflect the allocation of attention to a stimulus, with a greater amplitude indexing increased allocation of attention (Richards, 2002). In 7-month olds, fearful faces when compared to happy faces elicited a more negative-going waveform consisting of a decreased Pb and an enhanced Nc, indicating increased attention allocation to fearful expressions (Nelson and de Haan, 1996). Moreover, Peltola et al., 2009 found that 7-month olds showed an enhanced Nc to fearful faces whereas 5-month olds did not, suggesting that an enhanced sensitivity to fearful faces emerges between 5 and 7 months of age. Such an enhanced attention to fearful faces is also found in adults and is thought to be a fundamental mechanism to prioritize the processing of evolutionarily significant stimuli (Vuilleumier, 2006). Furthermore, in order to see whether the observed effects were specific to emotional face processing rather than related to general face processing, we analyzed the face-sensitive infant N170 (Halti et al., 2004) as a function of genetic variation at the two loci. Finally, we examined effects of genotype on infant temperament as measured by the Infant Behavior Questionnaire-R (Garstein and Rothbart, 2003). On the basis of the adult work discussed above, it would be predicted that both polymorphisms affect the processing of fearful expressions. However, it is also possible that these genetic polymorphisms might be associated with different effects in infancy than in adulthood, since effects of genetic variation observed in adulthood may be an outcome of developmental processes that have distinct origins and manifestations in infancy (Gottlieb, 2007; Karmiloff-Smith, 1998).

2. Methods

Participants. The final sample consisted of 48 7-month-old infants (24 females, M = 221 days, Range = 216–226 days). An additional five 7-month olds were tested but not included in the final sample due to fussiness. All infants were of European descent and born full-term (37–42 weeks gestation) with normal birthweight (>2500 g). This study
was approved by the Ethics Committee of Leipzig University. All parents gave informed consent before the study.

Stimuli. The neutral, happy and fearful stimuli were color portrait photographs of two actors taken from the Nimstim stimulus set (Tottenham et al., 2009; see also Fig. 1). Each infant saw only one face identity (half of the group of infants saw identity A and the other half saw identity B).

Procedure. The infants were seated on their mother’s lap in a dimly lit, sound-attenuated, and electrically shielded room. Happy and fearful facial stimuli were presented on the screen for 1000 ms with the constraint that each emotion was presented no more than twice in a row. Prior to each presentation of a facial expression, a neutral facial expression was presented for 1000 ms. This mode of presentation creates the impression that the face changes its expression from neutral to fearful or from neutral to happy and may thus be a more natural (ecologically valid) representation of an emotional facial expression. Using such dynamic rather than static presentations of facial motion have been shown to increase infants’ attention and cortical responses (Grossmann et al., 2008; Grossmann and Farroni, 2009). Indeed, the used presentation mode and its potentially positive effects on infants’ engagement with the stimuli may have contributed to the fairly low attrition rate observed in this ERP study. The inter-stimulus interval varied randomly between 800 and 1200 ms. All stimuli were projected in the centre of the screen on a black background, using a 70 Hz, 17 in. computer screen at a distance of 60 cm from the eyes. The image sizes were 27 cm × 22 cm and the vertical and horizontal visual angles were 12.12° and 10.07°, respectively. The stimuli were presented in random order. Mothers were instructed to look down at the infant rather than at the computer screen. The session continued until the infant had seen the maximum number of trials (80) or became fussy. A camera recorded a close-up view of the infant’s face to monitor attention to the stimuli.

EEG measurement and data analysis. The EEG was recorded with Ag–AgCl electrodes from 19 scalp locations of the 10–20 system (Fig. 1), referenced to Cz. Horizontal and vertical EOGs were recorded bipolarly. Sampling rate was 250 Hz. EEG data was re-referenced to the algebraic mean of the left and the right mastoid electrodes, and band-pass filtered with 0.3–20 Hz (1501 points). Data were baseline corrected by subtracting the average voltage in the 200 ms baseline period from each post-stimulus data point. For elimination of artifacts caused by eye and body movements, EEG data for the whole trial were rejected off-line whenever the standard deviation within a 200-ms gliding window exceeded 80 μV for the vertical or horizontal electro-oculogram and 50 μV at any electrode. In addition, video recordings were examined, and all trials in which infants did not look at the screen were rejected from the EEG. The mean number of successful trials was 12.3 (SD = 2.3) for happy and 11.8 (SD = 2.7) for fearful faces. The mean number of trials presented to the infant was 47 (SD = 8.4). For statistical analysis of mean amplitude effects, on the basis of previous work (Nelson and de Haan, 1996; Grossmann et al., 2007), time windows of 0–200 ms, 200–400 (Positivity before: Pb) and 400–600 ms (Negative component: Nc) were chosen. In addition, for statistical analysis of mean amplitude effects on occipital

![Fig. 1. This figure shows the event-related brain potentials (ERPs) in response to happy (blue) and fearful (red) facial expressions for the entire group of 48 seven-month-old infants.](image)
infant face-sensitive ERPs (Halit et al., 2004) a time window of 100–300 ms (infant N170) was chosen around the peak of this component. ERPs were evaluated by computing the following regions of interest (ROIs): frontal (F3, Fz, F4), central (C3, Cz, C4), parietal (P3, Pz, P4), temporal (T7, T8), occipital (O1, O2). For these ROIs variances were analyzed by univariate ANOVAs with COMT or 5-HTTLPR genotype as fixed factors.

Samples and DNA extraction. Buccal samples were collected from each infant with informed consent of a parent. Swabs were placed in a lysis buffer and DNA was extracted as described previously (Quinque et al., 2006).

DNA amplification and genotyping. PCR-amplifications and restriction enzyme digestions for genotyping the rs4680 COMT polymorphism were carried out in an MJ Research Thermal Cycler (MR Research, Waltham, MA, USA). Each 25 μl PCR reaction consisted of an initial DNA denaturation and Taq activation step at 95 °C for 15 min followed by 34 repeated cycles of denaturation at 95 °C for 1 min, an annealing step for 1 min at 58 °C and extension at 72 °C for 1 min. After amplification there was a final extension step at 72 °C for 10 min. The reactions included 20 ng of template DNA, 1 × PCR buffer A1 (Solis Biodyne, Tartu, Estonia), 200 μM dNTPs (Amersham Biosciences, Uppsala, Sweden), 400 nM of each primer (Biotec, Berlin, Germany) and 1 unit of Hot FirePol polymerase (Solis Biodyne, Tartu, Estonia). Primers (rs4680 F: ATCCAAGTTCCTCTCTCTC and rs4680 R: CTTTTTCAGGTCTGACAAAC) were designed as the aid of the Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_results.cgi). Following amplification of the 290 bp target, the products were digested with the Nla III restriction enzyme (New England BioLabs, Ipswich, MA, USA) for 2 h at 37 °C, followed by an enzyme inactivation step for 20 min at 65 °C. The resulting products were electrophoresed through a 4% SeaKem LE gel (Cambrex, Rockland, ME, USA) for 1 h at 120 V and stained with ethidium bromide, with two fragments (28 bp and 262 bp) visualized for the G allele, and three fragments (18 bp, 28 bp and 244 bp) for the A allele.

PCR-amplification for genotyping the rs4795541 5-HTTLPR indel polymorphism was carried out using the conditions described above, except after the initial denaturation and activation step, there were 34 repeated cycles of denaturation at 95 °C for 30 s, an annealing step for 30 s min at 66 °C and extension at 72 °C for 45 s. The reactions included 20 ng of template DNA, 1 × PCR buffer mix 1 (ABgene, Hamburg, Germany), 500 μM dNTPs (Amersham Biosciences, Uppsala, Sweden), 400 nM of each primer (Biotec, Berlin, Germany) and 1.25 unit of Extensor Long PCR Enzyme (ABgene, Hamburg, Germany). Primers (5-HTTLPR: Forw.: TCTCCCGCTTGGCGGCTCCTCC and Rev.: TGGGGTGTCCAGGGAGATCTGG) were those described previously (Wendland et al., 2006). Following amplification products were electrophoresed through a 2% SeaKem LE gel (Cambrex, Rockland, ME, USA) for 1.5 h at 120 V and stained with ethidium bromide, with a 512 bp product corresponding to the long (l) allele and a 469 bp product corresponding to the short (s) allele.

The distribution of genotypes at the COMT and 5-HTTLPR polymorphisms in the sample of 48 infants is given in Table 1.

<table>
<thead>
<tr>
<th>COMT/5-HTTLPR</th>
<th>met/met</th>
<th>met/val</th>
<th>val/val</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long/long</td>
<td>8</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Long/short</td>
<td>9</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Short/short</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 1. Genotype frequencies for both polymorphisms did not deviate significantly from Hardy–Weinberg expectations, nor was there a significant association between genotypes.

Infant Behavior Questionnaire (IBQ-R). To assess infant temperament, parents completed the IBQ-R (14 subscales: approach, vocal reactivity, high intensity pleasure, smile and laughter, activity level, perceptual sensitivity, sadness, distress to limitations, fear, rate of recovery from distress, low intensity pleasure, cuddliness, duration of orienting, sootheability). The items on the IBQ-R ask parents to rate the frequency of specific temperament-related behaviors observed over the past week (or sometimes 2 weeks). For example, parents were asked ‘How often during the last week did the baby smile or laugh when given a toy?’ (smile and laughter scale) or ‘When frustrated with something, how often did the baby calm down within 5 minutes?’ (rate of recovery from distress). Each response is recorded on a seven-point scale ranging from 1 (Never) to 7 (Always). Temperament scores for each subscale were analyzed by univariate ANOVAs with COMT or 5-HTTLPR genotype as fixed factors.

3. Results

As shown in Fig. 1, the ERP analysis performed on the data of all 7-month-old infants (N = 48) revealed that fearful faces elicited a more negative-going waveform when compared to happy faces. This difference had its maximum at parietal sites and was observed for the Positivity before (Pb) between 200 and 400 ms (F[1,47] = 7.866, p = 0.007, partial η² = 0.143), and for the Negative component (Nc) between 400 and 600 ms (F[1,47] = 5.876, p = 0.019, partial η² = 0.111). This replicates prior work with infants of the same age (Nelson and de Haan, 1996). No effects were observed for an early time window (mean amplitude from 0 to 200 ms). Gender did not have an effect on infants’ processing of facial expressions. In order to test whether in infancy, variation in COMT and 5-HTTLPR both affect the processing of fearful expressions as predicted on the basis of adult work or whether they influence the processing of emotional expressions differently, we examined whether previously identified variation at these loci had an effect on the processing of either happy or fearful facial expressions for frontal, central, temporal, parietal, and occipital regions of interest (see Section 2).

3.1. COMT analysis

ERPs (Pb component, 200–400 ms). As predicted based on adult work, variation at COMT was associated with the processing of fearful expressions at central ([F[2,45] = 3.919,
Fig. 2. This graph depicts how variation in COMT associates with brain responses (mean amplitude in microvolt) to fearful facial expressions during the time windows from 200 to 400 ms (Pb) and from 400 and 600 ms (Nc) at central and parietal electrode sites, and provides the results of the post hoc comparisons between the three genotypes, *p < 0.05.

$p = 0.027$, partial $\eta^2 = 0.143$) and parietal electrodes ($F_{[2,45]} = 4.346, p = 0.019$, partial $\eta^2 = 0.162$) from 200 to 400 ms. Specifically, infants with met/met or met/val genotypes showed an increased negativity to fearful expressions, whereas infants with the val/val genotype showed a positivity in response to fearful expressions. Post hoc comparisons revealed that the mean amplitude for the met/met and met/val genotypes differed significantly from the val/val genotype at central and parietal electrodes (see Fig. 2 and Supplementary Figure S1). The processing of happy expressions at central ($F_{[2,45]} = 0.025, p = 0.975$, partial $\eta^2 = 0.001$) and parietal ($F_{[2,45]} = 0.973, p = 0.386$, partial $\eta^2 = 0.041$) sites during this time window did not differ between COMT genotypes. No effects of COMT were observed over frontal, temporal and occipital regions.

ERPs (Nc component, 400–600 ms). Similar to the Pb component, and as predicted based on adult work, variation at COMT was associated with the processing of fearful expressions at central ($F_{[2,45]} = 3.644, p = 0.034$, partial $\eta^2 = 0.139$) and parietal electrodes ($F_{[2,45]} = 4.423, p = 0.018$, partial $\eta^2 = 0.164$) from 400 to 600 ms. Specifically, infants with met/met or met/val genotypes showed an increased negativity to fearful expressions, whereas infants with the val/val genotype showed a positivity in response to fearful expressions. Post hoc comparisons revealed that the mean amplitude for the met/met and met/val genotypes differed significantly from the val/val genotype at central and parietal electrodes (see Fig. 2 and Supplementary Figure S1). The processing of happy expressions at central ($F_{[2,45]} = 0.642, p = 0.531$, partial
observed over frontal, temporal and occipital regions. No effects of COMT were observed over frontal, temporal and occipital regions.

No effects of COMT on the ERPs were observed for an early time window (0–200 ms), showing that the effects were restricted to components that occurred later than 200 ms.

**IBQ-R (parental questionnaire).** COMT variation was associated with the score of the rate of recovery from distress scale (F[2,45] = 3.417, p = 0.042, partial \( \eta^2 = 0.132 \)), with infants with the met/met genotype scoring higher, meaning that parents judged their infants as recovering quicker and better from distress, than infants with the met/val genotype, who in turn scored higher than infants with the val/val genotype (see Table 2).

### 3.2. 5-HTTLPR analysis

**ERPs (Pb component, 200–400 ms).** Unlike COMT, variation at 5-HTTLPR was associated with the processing of happy expressions at frontal (F[2,45] = 3.989, p = 0.025, partial \( \eta^2 = 0.151 \)) and temporal electrodes (F[2,45] = 7.351, p = 0.002, partial \( \eta^2 = 0.246 \)) from 200 to 400 ms. Specifically, infants with long/long or long/short genotypes showed a negativity in response to happy expressions, whereas infants with the short/short genotype showed a positivity in response to happy expressions. Post hoc comparisons revealed that the mean amplitude for the long/long and long/short genotypes differed significantly from the short/short genotype at frontal and temporal electrodes (see Fig. 3 and Supplementary Figure S2). The processing of fearful expressions at frontal (F[2,45] = 0.047, p = 0.955, partial \( \eta^2 = 0.002 \)) and temporal (F[2,45] = 0.239, p = 0.788, partial \( \eta^2 = 0.011 \)) sites during this time window did not differ between 5-HTTLPR genotypes. No effects of 5-HTTLPR were observed over central, parietal and occipital regions.

**ERPs (Nc component, 400–600 ms).** 5-HTTLPR variation was associated with the processing of happy expressions at temporal electrodes (F[2,45] = 5.45, p = 0.008, partial \( \eta^2 = 0.195 \)) from 400 to 600 ms. Specifically, infants with long/long or long/short genotypes showed a negativity in response to happy expressions, whereas infants with the short/short genotype showed a positivity in response to happy expressions. Post hoc comparisons revealed that the mean amplitude for the long/long and long/short genotypes differed significantly from the short/short genotype at temporal electrodes (see Fig. 3 and supplementary Figure S2). The processing of fearful expressions at temporal sites (F[2,45] = 0.744, p = 0.481, partial \( \eta^2 = 0.032 \)) during this time window did not differ between 5-HTTLPR genotypes. No effects of 5-HTTLPR were observed over central, parietal and occipital regions.

No effects of 5-HTTLPR on ERPs were observed for an early time window (0–200 ms), showing that the effects were restricted to components that occurred later than 200 ms.

**IBQ-R.** 5-HTTLPR variation was associated with the score of the smiling and laughter scale (F[2,45] = 3.068, p = 0.056, partial \( \eta^2 = 0.12 \)), with infants with short/short genotypes scoring significantly lower, that is, parents of short/short genotypes judged their infants as smiling much less in various contexts than did parents of infants with long/short or long/long genotypes (see Table 2). 5-HTTLPR also had a significant effect on the score of the duration of orienting scale (F[2,45] = 4.482, p = 0.017, partial \( \eta^2 = 0.166 \)), with infants with the short/short genotype scoring significantly lower, meaning that parents of infants with the short/short genotype judged their infants as being less able to devote their attention to a single object for an extended period of time than did parents of infants with long/short or long/long genotypes (see Table 1).

**ERPs (general face processing).** The ERPs elicited by the neutral face did not differ depending on COMT or 5-HTTLPR genotypes for any of the time windows and regions. Moreover, no effects of COMT and 5-HTTLPR genotypes were observed on the face-sensitive infant N170 (100–300 ms) at occipital electrodes.

There were no significant interaction effects between genes, neither on the brain measures nor on the behavioral temperament measures.

### 4. Discussion

We examined the association of genetic variation in COMT and 5-HTTLPR with infants’ brain responses to facial expressions of emotion. The results revealed that variation in these genes is differentially associated with how infants process facial expressions of emotion. Specifically, variation at the COMT locus is associated with the processing of fearful facial expressions, whereas variation at the 5-HTTLPR locus is associated with the processing of happy facial expressions. These differences were also reflected in the distinct topography of the ERP effects, suggesting the involvement of distinct brain processes: COMT variation was associated with centro-parietal processing of fearful faces, whereas 5-HTTLPR was associated with fronto-temporal processing of happy faces. These genetic associations were specific to the processing of emotional faces as no such effects were observed for the processing of neutral facial expressions. This pattern suggests that, early in postnatal development, variations of these genes affect distinct brain systems involved in the processing of positive versus negative facial expressions.

In line with findings from adults, COMT variation was associated with processing negative (fearful) emotions in infants (Drabant et al., 2006). More specifically, the carriers of the met allele showed an enhanced negativity to fearful expressions at central and parietal electrodes, indicating increased attentional sensitivity to these expressions, whereas infants with the val/val genotype responded with an increased positivity to fearful expressions, suggesting that this genotype processes fearful expressions less sensitively. This finding might have important implications for clinical disorders insofar as work with patients with schizophrenia has found that these patients are impaired in the recognition of fearful faces, and there is evidence to suggest that schizophrenia is more common among individuals with the val/val COMT genotype (Egan et al., 2001;
Fig. 3. This graph depicts how variation in 5-HTTLPR associates with brain responses (mean amplitude in microvolt) to happy facial expressions during the time window from 200 to 400 ms (Pb) at frontal and temporal electrode sites, and from 400 to 600 ms (Nc) at temporal electrode sites, and provides the results of the post hoc comparisons between the three genotypes, *p < 0.05.

Harrison and Weinberger, 2005; Morris et al., 2009). The increased attentional sensitivity to fearful faces associated with the met allele has also been reported in neuroimaging studies with adults (Drabant et al., 2006), thus suggesting developmental continuity in the influence of COMT on the processing of facial expressions.

In contrast to what has been shown in adults (where variation in 5-HTTLPR like variation in COMT is associated with increased attentional sensitivity to fearful faces), the results of the post hoc comparisons between the three genotypes, *p < 0.05.

Table 2
Summarizes how genetic variation in COMT and 5-HTTLPR associate with infants’ temperament scores as measured by parental questionnaire (IBQ-R).

<table>
<thead>
<tr>
<th>Trait</th>
<th>COMT met/met Mean (SE)</th>
<th>COMT met/val Mean (SE)</th>
<th>COMT val/val Mean (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery from distress</td>
<td>5.39 (0.19)</td>
<td>4.96 (0.19)</td>
<td>4.49 (0.29)</td>
</tr>
<tr>
<td>5-HTTLPR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long/long Mean (SE)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smiling and laughter</td>
<td>4.59 (0.20)</td>
<td>4.56 (0.22)</td>
<td>3.65 (0.38)</td>
</tr>
<tr>
<td>Duration of orienting</td>
<td>3.93 (0.24)</td>
<td>3.43 (0.21)</td>
<td>2.72 (0.28)</td>
</tr>
</tbody>
</table>
with the processing of negative [fearful] affect), the current infant data revealed that 5-HTTLPR variation is associated with the processing of positive (happy) affect. Specifically, carriers of the l allele showed a negativity in response to happy expressions at frontal and temporal electrodes, whereas infants with the s/s genotype showed a positivity in response to happy expressions, suggesting that s/s genotype infants process happy expressions differently and might be less sensitive to positive affect. Thus, our findings suggest that there are differences as to how 5-HTTLPR variants influence emotion processing in the human brain depending on age. It is important to note that fMRI work comparing children (average age of 11 years) and adults has revealed that adults but not children show increased amygdala activity to fearful faces when compared to neutral faces (Thomas et al., 2001). This late development of amygdala sensitivity to fearful faces reported in the fMRI work might help explain the difference between the current findings with infants and the adult work. That is, if older children do not show specific amygdala sensitivity to fear, it seems unlikely that infants' processing of fearful faces will be influenced by a gene that affects amygdala sensitivity only in adults. Furthermore, it should be noted that we measured ERPs from the scalp, and these potentials might not be sensitive to amygdala activity.

Nonetheless, one intriguing developmental hypothesis derived from the current findings is that early in postnatal development, variation in 5-HTTLPR may critically alter the processing of positive emotion, which later in development has effects on how adults respond to negative emotions. One mechanism that has been proposed is that infants have been responding sensitively to positive emotions from birth, which has established a positive default (or background) mode against which negative emotions stand out (see Vaish et al., 2008). It is possible that less sensitive responding to positive emotion in early development due to a specific genotype impairs the way in which positive affect becomes the background mode. According to this scenario, hypersensitivity in the processing of negative affect in adults could thus partly be a consequence of a reduced or impaired acquisition of positive affective stability during infancy and childhood (see Sprangler et al., 2009). Future research investigating this hypothesis across the lifespan is needed to understand the impact of 5-HTTLPR on the developmental trajectory of emotional sensitivity.

Further support for distinct influences of COMT and 5-HTTLPR on emotional processes in infancy comes from our analysis of infant temperament as judged by their parents. The results showed that while COMT variation is associated with reported recovery from distress, 5-HTTLPR variation was associated with reported smiling and laughter and duration of orienting. It is interesting to note that infants with the short/short genotype of 5-HTTLPR, who were judged as smiling and laughing significantly less than infants with the other 5-HTTLPR genotypes, also showed a different brain response to watching others’ happy facial expressions. This may point to a link between infants’ own experience of positive affect and processing positive affect from facial expressions in others, raising the possibility that so-called mirroring or simulation mechanisms could be influenced by temperament and genotype. The finding that COMT was associated with infants’ recovery from distress is in line with work implicating this gene in prefrontal control and regulatory brain mechanisms (Goldberg and Weinberger, 2004; Heinz and Smolka, 2006). Surprisingly, the met allele appeared to be associated with better emotion regulation (recovery from distress) in infants, which seems to contradict findings with adults indicating that the met allele might be linked to anxiety and difficulties in emotion regulation (Heinz and Smolka, 2006). However, the met allele has also been linked to better cognitive control, a notion that is also supported by behavioral work with children and infants (Diamond et al., 2004; Holmboe et al., 2010). Thus, better recovery from distress associated with the met allele as found in our infant sample might relate to generally improved control processes across cognitive and emotional domains, at least at this young age.

With respect to the timing of the brain processes that were found to be affected by variation in COMT and 5-HTTLPR, our ERP analysis revealed that both genes are associated with infants' brain responses as early as 200 ms after face onset. The timing of these effects is in line with the adult ERP work (Herrmann et al., 2006, 2009). However, in the adult ERP work, both genotypes were associated with posterior brain processes at occipital sites, whereas there were no associations with occipital sites in the current infant ERP data, suggesting that there might be a change during development in the topography of the effects. However, we cannot further interpret these topographic differences between infants and adults because in the adult work the analysis of genetic effects was focused only on posterior (occipital) sites and no data for other regions were presented (Herrmann et al., 2006, 2009). This is problematic because, in adults, ERP effects can be obtained at frontal and central electrodes in response to fearful faces (see, e.g., Eimer and Holmes, 2002).

In conclusion, to our knowledge this is the first study that investigated genetic variation associated with infants’ brain responses. Taking such a genetic imaging approach has been shown to be of great value for our understanding of individual differences in adults, and studying the association of genetic variation with brain responses as intermediate phenotypes, or so-called endophenotypes, has been argued to be a more powerful approach than studying gene effects on behavior (or personality traits) (Goldberg and Weinberger, 2004). Applying this approach to infants in the current study has revealed novel insights by adding a developmental component to the complex picture of how genetic variation may affect human emotion. The finding that, in infancy, COMT and 5-HTTLPR variation are associated with emotion processing in distinct ways raises interesting hypotheses about how genetic variation may bias certain brain mechanisms and thereby give rise to early individual differences that ultimately contribute to complex phenotypes such as temperament and personality (Kagan and Snidman, 2004). This might be a promising novel approach to the study of early emotional development, but it is only a first step. To gain a fuller understanding of the relationship between genetic variation, brain and emotion in development, we will need to examine genetic influences longitudinally in a larger sample of infants. This future work should also include
measures of parental genotype and personality as well as measures of structural brain development, because all of these factors may contribute in critical ways to the individual differences seen in the present study.

Acknowledgements

T.G. was supported by a Sir Henry Wellcome Fellowship awarded by the Wellcome Trust (082659/Z/07/Z). M.J. was supported by the UK Medical Research Council (G0701484). A.V. was supported by a grant from the Excellence Cluster 302: Languages of Emotion. The work by D.A.H., D.Q., and M.S. was funded by the Max Planck Society. The work was conducted at the Max Planck Institute for Human Cognitive and Brain Sciences. We thank the children and parents who participated in the study, and Kristiane Werrman for help with data collection.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.dcn.2010.07.001.

References


Stim set of facial expressions: judgements from untrained research participants. Psychiatry Research 168, 242–249.

