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RELATIONSHIPS AMONG 5-HTT GENOTYPE, LIFE EVENTS AND GENDER IN THE RECOGNITION OF FACIAL EMOTIONS

N. ANTYPA,[a]* H. CERIT,[a] A. W. KRUIJT,[a] F. E. A. VERHOEVEN[a] AND A. J. W. VAN DER DOES[a,b]

[a]*Institute of Psychology, Leiden University, The Netherlands*

[b]*Department of Psychiatry, Leiden University Medical Centre, The Netherlands*

Abstract—Accumulating evidence has shown that a polymorphism in the promoter region of the serotonin-transporter (5-HTTLPR) modulates neural activation during the perceptual processing of emotional facial expressions. Furthermore, behavioral research has shown that attentional bias for negative information is increased in s allele carriers. We examined the interactions among 5-HTTLPR (including SNP rs25531), life events and gender on the detection of facial emotions. We found a main effect of genotype, as well as moderating effects of childhood emotional abuse (CEA) and recent life events (RLE). S homozygous participants recognized negative facial expressions at a lower intensity than the other genotype groups. This effect was more evident in female participants and in participants who had experienced life events. The 5-HTTLPR genotype affects facial emotional perception, a process which is linked to a neurobiological response to threat and vulnerability to emotional disorders.

Key words: serotonin transporter gene, stressful life events, facial emotion recognition, gender, gene-environment interaction.

Mood disorders are associated with impairments and biases in the processing of emotional and social stimuli. These impairments may underlie reduced affect regulation and social interaction, and therefore contribute to the development and maintenance of such disorders (Leppanen, 2006). Biases in the perception of emotional face expressions constitute a measurement with face validity since these biases influence social and emotional adaptation. Facial stimuli have also been used in many neuro-imaging studies since they reliably engage the amygdala, a brain region involved in emotional arousal and vigilance (Hariri et al., 2000).

Research has demonstrated that compared with healthy controls, depressed individuals show a bias in the processing of negative emotions in facial recognition tasks (Bouhuys et al., 1999; Gollan et al., 2008; Gur et al., 1992; Mikhailova et al., 1996; Surguladze et al., 2004). Facial emotion recognition bias has also been observed in the remitted state of depression (Bhagwagar et al., 2004; Hayward et al., 2005; Joormann and Gotlib, 2007; Merens et al., 2008b). Furthermore, experimental manipulations of serotonin affect the recognition of emotional face expressions, both in healthy volunteers (Harmer et al., 2003a,b, 2004; Hayward et al., 2005) and in remitted depressed patients (Merens et al., 2008a).

Hasler et al. (2004) have suggested that biased processing of emotional stimuli is a plausible endophenotype for major depression. With respect to the endophenotype criteria, there is evidence for specificity for depression, state-independence and familial association (Hasler et al., 2004). Neurobiological research has examined the association between emotional cognition and a polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR) (Canli and Lesch, 2007). The serotonin transporter (5-HTT) is known to be a key regulator of serotonergic neurotransmission (Heils et al., 1996; Lesch et al., 1994). 5-HTTLPR has two variants: short allele (*s*) carriers have reduced transcriptional efficiency of serotonin compared with individuals with two copies of the long allele (*ll*) (Heils et al., 1996). More recently, an A/G single nucleotide polymorphism (rs25531) within 5-HTTLPR has been described (Wendland et al., 2006). The G allele within the *l* variant (L_G) shows lower 5-HTT mRNA expression, similar to the *s* allele (Hu et al., 2006).

Hariri and colleagues (2002) assessed neural activation during perceptual processing of fearful and angry human facial expressions, and found that *s* allele carriers exhibited greater amygdala activity, than *ll* homozygotes (Hariri et al., 2002). This finding has been replicated with larger samples (Hariri et al., 2005), and by independent groups (Canli et al., 2008; Munafo et al., 2008; Pezawas et al., 2005). *S* homozygotes also show greater activation within other brain regions (fusiform gyrus, ventral, lateral prefrontal cortex) in response to fearful faces than *l* carriers (Surguladze et al., 2008).

These studies imply that the short variant of the serotonin transporter gene leads to enhanced reactivity to negative stimuli, which may indicate a genetic-susceptibility mechanism for depression (Pezawas et al., 2005). Parallel behavioral research has shown similar results. In a mixed inpatient psychiatric sample (n=27), *s* carriers showed a stronger attentional bias for anxious word stimuli than participants with two long alleles (Beevers et al., 2007). In a healthy sample (n=144), *s* homozygotes displayed greater difficulty disengaging attention from sad, happy and fearful facial expressions than *ll* homozygotes (Beevers et al., 2009). In another study, healthy individuals homozygous for the *l* allele (n=97) were found to selectively attend to

*Corresponding author. Tel: +31-71-527-3991; fax: +31-71-527-4678.
E-mail address: nantypa@fsw.leidenuniv.nl (N. Antypa).

Abbreviations: ANOVA, analysis of variance; CEA, childhood emotional abuse; HADS, hospital anxiety and depression scale; RLE, recent life events; 5-HTTLPR, serotonin-transporter-linked promoter region.

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positive affective pictures and avoid negative ones, whereas this pattern was absent among *s* allele carriers (Fox et al., 2009). Among an adolescent sample (n=112), bias for angry faces increased progressively according to 5-HTTLPR status in a dot-probe task, with the *ss* group showing the highest levels of bias. For happy faces, the reverse pattern was found (Perez-Edgar et al., 2009). Children with familial history of depression had greater attentional avoidance of sad faces; a bias which was stronger among children carrying the *s* allele (Gibb et al., 2009). The effects of acute tryptophan depletion on the processing of facial emotional expressions also vary as a function of 5-HTTLPR genotype: depletion impaired the recognition of fear in *s* carriers, but not in *l* homozygotes (Marsh et al., 2006).

In contrast, some studies have shown effects inconsistent with those mentioned above. In an eye-tracking paradigm, healthy *s* allele homozygotes displayed an attentional bias to positive images compared to the other genotype groups (n=45) (Beevers et al., 2010). In another study, among a sample of individuals with familial risk of depression, no effects of allelic variation in the 5HTTLPR were found on measures of facial emotional processing (Mannie et al., 2007).

Most studies so far have used tasks displaying facial expressions of various emotional intensities for only brief periods of time. In daily life however, facial expressions are not seen as static and brief, but as varying in intensity. The ease with which people detect subtle, rather than full-blown, emotional expressions may be related to depression vulnerability. From this perspective, Joormann and Gotlib (2006) introduced a task using real faces that change progressively from a neutral expression to a full emotional expression. They found that depressed participants, in comparison with social anxiety disorder patients or healthy controls, required a greater intensity of happy emotion to correctly identify it as happy. Additionally, social anxiety disorder participants correctly identified angry expressions at a lower intensity than did depressed participants or healthy controls.

The purpose of the present study was to further examine the relationship between the 5-HTTLPR and identification of emotional facial expressions. We used the task introduced by Joormann and Gotlib (2006), which allows for evaluations of facial emotions at varying intensities, as this more closely reflects perceptual communication in real life interpersonal situations. Secondly, previous research involving the 5-HTTLPR and emotional information processing has not explored gender effects (except Beevers et al., 2010, who report no effects). Research has shown differential performance between males and females in facial emotion recognition paradigms. For example, 5-HT depletion impaired the recognition of facial expressions of fear in healthy female volunteers, but not in males (Harmer et al., 2003b). Neuroimaging studies have also reported gender differences in neural responses to facial emotion recognition (Kesler-West et al., 2001; Williams et al., 2005). Men performed worse than women on a task measuring the perception of facial emotional expressions (Montagne et al., 2005). Further, in a study examining gene-environment interaction for depression, males and females showed opposite responses to environmental stressors: *s* allele homozygous females were affected by traumatic conflicts and were more prone to develop depressive symptoms, but *s* allele homozygous males were protected from depression (Sjoberg et al., 2006).

There is mounting research examining gene-environment interactions on depression outcomes, as well as on intermediate phenotypes that are indicators of stress sensitivity (stress hormones, amygdala reactivity) (Caspi et al., 2010). We aimed to examine such a gene-environment interaction on facial emotion perception. We focused on childhood emotional abuse as an environmental stressor, since this type of abuse has been uniquely linked with depression outcomes (Brown and Harris, 2008; Gibb, 2002; Gibb et al., 2001). Emotional problems in adolescents have also been associated with biased recognition of angry and sad faces (Leist and Dadds, 2009). We also examined the influence of recent life events.

We investigated the association between facial emotion identification and 5-HTTLPR, gene-environment interactions and gender differences in these associations. We hypothesized that the *ss* allele group would identify negative emotions (sad, anger, fear) earlier in the emotion intensity sequence than participants in the *sl* and *ll* groups, and that this pattern would be more dominant among females. Furthermore, we hypothesized that life events would moderate this relationship: the *ss* genotype group would identify negative emotions earlier than other genotypes when having had adverse life experiences (early or late). Finally, we aimed to explore the effects of recent life events upon the relationship between 5-HTTLPR and facial emotion perception, to determine whether they (a) have an additive or an inoculation effect upon this relationship among participants with prior childhood emotional abuse, and/or (b) act as a sole moderator of this relationship among participants without a history of childhood emotional abuse.

EXPERIMENTAL PROCEDURES

Participants and procedure

Two hundred and fifty university students of European ancestry were recruited at various sites at Leiden University through advertisements. Participants were included only if both their parents were European. Age range was 18–45 years. On arrival to the laboratory, participants provided written informed consent and completed a number of questionnaires (data reported elsewhere: Antypa and Van der Does, 2010). The participants subsequently provided saliva samples, and finally performed the facial morphing computer task. The procedure lasted about 45 min, and participants received a small monetary reward or course credits for their participation. The research was approved by the Ethics Committee of the Leiden University Medical Center in The Netherlands.

Assessments

Genetic assessment. DNA was obtained using the Oragene Self-Collection Kit—DISC format (DNA Genotek Inc., Ottawa, ON, Canada). 200 μl of saliva was collected in lysis buffer (100 mM NaCl, 10 mM EDTA, 10 mM Tris pH 8, 0.1 mg/ml

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proteinase K and 0.5% w/v SDS) until further processing. Genomic DNA was isolated from the samples using the Chemagic kit on a Chemagen Module I workstation (Chemagen Biopolymer-Technologie AG, Baesweiler, Germany). DNA concentrations were quantified by OD260 measurement and by agarose gel electrophoresis. The average yield was approximately 4 μg of genomic DNA per sample.

Polymerase chain reaction (PCR) amplification. The region of interest from the 5-HTT gene was amplified by triplex PCR using the following primers: a FAM-labeled primer HTTLPR-FW-FAM 5′-TCCTCCGCTTTGGCGCCTCTTCC-3′, and a reverse primer HTTLPR-RV 5′-TGGGGGTTGCAGGGGAGATCCTG-3′. Typical PCR reactions contained between 10 and 100 ng genomic DNA template, and 10 pmol of forward and reverse primer. PCR was carried out in the presence of 5% DMSO with 0.5 U of BioThermAB polymerase (GeneCraft, Munster, Germany) in a total volume of 30 μl using the following cycling conditions: initial denaturation step of 5 min at 95 °C, followed by 40 cycles of 30 s 96 °C, 30 s 61 °C, 60 s 72 °C and a final extension step of 10 min 72 °C. After PCR, 5 μl of the sample was subjected to restriction digestion with the enzyme HpaII in a total volume of 20 μl. Restriction was incubated for 3 h at 37 °C.

Analysis of PCR products. One μl of PCR product before and after restriction digestion was mixed with LIZ-500 size standard and formamide and run in two separate lanes on a AB 3100 genetic analyser set up for genotyping with 50 cm capillaries. Results were analysed using Genescan software version 3.7 (Applied Biosystems) and alleles were scored visually according to the following scheme: Uncut: S: 469 bp, L: 512 bp. Cut: Sg: 402+67 bp, Lg: 402+110 bp.

The facial morphing expression task

Faces from Ekman and Friesen's (1976) series of facial affect were morphed from a neutral expression to a fully emotive expression in 2% intervals; yielding 50 unique expressions (see Fig. A). One male and one female face expressing sadness, anger, happiness, and fear were used. For practice trials, the faces of the same actors expressing disgust were used. Using E-Prime software Version 2.0 we presented each face for 500 ms, which created the impression of an animated clip of the development of an emotional facial expression. The black-and-white faces were 18.5×13 cm in size, and were presented in the middle of the screen with a black background.

For each sequence, participants were instructed to watch the face change from neutral to an emotion, and to press the space bar as soon as they saw an emotion they could identify. After pressing the space bar, the sequence stopped, and participants were presented with a rating screen asking them to identify the emotion as happy, sad, fearful, or angry by pressing the button 1, 2, 3 or 4 respectively. The intensity of the emotion being expressed on the face when the participants pressed the space bar was recorded. In this way, both data on accuracy (final judgments) and level of emotion intensity (required before accurate judgment) were collected.

After responding to two practice trials to familiarize themselves with the procedure and the stimuli, participants were shown 40 morphed sequences in random order: each emotion was presented five times with a male face and five times with a female face. To reduce the perfect correlation between expression intensity and time (and to increase task difficulty), faces of the same intensity were repeated in some sequences. For example, the 14% intensity of an emotional face was repeated twice, or three times in some sequences, whereas in other sequences 14% was immediately followed by a 16% intensity of the emotional face. Thus, there were 50 unique emotional faces in each sequence, but 70 presentations possible (if the subject did not respond till the end of the sequence). Total task duration was about 20 min.

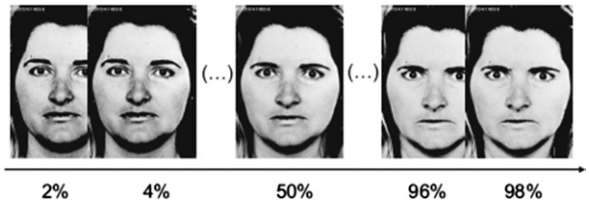

Fig. A. Example of emotional sequence of anger used in the facial morphing task.

Environmental adversity. Childhood emotional abuse (CEA) was measured using the Childhood trauma tuestionnaire (CTQ), a valid screening measure for maltreatment histories in both clinical and non-referred groups (Bernstein and Fink, 1998).

The instrument measures abuse during childhood and adolescence. A validated Dutch translation is available. An example of an item from the emotional abuse subscale is: "I thought that my parents wished I had never been born."

Recent life events (RLE) were measured using the list of threatening experiences (Brugha et al., 1985). This is a list of 12 commonly reported life events known to have moderate or marked long-term threat. Participants had to respond whether they had experienced any of these events during the past 6 months, with a "yes" or "no" answer.

Depression. We used the major depression questionnaire (MDQ) to assess the presence of current and past depression, in order to have an estimate of depression diagnosis in our sample. The measure covers all DSM-IV diagnostic criteria for current and past major depression. Consistency of this questionnaire with diagnoses based on SCID interviews has been examined in a sample of 39 individuals: sensitivity=100%, specificity=75%, positive predictive value =79%; negative predictive value =100%; overall kappa=0.75 (Williams et al., 2008).

In order to control for current mood state, we measured current symptoms of anxiety and depression using the hospital anxiety and depression scale (HADS; Zigmond and Snaith, 1983); Dutch translation: (Spinhoven et al., 1997). The HADS is a 14 item self-report scale developed to assess the presence of current anxiety or depressive states.

Statistical analyses

The intensity scores of correctly identified emotions were the primary outcome measures. Accuracy rates were also analyzed to avoid confounding with group differences in the response criterion used to identify and label an emotion. Accuracy data and intensity score (at the time of the key press) were analyzed by a repeated measures analysis of variance (ANOVA) with emotion (happy, sad, angry, fearful) as a within-subjects factor, and triallelic genotype, environmental contributor, and gender as between-subject factors. Participants were allocated into high and low abuse groups based on CEA scores (median split: above score six coded as one, below or equal to score six coded as 0; scores range from 5 to 25). Another division was created based on RLE outcome: 0 or 1 recent life event vs. >1 life event. Partial eta squared (η_p^2) is reported as an estimate of effect size. If significant interactions were detected, separate ANCOVAs were conducted in which accuracy rates of each emotion were added as a covariate. Main effects and interaction effects were followed by one-way ANOVA's including post hoc tests (Tukey HSD). Only significant post hoc tests are reported. Finally, we checked if the results remained the same after controlling for current symptoms of depression and anxiety (HADS).

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Table 1. Participant characteristics by 5-HTTLPR genotype (n=245) (means±standard deviations)

Genotype	S′S′ (n=54)	S′L′ (n=114)	L′L′ (n=77)	P-value
Age	23.2±6.2	22.5±4.2	22.0±4.5	0.34
Gender: females n(%)	42 (77.8%)	83 (72.8%)	61 (79.2%)	0.56
MDD diagnoses				
No lifetime MDD	53.7%	61.4%	63.6%	0.68
Past MDD	40.7%	31.6%	28.6%	
Current MDD	5.6%	7.0%	7.8%	
Current symptoms				
HADS total	9.48±5.9	8.78±5.9	9.1±5.3	0.76
HADS depression	2.96±3.1	2.51±2.8	2.83±3.0	0.58
HADS anxiety	6.52±3.4	6.27±3.7	6.22±3.0	0.87
Life Events				
Childhood emotional abuse	8.28±3.2	7.45±3.4	6.99±2.9	0.08
Recent life events	1.20±1.3	1.20±1.2	1.16±1.2	0.96

HADS, hospital anxiety depression scale, MDD, major depressive disorder.

RESULTS

Genotype analysis

Genotype analysis failed for two participants, yielding 248 samples for association analysis. Participants were divided on the basis of the triallelic classification. Lg alleles were collapsed with *s* variants according to evidence of similar functionality (Wendland et al., 2006), forming three genotype groups: S′S′ (n=57); L′S′ (n=114); L′L′ (n=77)[1]. Genotype frequencies in the present sample were as follows: SS: 16.9%, SLg: 5.2%, LgLg, 0.8%, LLg: 8.9%, SL: 37.1%, LL: 31.1%. Genotype frequencies were consistent with Hardy–Weinberg Equilibrium, $\chi^2(1)=1.55$, $P=0.21$.

Data screening

Prior to analysis all variables were examined for accuracy of data entry, missing values and normal distribution. Performance data were not available for three participants, due to computer failure, yielding a sample of 245 participants for analysis. The emotion intensity scores were normally distributed with no outliers. The emotion accuracy scores for the sad and happy faces were skewed. Arcsine transformations of the proportion accuracy scores improved skewness and kurtosis. Tables and figures report untransformed values.

Participant characteristics

Table 1 displays means and standard deviations of the participant characteristics. Among genotype groups, no differences were found with respect to age [$F(2,242)=1.08$, $P=0.34$], gender [$\chi^2(2)=1.17$, $P=0.56$] and depression diagnosis [$\chi^2(4)=2.3$, $P=0.68$]. There were also no differences between genotypes in current levels of anxiety (HADS anxiety subscale) [$F(2,242)=0.13$, $P=0.87$], depression (HADS depression subscale) [$F(2,242)=0.54$, $P=0.58$], and total symptomatology (HADS total) [$F(2,242)=0.28$, $P=0.76$]. There were no significant differences among the genotype groups on recent life events [$F(2,242)=0.4$, $P=0.96$]. The S′S′ homozygotes reported the highest rates of childhood emotional abuse, followed by the L′S′ genotype and the L′L′ homozygotes, but these differences reached a trend [$F(2,242)=2.62$, $P=0.08$].

Facial morphing task

Genetic interaction with childhood emotional abuse. Accuracy. The primary outcome measure was the intensity score at which participants correctly recognized each emotion. We analyzed accuracy to ensure that any group differences in required intensity are not due to response bias. If one of the groups was characterized by a general response bias (e.g. the participants are concerned about making errors and wait to respond until they are absolutely sure that they have correctly identified the face), we would have expected this group to need more intense expressions to respond and to be more accurate than the other participants in their expression identification. Overall identification accuracy was high. Repeated measures ANOVA showed a significant main effect of emotion type [$F(3,699)=143.9$, $P<0.001$] with the following differences between accurate emotion identification (mean percentage±standard deviation (SD)): happy: 97.8±0.4, sad: 88.5±0.1, anger: 70.5±1.3, fear: 83.3±1.1. No main effect of genotype group and no significant interactions between genotype, CEA and gender were found on the correct identification of any of the emotions (all P's>0.10).

Intensity. Analyses were restricted to the intensity of correctly identified emotions. A repeated measures ANOVA showed the following results. A significant emotion×genotype×CEA interaction was found [$F(5.6,655.1)=2.19$, $P=0.046$] ($\eta_p^2=0.018$)[2]. The emotion×genotype×gender interaction was marginally significant [$F(5.6,699)=2.12$, $P=0.05$] ($\eta_p^2=0.018$), but yielded a significant quadratic contrast [$F(2,233)=5.82$, $P=0.003$] ($\eta_p^2=0.048$). The four-way emotion×genotype×CEA×gender interaction was not significant ($P=0.19$). The following two-way interactions were also significant: emotion×genotype [$F(5.6,655.1)=2.63$, $P=0.02$], genotype×gender [$F(2,233)=5.92$, $P=$

[1] These group abbreviations will be used when referring to the data of the present study in order to indicate the triallelic classification.

[2] 0.01 = small effect, 0.06 = moderate effect, 0.14 = large effect.

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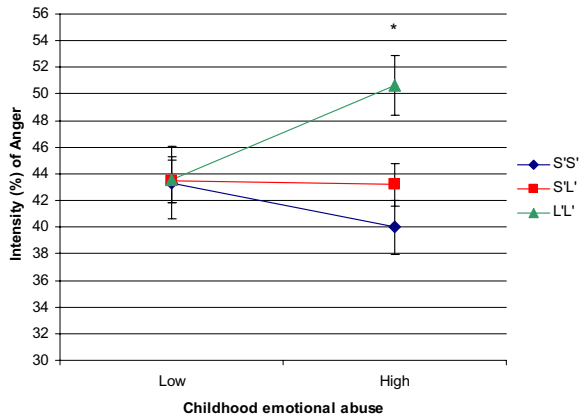


* $p < 0.05$

Fig. 1. Childhood emotional abuse moderates the relationship between the 5-HTTLPR genotype and recognition of anger. * $P<0.05$. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

0.003], and emotion×gender [$P<0.001$]. Significant main effects of emotion type [$F(2.8,655.1)=217.0$, $P=<0.001$] and gender [$F(1,233)=15.97$, $P<0.001$] were also found.

First, we decomposed the significant emotion×genotype×CEA interaction. Separate ANCOVAs for each emotion, with accuracy of the emotion as a covariate, showed a significant G×CEA interaction only on angry faces [$F(2,238)=3.31$, $P=0.04$] ($\eta_p^2=0.027$) (Fig. 1). A trend was found for sad faces: [$F(2,238)=2.56$, $P=0.08$] ($\eta_p^2=0.021$), and a non-significant interaction for fearful and happy faces ($P>.10$). Examining the significant interaction on anger further, we found no differences between genotype groups when participants had experienced low CEA. In the high CEA group, there was a significant difference among genotype groups [$F(2,111)=5.79$, $P=0.004$]. Post hoc tests (Tukey HSD) showed that the S′S′ and the S′L′ genotype groups recognized angry faces significantly earlier compared to the L′L′ group (P′s<0.05).

Furthermore, the ANCOVA yielded a significant main effect of genotype on angry faces [$F(2,238)=3.5$, $P=0.03$] ($\eta_p^2=0.029$). Means±standard error (SE): *S′S′*: 41.7±1.7, *S′L′*: 43.4±1.1, *L′L′*: 47.1±1.4. Post hoc tests showed that the the S′S′ group recognized anger at a lower intensity level compared to the L′L′ genotype but this difference was significant at only at trend level ($P=0.07$).

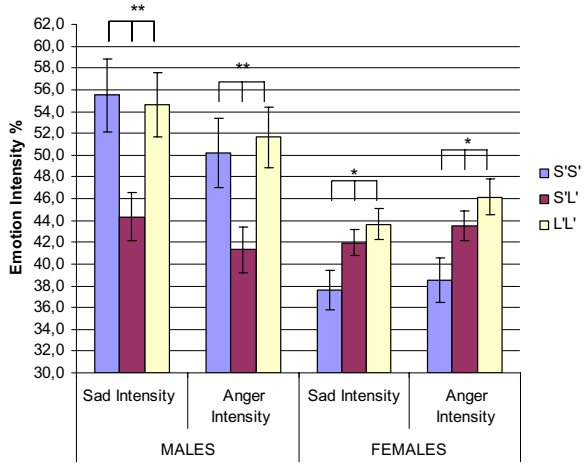
Next, we decomposed the emotion×genotype×gender interaction. Separate ANCOVAs for each emotion, with accuracy rate of the emotion as a covariate, showed a significant genotype×gender interaction on the perception of sad faces [$F(2,238)=6.23$, $P=0.002$] ($\eta_p^2=0.05$) and angry faces [$F(2,238)=3.35$, $P=0.04$] ($\eta_p^2=0.027$). A main effect of genotype was found on sad faces [$F(2,238)=3.49$, $P=0.03$] ($\eta_p^2=0.028$). A linear pattern was shown in which the S′S′ genotype recognized sadness earlier than the other genotypes but post hoc tests were not significant ($P>0.10$).

We investigated the significant interaction further for each gender. Analysis with only the male sample ($n=59$; S′S′=12, S′L′=31, L′L′=16), showed significant main effects of genotype on sad intensity [$F(2,53)=5.73$, $P=0.006$] ($\eta_p^2=0.178$) and on anger intensity [$F(2,53)=5.39$, $P=0.007$] ($\eta_p^2=0.169$) (Fig. 2). Post hoc tests showed that the S′L′ genotype recognized sadness significantly earlier compared to the other two genotype groups ($P<0.05$); the S′L′ group recognized anger earlier than L′L′ ($P=0.02$) and than S′S′ (but at trend level of significance $P=0.08$). Analysis with the female sample ($n=186$; S′S′=42, S′L′=83, L′L′=61) showed a main effect of genotype also on sad intensity [$F(2,180)=3.50$, $P=0.03$] ($\eta_p^2=0.037$) and on anger intensity [$F(2,180)=4.17$, $P=0.02$] ($\eta_p^2=0.044$) (Fig. 2). Post hoc tests showed that the S′S′ group recognized anger ($P=0.04$) and sadness ($P=0.058$) at lower intensities than the L′L′ genotype. Repeated Measures ANOVA with the HADS as a covariate showed the same results as the original analyses.

Genetic interaction with recent life events. Accuracy. A repeated measures ANOVA showed no main effect of genotype and no significant interactions between genotype, RLE and gender on the correct identification of any of the emotion faces (all P′s>0.10). Participants were more correct in identifying emotions if they had experienced >1 recent life events, compared to those with ≤1 recent life events [$F(1,233)=6.22$, $P=0.01$].

Intensity. A repeated measures ANOVA showed a significant four-way emotion×genotype×RLE×gender interaction [$F(5.7,666.4)=3.76$, $P=0.001$] ($\eta_p^2=0.031$). The three-way emotion×genotype×gender interaction was also significant [$F(5.7,666.4)=2.19$, $P=0.045$] ($\eta_p^2=0.018$). The emotion×genotype×RLE interaction was not significant [$F(5.7,666.4)=1.33$, $P=0.25$]. The emotion×gender interaction was also significant ($P<0.001$) and a main effect of RLE was found ($P<0.05$).

First, we decomposed the four-way interaction. Separate ANCOVAs for each emotion, with accuracy rate of the emotion as a covariate, yielded the following results. On the recognition of sad faces, a significant genotype×



* $p < 0.05$, ** $p < 0.01$

Fig. 2. Recognition pattern of sadness and anger across genotype groups, in males and females. * $P<0.05$, ** $P<0.01$. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

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gender interaction [$F(2,233)=3.93$, $P=0.02$] ($\eta_p^2=0.033$) and a marginally significant genotype×RLE interaction [$F(2,233)=2.94$, $P=0.055$] ($\eta_p^2=0.025$) were found. On the recognition of angry faces, significant genotype×RLE×gender [$F(2,233)=3.10$, $P=0.047$] ($\eta_p^2=0.026$) and genotype×gender [$F(2,233)=4.41$, $P=0.01$] ($\eta_p^2=0.036$) interactions were found. No significant interactions or main effects of genotype were found for the happy and fearful emotions.

We further decomposed the interactions involving RLE on sad and angry emotion perception (the genotype×gender interactions are the same as with the first analysis—see Fig. 2). For the recognition of sadness, we decomposed the two-way G×RLE interaction: we found no significant differences between genotype groups when they had experienced ≤1 recent life events; the genotype groups differed significantly when they had experienced >1 recent life event in the intensities of recognizing sadness [$F(2,79)=3.86$, $P=0.03$]. Post hoc tests showed that the S′S′ group recognized sadness significantly earlier compared to the S′L′ and L′L′ genotype groups (P′s< 0.05) (Fig. 3A).

For the recognition of anger, we decomposed the 3-way genotype×RLE×gender interaction by analyzing each gender separately. Analysis with males only ($n=59$) showed no significant G×RLE interaction and only a main effect of genotype on anger ($P=0.04$) ($\eta_p^2=0.111$) and on sadness ($P=0.058$) ($\eta_p^2=0.102$) (as in previous analysis: Fig. 2). Analysis with females only ($n=186$) showed a significant G×RLE interaction on anger [$F(2,180)=4.62$, $P=0.01$] ($\eta_p^2=0.049$), and a main effect of genotype on anger ($P=0.007$) ($\eta_p^2=0.054$) and sadness ($P=0.03$) ($\eta_p^2=0.037$) (as in Fig. 2). Examining the G×RLE interaction on anger further, we found no differences between genotypes in participants with ≤1 recent life events; the genotype groups differed significantly in their recognition of anger when they had experienced >1 recent life event [$F(2,62)=8.26$, $P=0.001$]. Post hoc tests showed that the S′S′ genotype recognized the emotions of anger at significantly lower intensities compared to the S′L and L′L′ genotypes (all P′s<0.05) (Fig. 3B). A Repeated measures ANOVA with HADS as a covariate showed the same results as the initial analysis.

Genetic interaction with recent life events—with and without the influence of childhood emotional abuse. Since we found an interaction between genotype and RLE, we were interested to investigate whether this interaction was dependent on the presence or absence of prior CEA. We examined participants with and without CEA as two separate groups; however, we could not examine the genotype×RLE×gender interactions since the male sample size was too small. Consequently we investigated genotype×RLE interactions only in females[3].

In the female group without history of childhood emotional abuse ($n=95$) a repeated measures ANOVA showed a significant G×RLE interaction [$F(2,89)=4.21$, $P=0.02$]

[3] Analyses with all participants yielded same results for both groups (with/without history of CEA).

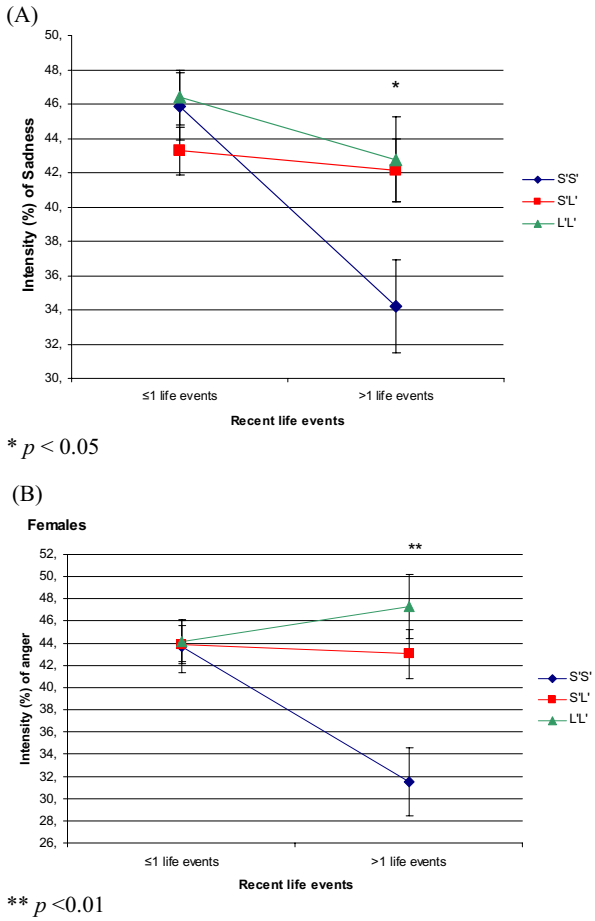

Fig. 3. Recent life events (RLE) as a moderator of 5-HTTLPR on emotion recognition. (A) Recognition of sadness across genotype groups in the whole sample (G×RLE interaction), * $P<0.05$. (B) Recognition of anger across genotype groups in *females* (G×RLE×gender interaction), ** $P<0.01$. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

($\eta_p^2=0.086$) and a significant main effect of genotype [$F(2,89)=4.14$, $P=0.02$] ($\eta_p^2=0.085$). Investigating these effects for each emotion, we found a significant G×RLE interaction on sadness [$F(2,89)=3.12$, $P=0.049$] ($\eta_p^2=0.066$), anger [$F(2,89)=3.71$, $P=0.03$] ($\eta_p^2=0.077$) and fear intensities [$F(2,89)=3.93$, $P=0.02$] ($\eta_p^2=0.081$). A significant main effect of genotype was found on sadness [$F(2,89)=3.12$, $P=0.049$] ($\eta_p^2=0.066$), anger [$F(2,89)=3.37$, $P=0.04$] ($\eta_p^2=0.070$) and fear [$F(2,89)=3.37$, $P=0.04$] ($\eta_p^2=0.070$); post hoc tests showed no significant differences among the genotype groups.

Examining the G×RLE interaction further, we found no significant differences among genotypes when participants had experienced ≤1 life events. When females had experienced >1 recent life events, there were significant differences between genotype groups in the recognition of sadness [$F(2,24)=4.84$, $P=0.02$], anger [$F(2,24)=7.87$, $P=0.002$] and fear intensities [$F(2,24)=5.33$, $P=0.01$] (Fig. 4A). Post hoc tests showed that the S′S′ genotype recognized sad faces at a lower intensity than the S′L′

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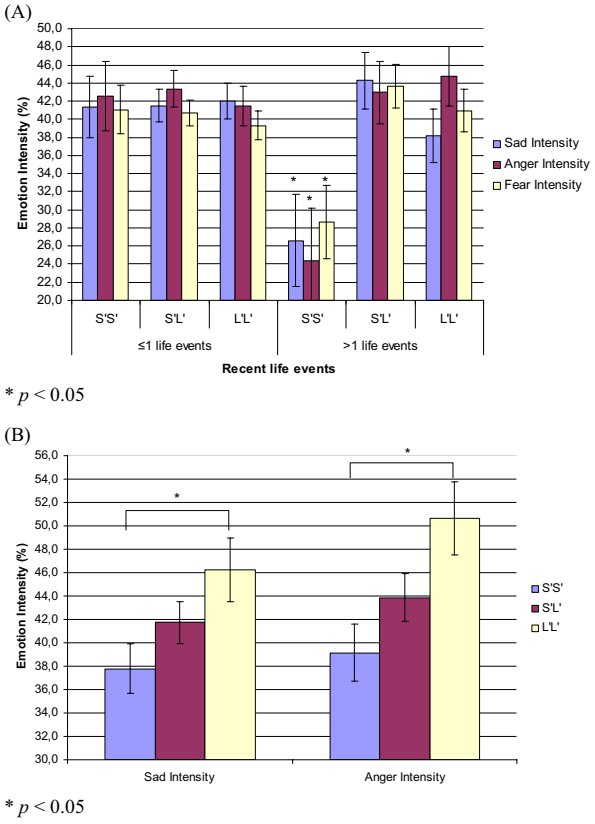

Fig. 4. (A) Females without childhood emotional abuse history: interaction between genotype and recent life events on sadness, anger and fear recognition, * $P<0.05$. (B) Females with childhood emotional abuse history: recognition of sadness and anger across genotype groups, * $P<0.05$. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

genotype ($P=0.01$) but the difference with the L′L′ genotype fell short of significance ($P=0.12$). The S′S′ genotype recognized anger and fearful expressions at significantly lower intensities compared to the other genotype groups (all P's<0.05).

In females with emotional abuse ($n=91$) a repeated measures ANOVA showed no significant emotion×genotype×RLE interaction as well as no genotype×RLE interaction (all P's>0.10). The genotype×emotion interaction was significant [$F(5.2,219.5)=2.76$, $P=0.02$] ($\eta_p^2=0.061$). A one-way ANOVA showed significant differences between genotypes on the recognition of anger [$F(2,88)=3.51$, $P=0.03$] and sadness [$F(2,88)=3.23$, $P=0.04$] (Fig. 4B). Post hoc tests showed that the S′S′ genotype recognized sad and anger expressions earlier in the intensity sequence than the L′L′ genotype group (P's<0.05).

Effects of depression diagnostic status on emotion intensity. We examined whether participants with current, past or no depression diagnosis recognized emotions at different intensities. Although a repeated measures ANOVA with diagnostic group (current MDD, past MDD, no MDD) as between-subject factor and emotion (happy, sad, anger, fear) as within subject factor showed a non-significant group×emotion interaction [$F(5.4,663.4)=1.35$, $P=0.24$] and no main effect of group ($P=0.55$), we probed into potential effects of diagnostic status by investigating group differences on the intensity of each emotion in separate ANCOVAs. Using accuracy rate of the emotion as a covariate, currently depressed participants required a more intense emotional expression to identify happy faces [$F(2,243)=3.36$, $P=0.04$] ($\eta_p^2=0.027$). No significant differences were found among diagnostic groups on recognition of the other emotions (all P's>0.33). Furthermore, we examined whether the above mentioned effects involving genotype, life events and gender could be modulated by depression status. Repeated measures ANOVA showed no significant two-, three- or four-way interactions of diagnostic status (lifetime depression vs. never-depressed) with genotype, gender and life events (CEA or RLE) (all P's>0.05). The significant interactions reported earlier between genotype, life events and gender remained statistically significant when adding diagnostic status in the analyses.

DISCUSSION

The present study found a main effect of the 5-HTTLPR genotype on emotion recognition, as well as a gene-environment interaction, with childhood emotional abuse and recent life events as contributors. Some of these effects were modulated by gender.

Firstly, we found that S′ homozygotes who had experienced high CEA recognized anger earlier than the L′L′ genotype. Secondly, we found that genotype affects recognition of negative facial emotions. In the whole sample, the S′S′ allele group showed earlier recognition of anger compared to the other genotype groups. This finding is in accordance with previous research, which showed an attentional bias for negative emotional stimuli of the low-expressing genotype (Beevers et al., 2007; Perez-Edgar et al., 2009). Furthermore, we found that this effect is different for males and females. Specifically, female S′ homozygotes recognized facial emotions of sadness and anger quicker than the other genotype groups. Among males however, the heterozygous genotype was quicker to recognize these same emotional expressions than the other genotypes, and this was a large effect.

Finally, we found that the S′S′ genotype group recognized sad and angry facial expressions earlier if they had recently experienced negative life events. Among other genotypes, recent adversity did not influence the identification of emotions. This effect (together with early fear perception) was mainly evident among female S′ homozygous participants who had not experienced childhood emotional abuse, but had experienced more than one recent life event. This group recognized all negative facial emotions quicker than the other genotype groups, yielding moderate to large effect sizes. On the contrary, recent life events did not have an additive effect among females who had experienced childhood emotional abuse. Among early abused females, the S′ homozygous genotypes recognized sad and angry facial emotions at lower intensities

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than the other genotypes, regardless of recent life event history.

In most analyses, the S′ homozygous genotypes recognized sad and angry, but not fearful, emotions earlier in the emotion sequence than the other genotype groups. Careful inspection of our data showed that, compared with the other emotions, fear recognition had the lowest variance in intensity (Variance: fear: 70, anger: 155, sadness: 144). The lack of effect on fear recognition might be attributed to the lower variance in responses. Other studies have excluded the fearful emotion from the task (LeMoult et al., 2009) or have used it only to increase task difficulty (Joormann and Gotlib, 2006). Other paradigms may be more sensitive in detecting effects on fear recognition; for example, genetic variation in the 5-HTTLPR is associated with increased amygdala response to fearful faces (e.g. Hariri et al., 2002, 2005).

Diagnostic status had an effect on the perception of happy faces, which is consistent with previous literature (Joormann and Gotlib, 2006). No differences were found among diagnostic groups on the intensity levels required for identifying sad, angry and fearful faces, indicating that the current findings have not been affected by diagnostic status. Including current symptoms as a covariate also did not change our results.

Our findings indicate that the low-expressing genotype influences facial emotion perception, primarily among females. Most prior studies have not examined gender by genotype interactions on emotion recognition, probably due to insufficient power. There is some evidence of modulation by gender in studies examining gene-environment interactions to predict depression. In a young sample (16–19 years-old; $n=200$), homozygous *s* allele females had higher depressive symptom scores when exposed to environmental stress, whereas homozygous *s* allele males seemed to be protected from the effects of stress (Sjoberg et al., 2006). It was also found that males and females responded to different environmental factors: females were more affected by traumatic conflicts in the family, whereas males were more affected by separated family or living outside the home. Our results are also in accordance with other previous research showing gene effects (Brummett et al., 2008b) and G×E effects interacting with gender on depression-related phenotypes (Aslund et al., 2009; Brummett et al., 2008a; Eley et al., 2004).

The finding that heterozygous males recognized anger and sadness earlier than both homozygous genotypes was unexpected. Females did show the expected main effect of genotype. We found one animal study that found a similar pattern. Heterozygous male rhesus macaques had a higher adrenocorticotropic hormone (ACTH) response to separation than both homozygous groups, whereas females carrying the *s* allele had increased stress-induced release of ACTH and decreased cortisol levels after separation (Barr et al., 2004).

Our findings add to the growing body of research showing that the effects of the 5-HTTLPR may be different between men and women. This is not surprising since the serotonin system is known to act differently in each gender (Biver et al., 1996; Nishizawa et al., 1997; Williams et al., 2003), and the prevalence of depression is also higher among females (Kessler et al., 1993). Dysregulation in the emotion information processing circuitry is evident in studies examining gender and depression. In a sample of young adults, women with depression made more errors when identifying fearful and sad faces than did non-depressed women or men with depression (Wright et al., 2009). Since males are more likely to develop other kind of psychopathologies (aggression-related disorders for example), other kinds of processing biases or other genes may come into play when examining gene and gene-environment interactions in males.

The mechanisms that underlie the susceptibility of the short allele genotype to early detection of negative emotional information are yet unknown. There is growing neurobiological evidence however, that the *s* allele shows heightened amygdala activation to emotional stimuli relative to neutral stimuli, a key process reflecting physiological arousal to environmental threat (Munafo et al., 2008). Prior research investigating the effects of the 5-HTTLPR has shown that resting activation in the hippocampus and amygdala increased with increasing life stress for *s* carriers, but decreased with increasing life stress in the *ll* group (Canli et al., 2006). It appears that the short allele is related to poorer prefrontal cortical control over automatic activation of the amygdala, which may result in increased vigilance for environmental emotional stimuli, and for negative emotional information in particular. Further, life stress may induce more vigilance for negative environmental stimuli in some genotype groups (*ss*), whereas it may reveal resilience to such negative information in others (*ll*).

The issue of the homozygous versus heterozygous effect of the *s* allele remains unclear. We found in most of our analyses that the S′S′ homozygous genotype consistently recognized negative emotional faces earlier than the other genotypes. The heterozygous genotype performed comparably to the L′L′ genotype in some cases, and in between the two homozygous genotypes in other. Differences between carriers of one or two copies of the *s* allele have been found before, in various studies involving neural responses to emotional stimuli (Surguladze et al., 2008), selective attention for emotional stimuli (Beevers et al., 2009), stress response (Gotlib et al., 2008), and in gene-environment interactions with depression as an outcome (Caspi et al., 2003). Subsequently, future research should continue to examine differential effects of the three genotypes separately.

There are several limitations to be considered in the present research. Since participants were not forced to make a judgment at a specific point in time, groups may differ in the perception or detection of the onset of an emotion or in the response criterion (Joormann and Gotlib, 2006). However, if participants in different groups were adopting more conservative response criteria, we would expect differences in accuracy errors too. This was not the case. We based our analyses on ratings of correct responses, and used individual differences in accuracy rates as a covariate (as previously suggested by Joormann and

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Gotlib, 2006). Further, we need to acknowledge that the male sample size is quite small to draw firm conclusions from our findings about this subgroup. We also have no data on other psychiatric diagnoses of the sample and no information about any current treatments. Such unmeasured factors may have influenced the results and should be taken into account in future studies. For example, presence of social anxiety disorder is known to facilitate the perception of angry emotional expressions (Joormann and Gotlib, 2006). Another limitation is that environmental adversities were assessed via self-report. Reports of early trauma are likely dependent on subjective retrospective interpretation, and should be interpreted with caution (Offer et al., 2000). Our study population might have been suboptimal for detecting G×E interactions, as individuals with stressful life events (recent or in childhood) were probably under-represented in our young student sample. Future studies examining samples with higher exposure to adversity may detect genotype vulnerability effects of greater magnitude.

Recently it was shown that environmental moderators such as childhood environment and life events seem to be under genetic influence, as they are partly heritable (Vinkhuyzen et al., 2009). In the present study we also observed a linear relationship between childhood emotional abuse and genotype: the S′S′ group reported higher levels of abuse, followed by the S′L′ group and the L′L′ group. A review on such "gene-environment correlations" showed that the estimates of heritability of the environment are not solely the result of subjective perceptions (also when measured by self-report), but reflect actual environmental experiences (Kendler and Baker, 2007). Ignoring genetic effects on the measured environmental factor may lead to overestimating its effect as a moderator in gene-environment interaction research (Purcell, 2002). In our case, we found a moderating effect not only of childhood emotional abuse but also of recent life events, thereby increasing our confidence in the value of the adverse environment as a moderating factor. We also found a direct effect of genotype on our outcome. Future research should take into account G×E correlations when investigating G×E interactions.

The *s* allele seems to be involved in a perceptual bias for negative emotional stimuli (in this case, vigilance for negative facial expressions), which may be linked to a neurobiological response to threat. A novel aspect of the present research involves the assessment of attentional and perceptual processing across an emotion intensity continuum, in a paradigm with high ecological validity. Our findings shed light on the interplay between genes, environment and gender, and their effects on emotional processing.

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