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5-HTTLPR Biases Amygdala Activity in Response to Masked Facial Expressions in Major Depression

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The amygdala is a key structure in a limbic circuit involved in the rapid and unconscious processing of facial emotions. Increased amygdala reactivity has been discussed in the context of major depression. Recent studies reported that amygdala activity during conscious emotion processing is modulated by a functional polymorphism in the serotonin transporter gene (5-HTTLPR) in healthy subjects. In the present study, amygdala reactivity to displays of emotional faces was measured by means of fMRI at 3T in 35 patients with major depression and 32 healthy controls. Conscious awareness of the emotional stimuli was prevented via backward-masking to investigate automatic emotion processing. All subjects were genotyped for the 5-HTTLPR polymorphism. Risk allele carriers (S or L_G) demonstrated increased amygdala reactivity to masked emotional faces, which in turn was significantly correlated with life-time psychiatric hospitalization as an index of chronicity. This might indicate that genetic variations of the serotonin transporter could increase the risk for depression chronification via altering limbic neural activity on a preattentive level of emotion processing.
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INTRODUCTION

Converging evidence suggests that the serotonin system plays a critical role in the pathophysiology of major depression (Wong and Licinio, 2001). During the last decade, genetic variations in the serotonin system have been discovered that might constitute susceptibility factors for the development of depression. The serotonin transporter (5-HTT) facilitates re-uptake of serotonin from the synaptic cleft and is the target of most antidepressant drugs. In the promoter region of the serotonin transporter gene, mapping to chromosome 17q11.1-12, a functional variable repeat sequence polymorphism (5-HTTLPR) resulting in a short (S) and a long (L) variant has been identified (Lesch *et al*, 1996). It was repeatedly found that S allele carriers are more prone to the depressogenic effect of stressful life events than homozygotes for the L allele (Caspi *et al*, 2003; Kendler *et al*, 2005). Furthermore, 5-HTTLPR 'risk alleles' were reported to be associated with depression severity (Zalsman *et al*, 2006) and reduced therapy response (Smits *et al*, 2004). However, other studies failed to find associations of 5-HTTLPR with major depression (Willis-Owen *et al*, 2005). We investigated 5-HTTLPR in combination with a recently discovered single nucleotide polymorphism (SNP) with an A–G substitution (rs25531). Since the S_G allele seems to be a rare variant (Wendland *et al*, 2006; and was not found in the present sample at all) several previous reports treated 5-HTTLPR/rs25531 as a triallelic polymorphism. The L_G allele was reported to behave comparable to the low expressing S allele (Hu *et al*, 2005). Furthermore, a recent PET study demonstrated *in vivo* that human L_A/L_A carriers show higher 5-HTT binding potential in the putamen as an index of 5-HTT density (Praschak-Rieder *et al*, in press).

Associations of single polymorphisms with clinically defined phenotypes suffer from methodological difficulties (Malhotra and Goldman, 1999) and the heterogeneity and complexity of clinical phenotypes necessitate large sample sizes to detect rather small effects. A strategy to overcome such methodological problems is the endophenotype approach (Hasler *et al*, 2004). Hariri *et al* (2006) suggested that the examination of regional brain activation during emotion processing represents an innovative approach to link neural dysfunction to genes involved in the pathogenesis of depression with effect sizes 10–20 times larger than in classical association studies. The amygdala as a central processor of emotional significance has most intensively been studied in this emerging field called 'imaging genetics'. Increased amygdala reactivity is a frequent finding in major depression (eg Sheline *et al*,

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2001; Siegle *et al*, 2007) and a potential neural substrate of altered emotion processing in patients suffering from depression (Whalen *et al*, 2002). Several studies have demonstrated that healthy S allele carriers show increased amygdala reactivity to emotional stimuli (Bertolino *et al*, 2005; Hariri *et al*, 2002, 2005; Heinz *et al*, 2005, Pezawas *et al*, 2005). Thus, it was speculated that the S allele could increase the risk for depression via alterations of amygdala activity during emotion processing (Hariri *et al*, 2005). In a recent study, we have demonstrated that depressed patients carrying 5-HTTLPR risk alleles also show increased amygdala activity compared with depressed non-risk allele carriers (Dannlowski *et al*, in press).

However, all previous studies reporting influence of genetic variations on brain activity have employed paradigms using *overt* stimulus presentation with conscious processing of emotional stimuli. It remains unclear if the 5-HTTLPR effect on amygdala activity is restricted to the conscious level of emotion processing or if it can already be found at an automatic, pre-attentive stage. According to neurobiological theories of emotions, the amygdala is particularly implicated in the rapid and automatic processing of emotional significance preceding conscious awareness (LeDoux, 1996). In healthy subjects, several studies have confirmed that the amygdala is engaged during processing of emotional stimuli, even if presented briefly (<40 ms) and backward-masked, and thus without conscious awareness (eg Whalen *et al*, 1998). Amygdala reactivity to covertly but not to overtly presented fear faces was associated with individual differences in trait anxiety (Etkin *et al*, 2004). Thus, using *covert* stimulus presentation might be a more appropriate approach to investigate the role of the amygdala in dispositional emotional reactivity. So far, however, there has not been any report of a genetic effect on neural activity to *masked* emotional stimuli neither in healthy controls nor in patients with depression.

In the present study, we therefore have investigated amygdala activity in response to masked displays of emotional faces in relation to the 5-HTTLPR genotype in both, a sample of depressed inpatients and healthy subjects. Success of the masking procedure was assessed by means of a forced choice detection task. We speculated that 5-HTTLPR confers a change in amygdala activity at a pre-attentive stage of emotion processing, at which no conscious processing of the emotional faces has taken place.

PATIENTS AND METHODS

Subjects

Datasets of 35 inpatients with major depression, diagnosed with the SCID-I interview (Wittchen *et al*, 1997), and 32 healthy subjects were analyzed. Sociodemographic and clinical details of the study groups are shown in Table 1. Exclusion criteria were neurological abnormalities, substance abuse, former electroconvulsive therapy, age of 60 and above, and benzodiazepine treatment. Healthy subjects had no history of psychiatric disorders or psychotropic medication. All patients were under antidepressant medication, which was coded in terms of dose and treatment duration into medication levels from 1 to 4, according to the suggestions of Sackeim (2001). Details of the antidepressant medication are listed in Table 2. No patient received lithium or anti-epileptic drugs. Two patients (both S/L_A genotype) received additional antipsychotic drugs (amisulprid and quetiapine). Excluding these two patients would not change the results. The experiments were conducted in accordance with the Declaration of Helsinki. Approval was obtained from the ethics committee at the University of Münster. After a comprehensive description of the study to the subjects, written informed consent was obtained.

Genotyping

All subjects were genotyped for the 5-HTTLPR polymorphism, including SNP rs25531 (A/G), according to published protocols (Deckert *et al*, 1997; Wendland *et al*, 2006) with minor variation. Primers 5′-GGCGTTGCCGCTCTGAATGC-3′ and 5′-GAGGGACTGAGCTGGACAACCAC-3′ (10 pM each) were used for 20 μl PCR containing 60 ng DNA, 200 μM dNTPs, H_2O and 0.5 U HotStar Taq Polymerase with 1.5 mM $MgCl_2$, 1 × Q-Solution and 1 × Buffer (Qiagen), with an initial 15 min denaturation step at 95°C followed by 35 PCR cycles of 94°C (60 s), 64°C (60 s) and 72°C (120 s) and a final extension step of 10 min at 72°C. For RFLP analysis PCR products were digested with *Hpa*II at 37°C overnight, separated in 15% polyacrylamide gels (1 × TBE, 230 V/cm) for 3.5 h and visualized by silver staining, which resulted in fragments between 62 and 340 bp length allowing differentiation and assignment of all 5-HTT-LPR and –rs25531 genotypes. For genotyping quality control about one-third

Table 1 Clinical and Affective Characteristics of Study Participants after Exclusion of Subjects Performing Above Chance in the Detection Task

	Patients ($n=28$)	Controls ($n=28$)	Test statistic (two-tailed)
Age	38.6 (12.2)	36.8 (12.2)	$t(54)=0.55$, NS
Sex (m/f)	10/18	13/15	$\chi^2(1)=0.7$, $p=0.59$
5-HTTLPR/rs25531 ($L_AL_A/L_GL_A/SL_A/SL_G/SS$)	7/3/12/1/5	5/3/13/0/7	$\chi^2(4)=1.8$, $p=0.92$
HAMD[a]	22.2 (3.7)	1.0 (1.2)	$t(54)=28.6$, $p<0.001$
Number of episodes	4.7 (5.3)		
Life-time hospitalization (weeks)	7.8 (10.2)		
Duration of illness (months)	125.0 (125.5)		

[a]HAMD, Hamilton Rating Scale for Depression (Hamilton, 1960).

Table 2 List of Antidepressant Medication in the Genotype Groups

	5-HTTLPR/5-HT-rs25531	
	L_AL_A ($n=7$)	SS/SL/L_AL_G ($n=21$)
Venlafaxine	3	5
Venlafaxine+Mirtazapine	1	3
Escitalopram	1	5
Escitalopram+Mirtazapine	0	2
Duloxetine	0	1
Fluoxetine	0	1
Sertraline+Mirtazapine	1	0
Mirtazapine	1	3
Nortriptyline	0	1

Values reflect number of patients treated with the respective medication.

of probands were additionally genotyped by direct automated sequencing, which resulted in concordance rates of 100%. The genotype distribution of the 5-HTTLPR did not differ significantly from the expected numbers calculated according to the Hardy–Weinberg equilibrium. Following previous studies, subjects were grouped into risk (S) allele carriers and non-risk (L_A) allele carriers for each polymorphism. Since the L_G and S alleles have comparable levels of serotonin transporter expression (Hu *et al*, 2005), the L_G allele was treated as risk allele for the purpose of grouping. See Table 1, for a description of allele frequencies. According to *t*-tests or χ^2-tests, risk allele carriers and non-risk allele carriers were not significantly different concerning age, gender, education, verbal intelligence, and, for patients, medication level, medication type, depression severity, duration of illness, number of episodes or total hospitalization time (all $p>0.25$).

fMRI Methods

Technical details of the fMRI data acquisition and processing have been reported (Domschke *et al*, 2006). Briefly, facial stimuli consisted of sad, angry, happy and neutral expressions (Ekman and Friesen, 1976). Subjects were presented with alternating 30 s epochs of a face category or a no-face stimulus (a grey rectangle). In a passive viewing task facial stimuli were presented twice per second for 33 ms in a random sequence directly followed by a 467 ms mask depicting a neutral face of the same actor. Each emotion epoch was preceded by a no-face epoch and was presented twice, resulting in a total presentation time of 8 min. T2* functional data were acquired at a 3 T scanner (Gyroscan Intera 3.0T, Philips Medical Systems, Best, NL) using a single shot echoplanar sequence with parameters selected to minimize distortion in the amygdala while retaining adequate S/N and T2* sensitivity according to suggestions made by Robinson *et al* (2003). Volumes consisting of 25 axial slices were acquired (matrix 128*128, resolution 1.75*1.75*3.5 mm; TR = 3 s, TE = 30 ms, FA = 90°) 160 times in blocked design, 10 times per condition. To optimize the following normalization procedures, the same sequence-parameters were used to cover the whole brain with 43 slices. Additionally, two anatomical data sets were acquired: T1 weighted inversion recovery and a high resolution T1 weighted 3D sequence (isotropic voxel, 0.5 mm edge length).

Functional imaging data were motion corrected, using a set of six rigid body transformations determined for each image, spatially normalized to standard MNI space (Montreal Neurological Institute) with a voxel size of 2*2*2 mm, and smoothed (Gaussian kernel, 6 mm FWHM) using Statistical Parametric Mapping (SPM2, Wellcome Department of Cognitive Neurology, London, UK). The first (individual) level analysis was performed by modeling the different conditions (angry, sad, happy, and neutral) as variables within the general linear model (modeled with a standard hemodynamic response function), contrasting emotional faces (angry, sad and happy) with the neutral face condition. Voxel values of bilateral amygdala were extracted, summarized by mean and tested among the different conditions using the MarsBaR toolbox (Brett *et al*, 2002). The bilateral amygdala mask was defined according to a published anatomical atlas, (Tzourio-Mazoyer *et al*, 2002) which comprised 448 voxels (ie 3.6 ml) and included the grey nuclei at the rostral boundary of the hippocampus and the caudal boundary of the uncus.

Repeated measures analysis of variance (ANOVA) was conducted on mean amygdala activation parameters (contrast values). Since left and right amygdala activations were highly inter-correlated in all three emotion conditions (all $r>0.74$), an averaged measure for bilateral amygdala activity was used.

To explore genotype effects in brain regions outside the amygdala, a voxel-wise approach was employed. The first level contrast images were included in exploratory second level group analyses for group and genotype effects. In addition to the amygdala, the anterior cingulate cortex (ACC) was treated as region of interest (ROI), since the ACC has been implicated in the processing of masked emotional faces in a previous study (Killgore and Yurgelun-Todd, 2004) and was found to activate depending on 5-HTTLR genotype during emotion processing (Pezawas *et al*, 2005). The WFU PickAtlas Toolbox (Maldjian *et al*, 2003) was used to create an amygdala/ACC ROI mask for small volume correction according to the definitions of Tzourio-Mazoyer *et al* (2002). The statistical threshold was set at $p<0.05$, corrected for the amygdala/ACC volume. For the genotype comparison *within* each of the two subgroups, a more lenient threshold of $p<0.01$, uncorrected was chosen. Outside these anatomical areas with strong *a priori* hypothesis, a threshold of $p<0.05$, corrected for the entire brain was employed.

Detection Task

The detection task was designed to assess possible objective awareness of the masked emotional faces. After the experiment, the subjects were informed about the presentation of covert emotional faces in the MRI scanner. Then they were asked for subjective awareness of the emotional prime faces. In the following, the detection task was administered outside the scanner. Each of the 40 trials had the following routine: after a fixation cross lasting for 500 ms, a prime face was presented for 33 ms that was directly followed by a neutral target face. Each prime

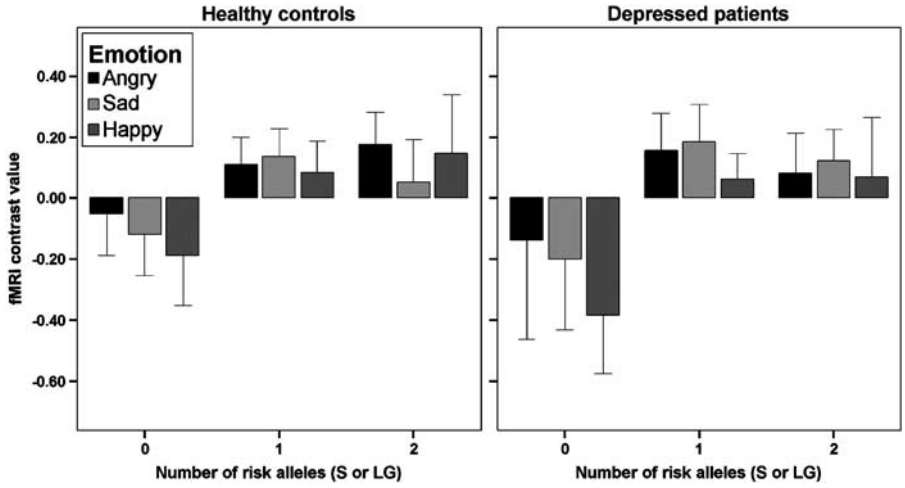

Figure 1 Bar graphs depicting mean amygdala activation to angry, sad and happy facial expressions in comparison to the neutral face condition dependent on group (patients *vs* controls) and number of risk alleles (S or L_G allele) in the 5-HTTLPR/5-HT-rs25531 polymorphisms.

emotion (angry, sad, happy and neutral) was presented 10 times and the order of trials was randomized for each subject. The instruction was to indicate which emotion quality was displayed briefly as prime. The chance level for correct answers was 25%. Binominal tests were used to explore whether a subject performed above chance.

RESULTS

Detection Task

One healthy subject (genotype S/S) reported having consciously detected brief displays of emotional faces inside the scanner. This subject also performed above chance in the detection task (45%, $p = 0.002$). All other participants reported not having seen any masked emotional faces. However, three more healthy subjects (S/S, S/L_A, and L_A/L_A) and seven patients (4 S/L_A, S/S, S/L_G, and L_A/L_G) performed above chance in the detection task. These subjects were considered 'aware' and consequently they were removed from further analysis. The remaining subjects (28 patients and 28 controls) performed at or below chance level with no differences between patients and controls (mean hit rate = 26.4%; range: 12.5–35%). However, inclusion of the eliminated data would not change the pattern of results.

fMRI Results

Dependent variables were mean amygdala contrast values in response to masked angry, sad and happy faces versus neutral faces (Figure 1). A 3 (emotion type: angry, sad, happy) $\times 2$ (group: patients, controls) $\times 2$ (genotype: risk, non-risk) analysis of variance was conducted on the mean amygdala responses. Box's test indicated equality of covariance matrices (Box's $M = 33.5$, NS) and Mauchly's test yielded no violation of the sphericity assumption ($W = 0.95$, NS). A main effect of 5-HTTLPR genotype was found, $F(1,52) = 6.2$, $p = 0.016$, Cohen's $d = 0.79$. As expected, risk allele carriers (S allele or L_G allele carriers, $n = 44$) demonstrated increased amygdala activity compared with L_A/L_A homozygotes ($n = 12$, see Figure 2). The

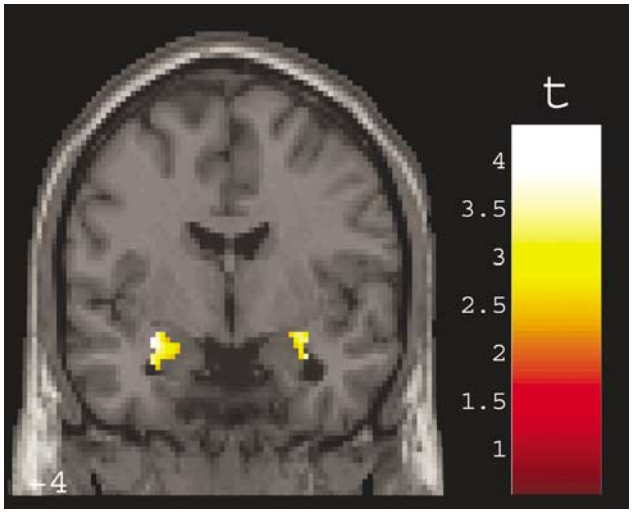

Figure 2 Coronal view ($y = -4$) depicting the main effect of 5-HTTLPR/5-HT-rs25531 on amygdala reactivity. Amygdala reactivity differences are shown between risk allele carriers (S or L_G allele, $n = 44$) and non-risk allele carriers (L_A/L_A, $n = 12$) in response to masked negative faces (angry and sad *vs* neutral). Voxel threshold was set at $p < 0.05$, with FDR (false discovery rate) correction for bilateral amygdala volume. Cluster maxima are located at MNI coordinates $x = -30$, $y = -4$, $z = -14$ (left amygdala, 92 voxels, $Z = 3.60$, $p_{corrected} = 0.017$) and $x = 30$, $y = -6$, $z = -14$ (right amygdala, 41 voxels, $Z = 3.35$, $p_{corrected} = 0.024$).

genotype effect was not modulated by group, $F(1,52) = 0.2$, $p = 0.6$. No other main effect or interaction of emotion type or group was recorded, although a power analysis with G-Power (Erdfelder *et al*, 1996) indicated sufficient power (0.83) to detect 'medium' size interactions (Cohen, 1988). In addition, the 5-HTTLPR effect was also found in the patient group alone, $F(1,26) = 4.4$, $p = 0.046$, Cohen's $d = 0.92$. However, in the control group, amygdala activity differences between risk and non-risk allele carriers did not reach significance, $F(1,26) = 2.1$, $p = 0.16$, Cohen's $d = 0.71$.

The voxel-wise analysis of the genotype effect in the whole sample confirmed the ANOVA result of stronger amygdala responses in risk allele carriers [left amygdala: k (cluster size) = 93 voxels, peak voxel at $x = -30$, $y = -4$,

$z=-14$, $Z=3.6$, $p_{corrected}=0.024$; right amygdala: $k=45$, $x=30$, $y=-6$, $z=-14$, $Z=3.55$, $p_{corrected}=0.024$]. Furthermore, a large and highly significant cluster in the ACC was found [$k=854$, $x=4$, $y=16$, $z=26$, $Z=3.98$, $p_{corrected}=0.001$, extending from the supragenual ACC to the perigenual area, $x=-2$, $y=44$, $z=2$]. The genotype effect was also found in the patient group alone [left amygdala: $k=35$ voxels, $x=-30$, $y=-2$, $z=-22$, $Z=3.14$, $p_{uncorrected}=0.001$; right amygdala: $k=5$, $x=28$, $y=-4$, $z=-12$, $Z=2.61$, $p_{uncorrected}=0.007$; ACC: $k=91$, $x=-6$, $y=28$, $z=30$, $Z=2.88$, $p_{uncorrected}=0.002$, extending to $x=4$, $y=30$, $z=-2$] and in the healthy control group [right amygdala: $k=6$, $x=30$, $y=-8$, $z=-12$, $Z=2.50$, $p_{uncorrected}=0.006$; ACC: $k=175$, $x=2$, $y=14$, $z=28$, $Z=3.48$, $p_{uncorrected}=0.001$, extending to $x=-2$, $y=50$, $z=2$]. No genotype effect was detected in brain areas without a specific *a priori* hypothesis.

An exploratory analysis of group differences yielded no significant results in the amygdala/ACC ROI or other brain areas.

Allele Load

A recent study reported a linear increase of amygdala responses to overt negative pictures with risk (S) allele load (Heinz *et al*, 2005). To explore whether a linear increase of amygdala activation is responsible for the observed risk *vs* non-risk group effect, we conducted a voxel-wise linear regression analysis of amygdala responses to masked negative faces in the whole sample. Genotype was coded as the number of risk alleles (S or L_G; 0, 1, or 2). Applying the same statistical threshold as in the group comparison ($p<0.05$, corrected for the amygdala/ACC ROI or the entire brain), no significant effect of allele load was detected, indicating a 'dominant' effect of risk allele carriage.

Amygdala Reactivity and Clinical Characteristics

Amygdala reactivity to any emotion quality was not significantly associated with number of episodes or duration of illness, although all correlations carried a positive sign. However, we observed significant associations of lifetime psychiatric hospitalization and amygdala reactivity to masked angry ($r=0.38$, $p=0.046$), sad ($r=0.44$, $p=0.020$) and happy ($r=0.40$, $p=0.037$) expressions. Thus, patients showing increased amygdala reactivity had a history of longer previous psychiatric hospitalization. There was no significant association of amygdala reactivity and current depressive symptoms (HAMD-score, all $r<0.12$).

Role of Medication

To explore the role of antidepressant medication, patients were grouped into a low-dose group (medication level 1–2, $n=12$) and a high-dose group (medication level 3–4, $n=16$) (Surguladze *et al*, 2005). High-and low-dose group did not differ with respect to 5-HTTLPR risk group ($\chi^2(1)=0.0$, $p=1.0$) and amygdala activity elicited by any masked emotion type (all $p>0.2$). Adding medication level as covariate did not alter the results.

DISCUSSION

The present data suggest that amygdala reactivity to emotional faces is modulated by the 5-HTTLPR polymorphism even in absence of conscious processing of the emotional stimuli. These findings replicate and extend the common observation in healthy subjects that 5-HTTLPR risk allele carriers demonstrate stronger amygdala activity in response to emotional faces and pictures (Bertolino *et al*, 2005; Hariri *et al*, 2002, 2005; Heinz *et al*, 2005; Pezawas *et al*, 2005). Recently, Smolka *et al* (2007) reported that the 5-HTTLPR effect on amygdala and anterior cingulate cortex activity in response to unpleasant pictures is stronger if the triallelic variant was considered compared with the 5-HTTLPR effect alone. However, the present study is the first imaging genetics investigation that used masked displays of facial expressions and examined patients with clinical depression. The biasing effect of 5-HTTLPR on amygdala activity was somewhat more pronounced in the patient group (although not significantly, as indicated by a non-significant genotype $\times$ group interaction). Furthermore, in this group, amygdala reactivity was associated with life-time psychiatric hospitalization, as an index of illness chronicity. Our data may thus suggest that 5-HTT genotype affects the course of major depression by biasing amygdala activity during automatic emotion processing.

Interestingly, the 5-HTTLPR effect was not restricted to negative (angry and sad) emotions. Masked happy expressions elicited stronger amygdala activity in risk allele carriers as well, a finding in line with a recent study in which amygdala activity in a happy face condition was found to be increased in S allele carriers suffering from panic disorder (Domschke *et al*, 2006). In addition, the only study so far that investigated amygdala activity in response to masked facial expressions in patients suffering from major depression demonstrated that unmedicated patients show increased amygdala activity compared to controls also for masked happy faces (Sheline *et al*, 2001). It could be argued that increased amygdala activity in response to emotional faces might indicate negatively biased emotion processing regardless of the stimulus valence, even processing of happy facial expressions. This corresponds well with a recent masked affective priming study (Dannlowski *et al*, 2006). It was found that acutely depressed patients demonstrate automatic negative evaluative biases elicited by masked emotional faces, including happy expressions, which predicted weak therapy outcome. However, since no evaluation data were collected in the present experiment this interpretation remains speculative. An alternative explanation of the observed 5-HTTLPR effect might be that risk allele carriers are in general more sensitive in the automatic detection of biologically and socially relevant information in the environment. There is substantial evidence that the human amygdala is involved in the appraisal of events relevant for the survival and well-being of the organism (Öhman, 2002; Sander *et al*, 2003).

According to our data 5-HTTLPR risk allele carriers show also an enhanced responsivity of the anterior cingulate cortex to masked facial emotions. The ventral ACC appears to have an important role in the production of affective states (Phillips *et al*, 2003) and in the detection of socially relevant visual stimuli encountered below the threshold of

conscious perception (Killgore and Yurgelun-Todd, 2004). Thus, it can be hypothesized that risk allele carriers might be more affectively responsive to facial emotions and bring potentially important stimuli more easily into the forefront of conscious awareness.

Certain limitations must be acknowledged. Although the combined sample provided sufficient power to detect main effects of genotype or interactions, the number of non-risk allele carriers was small within the two subgroups, limiting the statistical power to detect genotype effects in the two groups considered alone. No follow-up data were assessed and the individual history of stressful life events was not recorded. Thus, any conclusion with respect to a prognostic value or causal relationships of genetic susceptibility, amygdala reactivity and chronification remains speculative. The correlation analysis of amygdala reactivity and clinical characteristics was explorative and would not survive alpha correction for multiple comparisons. Furthermore, we did not find differences in amygdala activity between depressed patients and healthy controls. However, all of our patients were medicated. Hence, even in case of significant group differences it could not have been concluded whether any observed group effect was due to depression or a confounding effect of medication. Nonetheless, our data are in line with the findings of Sheline *et al* (2001) and Fu *et al* (2004). These previous studies found no differences between medicated patients and healthy controls with respect to their amygdala reactivity to emotional faces. Although we did not find any effect of current antidepressant medication level on amygdala reactivity or any clinical characteristic in our patient group, the present data cannot necessarily be generalized to unmedicated patients. Further studies should address these issues by investigating unmedicated patients in longitudinal designs.

In summary, our preliminary findings provide evidence that genetic susceptibility factors for major depression might be transported via altered limbic neural activity already at a preattentive level of emotion processing. The findings of the present study hopefully stimulate further investigations of depressed patients employing the imaging genetics approach. The consistency of previous studies and the present data underscore the power of direct assessment of regional brain activity in exploring the functional impact of genetic variation in clinical states.

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