Differential Effects of 5-HTTLPR Genotypes on Inhibition of Negative Emotional Information Following Acute Stress Exposure and Tryptophan Challenge

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Previous data suggest that a polymorphism at the serotonin (5-HT) transporter gene (5-HTTLPR) may influence stress resilience and stress-related depression symptoms due to interactions between brain 5-HT dysfunction and stress exposure. Although attentional bias for emotional information has been reliably observed in depression, the interaction between 5-HT transporter-linked promoter region (5-HTTLPR), brain 5-HT vulnerability, and acute stress on affective information processing has not yet been investigated. This study examines the effects of tryptophan (TRP) augmentation (indicating 5-HT manipulation) on inhibition of negative emotional information under stress in mainly female S′/S′- vs L′/L′-allele carriers. A total of 15 female homozygotic short-allele 5-HTTLPR (S′/S′ = S/S, S/L$_G$, L$_G$/L$_G$) and 13 female homozygotic long-allele 5-HTTLPR (L′/L′ = L$_A$/L$_A$) subjects were tested for mood and inhibition of emotional information in a double-blind, placebo-controlled design before and after stress exposure following TRP manipulation. Stress exposure significantly impaired inhibition of negative affective information only in S′/S′ carriers, whereas L′/L′ carriers even showed increased inhibition of negative information. The S′/S′ allele 5-HTTLPR genotype increases cognitive-attentional bias for negative emotional information under acute stress. As this bias is an important component of depression, this may be a mediating mechanism making S′/S′-allele carriers more vulnerability for stress-induced depression symptoms. Moreover, current data suggest that L′/L′-allele genotypes are more resilient, even increasing cognitive emotional (inhibitory) control after stress.

Neuropsychopharmacology (2011) **36,** 819–826; doi:10.1038/npp.2010.221; published online 8 December 2010

Keywords: 5-HTTLPR; emotion processing; serotonin; stress; tryptophan

INTRODUCTION

The identification of biological vulnerability factors for depression is an important challenge facing biological psychiatry today. Among the various biochemical processes involved, brain serotonin (5-HT) dysfunction appears to be a meaningful risk factor (Maes and Meltzer, 1995; Van Praag, 2004). 5-HT dysfunction manifests in depressed patients as lowered brain tryptophan (TRP) concentrations (eg, see Agren and Reibring, 1994; Maes *et al*, 1990); impaired 5-HT synthesis, release, reuptake, or metabolism (eg, see Maes and Meltzer, 1995; Malison *et al*, 1998; van Praag *et al*, 1970); or 5-HT receptor disturbances (eg, see Cowen *et al*, 1994; Sargent *et al*, 2000), whereas

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antidepressant drugs generally act by improving brain 5-HT function (Delgado *et al*, 1990; Delgado *et al*, 1993; Maes and Meltzer, 1995).

5-HT dysfunction in depression is promoted by a genetic vulnerability involving a polymorphism in the 5-HT transporter-linked promoter region (5-HTTLPR). This region encodes the 5-HT transporter (5-HTT) protein that controls 5-HT reuptake and function, and which is the main target mechanism for antidepressant drugs (Serretti *et al*, 2007). The short-allele (S) variant of 5-HTTLPR is associated with fewer 5-HTT binding sites compared with the long-allele (L) variant (Heils *et al*, 1996; Mann *et al*, 2000) and is involved in 5-HT dysfunction during depression (Jans *et al*, 2007). Recently, the presence of an A > G single-nucleotide polymorphism has been identified in the L allele, rendering an Lg variant (as opposed to La) that is functionally equivalent to the S-allele (Hu *et al*, 2005; Wendland *et al*, 2006). However, 5-HT dysfunction in depression is also promoted by stress due to interactions between 5-HT and the hypothalamus–pituitary–adrenal (HPA) system (Firk and Markus, 2007; Van Praag, 2004). Hence, depression is

often preceded by stress (Van Praag, 2004), and there is a positive association between the number of copies of the S-allele 5-HTTLPR and self-reports of past depressive responses to stressful events (Caspi *et al*, 2003; Jacobs *et al*, 2006; Wilhelm *et al*, 2006). However, attempts to replicate these findings have yielded mixed results (Risch *et al*, 2009).

Despite the suggestion from previous studies that stressful events might promote depression symptoms mostly in homozygous S-allele compared with homozygous L-allele 5-HTTLPR carriers, actual interactions between genetic 5-HT vulnerability, stress exposure, and depression have barely been investigated. A recent experimental study reveals evidence for enhanced HPA stress-responses exclusively in homozygous S-allele 5-HTTLPR carriers (Gotlib *et al*, 2008), but this experiment did not include the measurement of depression-specific cognitive-affective changes or of brain 5-HT vulnerability.

Depression specific changes can be adequately measured by negative affective priming (NAP) tasks that measure attentional bias for emotional information (Goeleven *et al*, 2006; Joormann, 2004). Hence, attentional bias for negative information is a hallmark feature of MDD (see Scher *et al*, 2005), which relates to impaired cognitive inhibition toward negative emotional material (Goeleven *et al*, 2006; Gotlib *et al*, 2005; De Raedt and Koster, 2010). In support, 5-HT is implicated in decreased attentional control over negative stimuli by modulating prefrontal-amygdala connectivity (see Cools *et al*, 2008), and S/S show enhanced emotional amygdala responses (Hariri and Holmes, 2006) and impaired disengagement to emotional information (Beevers *et al*, 2009).

Brain 5-HT vulnerability relates to an enhanced sensitivity to alterations in the 5-HT system (Jans *et al*, 2007). This can be measured by acute TRP depletion (Biggio *et al*, 1974; Carpenter *et al*, 1998; Nishizawa *et al*, 1997; Williams *et al*, 1999) that has been found to increase depression symptoms in individuals at risk for depression (Ruhe *et al*, 2007), including homozygous S-allele 5-HTTLPR carriers (Neumeister *et al*, 2002; Neumeister *et al*, 2006). However, because S/S-allele carriers may exhibit altered 5-HT sensitization to compensate lower 5-HTT expression (David *et al*, 2005; Moore *et al*, 2000), TRP challenge may be an alternative method to reveal 5-HT vulnerability associated with 5-HTTLPR. In support, TRP administration improved mood in S/S- not L/L-allele carriers (Markus and Firk, 2009).

The current study is the first to investigate the interaction between acute stress, 5-HTTLPR, and 5-HT vulnerability on the inhibition of negative emotional information. Based on previous assumptions, it is hypothesized that stress reduces the inhibition of negative information in S/S- not L/L-allele carriers and that TRP administration reduces this effect only in S/S-allele carriers.

PATIENTS AND METHODS

Participants

Students from Maastricht University completed a questionnaire package concerning general information (health, personal, or family history of medical or psychiatric complaints, smoking and drinking habits, caffeine consumption, weight and height, use of psychoactive drugs) and concerning relevant symptoms and psychopathology (Beck Depression Inventory, Inventory of College Students' recent life experiences (ICSRLE), Inadequacy/neuroticism Scale of the Dutch Personality Inventory, and the Social Complaints List). Exclusion criteria were health or medical complaints; personal or family history of psychiatric illness; history of medical illness; medication use; metabolic-, hormonal-, or intestinal diseases; irregular diet; deviant eating habits or excessive alcohol or drug use. Following this first selection, 90 participants attended a buccal sample extraction session to genotype for 5-HTTLPR (resulting in 26% S/S, 46% S/L, and 28% L/L). As brain 5-HT vulnerabilities for stress are mainly found in homozygotes S compared with homozygotes L-allele carriers (eg, see Neumeister *et al*, 2002; Caspi *et al*, 2003; Wilhelm *et al*, 2006; Zalsman *et al*, 2006), only homozygous S-allele (S/S, S/L$_G$, L$_G$/L$_G$; classified as S′/S′) and homozygous L-allele (L$_A$/L$_A$; classified as L′/L′) were then invited for a psychiatric structured interview (MINI: Sheehan *et al*, 1994) to double-check exclusion compliance and to receive information about the experiment. After invitation, before the psychiatric interview, a number of 8 S′/S′ and 12 L′/L′ subjects refrained from further participation because of shortage of time or other non-specified reasons.

A total of 15 S′/S′ and 13 L′/L′ female participants completed the experiment. Both groups included Caucasian participants with European (mainly Dutch) background. They all revealed normal body mass indexes (BMI between 20 and 25 kg/m^2; mean 22 ± 2), were non-smokers, and were requested not to use alcohol or any kind of drugs 24 h before and during the study. Both groups did not differ in age, BMI, and additional relevant symptoms. The study was approved by the Medical Ethics Committee of the Academic Hospital Maastricht (CTCM azM; Maastricht; The Netherlands) and the procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 1983. All subjects gave their written informed consent and were paid for participation.

Design and Procedure

A placebo-controlled, double-blind, crossover design was used. During two sessions, subjects were monitored for NAP before and after stress exposure following intake of TRP or placebo (PLC). The order of presentation of the treatment conditions was counterbalanced within groups; both sessions were separated by 1 week. All subjects were tested in the mid-late follicular phase of their menstrual cycle or when actually taking oral contraceptives. An illustration of the design is given in Figure 1.

All participants fasted overnight; except for water or tea without sugar. On each experimental day, three participants arrived at 0800, 0900, and 1000 hours, respectively. Capsules were consumed 1 h before arrival. After arrival and a subsequent 15 min rest period, mood and NAP was assessed. Then, participants were exposed to a stress-inducing procedure followed by a second mood and NAP measure.
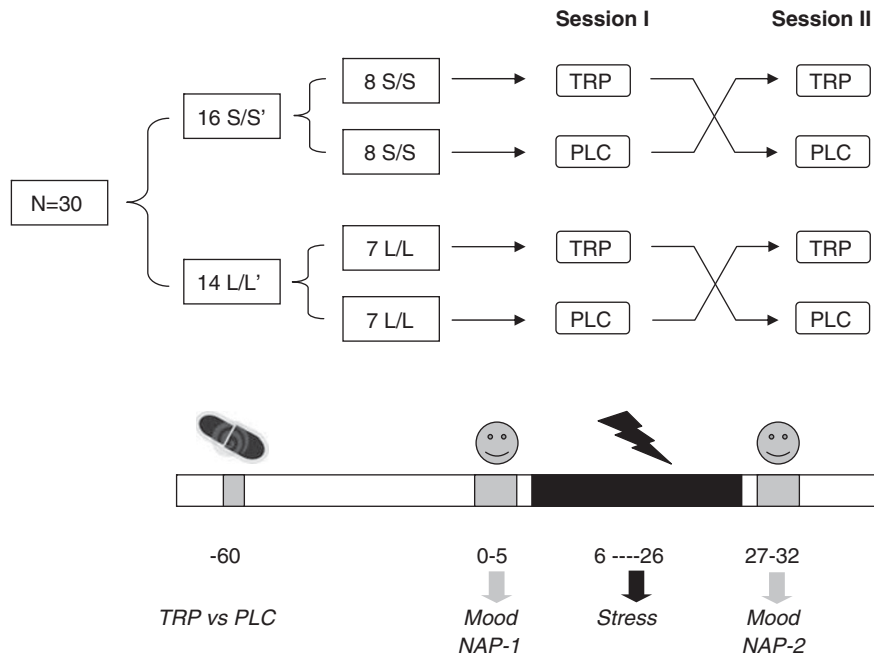
Figure 1 Schematic overview of the study design.

Stress Exposure

Subjects were placed in front of a camera for 20 min while completing 2-min serial subtraction sessions and cold-pressor exposures. At unpredictable occasions, they were signaled to start at a certain number and count backwards by seven as quickly as possible. If they made a mistake, they were interrupted by the experimenter and had to start over. In addition, at unpredictable occasions while continuing counting, they had to place their non-preferred hand on a 1.5 °C cold plate for as long as possible (with a maximum of 2 min). For cold-pressor exposure, a cooler device was used composed of a copperplate surface (150 × 230 mm) that was thermoelectrically cooled at a constant level by way of Peltier (TEC) devices. Backward counting was initiated at four different unpredictable 180 ± 30 s time intervals using different numbers (400, 425, 530, or 840) that were counterbalanced within-subjects. Cold-pressor signals appeared at six unpredictable 120 ± 60 s time intervals either during (85%) or between (15%) counting sessions.

TRP Challenge

To increase plasma TRP, 0.8 g TRP as compared with 0.8 g PLC (cellulose) capsules were used. Comparable intake of TRP has been shown to increase plasma TRP availability (TRP/LNAA ratio) with 190% 60–90 min after intake (Markus *et al*, 2008). Capsules were identical and subjects were instructed to swallow them whole with water.

## Positive and Negative Affect Scale (PANAS)

The PANAS (Watson *et al*, 1988) consists of two 10-item mood scales, a positive mood scale and a negative mood scale. These items are randomly presented and participants

have to respond on a scale ranging from 1 (totally disagree) to 5 (totally agree).

Negative Affective Priming

The NAP task included (5 × 5.5 cm) colored pictures of positive (*n* = 33), negative (*n* = 33), or neutral (*n* = 22) faces that were selected from the Karolinska Emotional Directed Faces database (Lundqvist and Ohman, 1998) and on the basis of previous validation (Goeleven *et al*, 2006). Pictures were divided into eight lists (based on stimulus type) of 11 randomly chosen pictures sharing the same valence: one negative and one positive prime target list, one negative and one positive prime distracter list, one negative and one positive probe target list, and two neutral probe distracter lists. Pictures were indicated as target or distracter by a black or gray frame. Participants were instructed to evaluate the valence of the target picture—indicated by the color of the frame—as accurately as possible by a response key while ignoring the distracter picture. Each trial started with a fixation cross (for 1000 ms) after which two emotional faces appeared in the upper and the lower half of the screen (one surrounded by a gray frame and one by a black frame) until responding (see Figure 2).

After training (including 32 trials), a sequence of 256 test trials (separated by a 1000 ms blank screen) followed that were divided into 8 blocks of 16 prime and probe trials. The response cue (gray or black frame) and key assignment was counterbalanced between subjects and the spatial position of the target and the distracter—as well as sequence of congruent and incongruent prime-probe trials—were randomly assigned from trial to trial. Reaction time (RT) to the probe trials for both the positive and negative trials were reordered (RT below 300 ms and above 2000 ms were excluded (see Goeleven *et al*, 2006) and NAP scores were
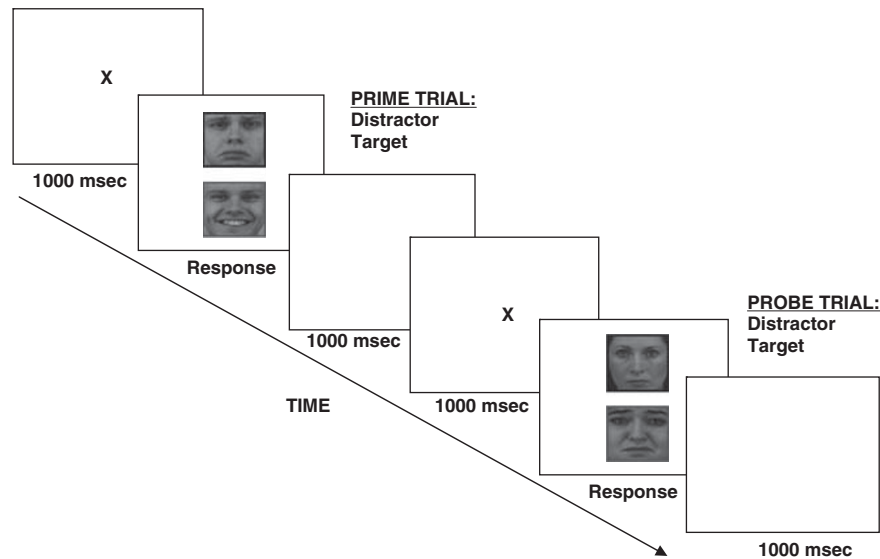
Figure 2 Negative affective priming design.

calculated by subtracting RT to control trials from RT to experimental trials.

Buccal Cells for Tri-Allelic 5-HTTLPR Polymorphism

Buccal cell samples for 5-HTTLPR genotype determination were obtained using sterile swabs (Omni Swabs, Whatman's, Hertogenbosch, The Netherlands). Genomic DNA was isolated by QIamp DNA Mini Kits from Qiagen (Westburg, Leusden, The Netherlands) and 5-HTTLPR genotyping was performed using the PCR protocol according to Glatz et al (2003). In compliance with previous work (eg, see Neumeister et al, 2006; Walderhaug et al, 2007; Zalsman et al, 2006), tri-allelic variants were reclassified into a bi-allelic model as follows: S/S, S/L$_G$, and L$_G$/L$_G$ were classified as S'/S' and L$_A$/L$_A$ as L'/L'.

Statistical Analysis

Data were first examined for accuracy of data entry, missing values, and normal distributions. Hardy–Weinberg equilibrium was determined on the original 5-HTTLPR database ($n = 90$) using χ^2 tests, revealing that the genotype frequencies of S'/S' ($n = 24$), S/L ($n = 40$) and L'/L' ($n = 26$) did not depart from Hardy–Weinberg equilibrium ($\chi^2_{(1)} = 1.45$; $p = 0.23$). Data were analyzed by means of repeated measures Anova (GLM: SPSS 12.0 for Windows) with one between-subjects factor *Genotype* (S'/S' *vs* L'/L') and two within-subjects factors, *Treatment* (TRP *vs* PLC) and *Stress* (prestress *vs* post-stress), on mood (PANAS) and NAP. Regarding NAP, repeated measures were conducted on difference RT scores between control and experimental probe trials, separately for the positive and negative trials. Although we counterbalanced for the order of treatment, *Order of Treatment* was preliminary taken as a between-subjects factor. Because *Order of Treatment* did not contribute to any of the scores, final analyses were performed with only *Genotype* as between-subjects factor.

All statistics are evaluated at a two-tailed significance level of 5%. Data are reported as means ± SD.

RESULTS

PANAS

Repeated measure analyses were conducted on the PANAS scores for positive and negative mood with *Genotype* (S'/S' *vs* L'/L') as between-subjects factor and *Treatment* (TRP *vs* PLC) and *Stress* (prestress *vs* post-stress) as within-subjects factors. Analysis revealed an effect of *Stress* on positive feelings ($F(1,26) = 13.49$, $p = 0.001$; power = 0.95) and negative feelings ($F(1,26) = 15.52$, $p = 0.001$; power = 0.94); indicating a reduction in positive feelings post-stress (2 ± 0.7) compared with prestress (2.6 ± 0.7) and an increase in negative feelings post-stress (1.3 ± 0.4) compared with prestress (1.0 ± 0.2). Regarding negative mood, analysis revealed an interaction of *Treatment* × *Genotype* ($F(1,26) = 6.01$, $p = 0.02$; power = 0.70), *Treatment* × *Stress* ($F(1,26) = 4.58$, $p = 0.042$; power = 0.60), and a marginally three-way interaction of *Treatment* × *Stress* × *Genotype* ($F(1,26) = 4.2$, $p = 0.041$; power = 0.50). As shown in Figure 3, S'/S' reported a modest increase in negative feelings post-stress (1.6 ± 0.9) compared with prestress (1.0 ± 0.12) after PLC that was absent after TRP (1.1 ± 0.3 post-stress compared with 1.1 ± 0.2 prestress).

Negative Affective Priming

Repeated measure analyses were conducted on the NAP scores for positive and negative trials with *Genotype* (S'/S' *vs* L'/L') as between-subjects factor and *Treatment* (TRP *vs* PLC) and *Stress* (prestress *vs* post-stress) as within-subjects factors. Analysis revealed an interaction of *Stress* × *Genotype* ($F(1,26) = 16.84$, $p < 0.001$; power = 0.95) for the negative trials; indicating an effect of stress exposure on inhibition of negative emotional information depending on

5-HTTLPR. As illustrated in Figure 4, stress exposure significantly impaired inhibition of negative emotional information in S′/S′ ($p < 0.0001$), whereas in L′/L′ inhibition of negative information improve (higher NAP) by stress exposure ($p = 0.027$).

DISCUSSION

The aim of the present study was to explore whether the S′/S′-allele variant compared with the L′/L′-allele variant of the 5-HTTLPR increases the negative effects of stress exposure on the inhibition of affective information. Assuming 5-HTergic vulnerability in carriers of the low-expressing 5-HTTLPR, S′/S′-allele carriers were also expected to be less affected by stress following TRP

Negative mood (PANAS)

Figure 3 S′/S′ allele reported an increase in negative feelings post-stress compared with prestress after PLC that was prevented after TRP (post-stress compared with prestress).

compared with PLC. Results revealed that stress exposure significantly reduced mood and that only in S′/S′-allele carriers did stress impairs the successful inhibition of negative affective information.

Stress and NAP

To induce acute stress, subjects completed serial subtraction sessions in combination with frequent cold-pressor exposures. Both the cold-pressor exposures (Isowa et al, 2006) and counting tasks (Dickerson and Kemeny, 2004) have been shown to be sufficient to induce stress; repeated exposures given in a variable and unpredictable manner have induced even more severe stress responses (Markus, 2007). In addition, the stress procedure in the current study was sufficient to induce negative mood responses.

In line with our expectation, exposure to the stress procedure increased the facilitation of negative information processing only in S′/S′-allele carriers. Hence, exclusively in S′/S′, acute stress speeded up recognition for negative facial expressions during experimental NAP conditions (responding to negative probe targets that are preceded by prime distracters with the same negative valence) compared with control conditions (responding to negative probe targets that are preceded by prime distracters with an opposite valence). Because this reduced NAP effect represents an impaired inhibitory function toward negative affective information (Joormann, 2004; Goeleven et al, 2006), the current findings suggest that S′/S′-allele carriers are more susceptible to the deteriorating effects of stress exposure on the successful inhibition of negative information. In contrast, L′/L′-allele carriers not only did not show these vulnerabilities, but also they were more successful at inhibiting negative information under stress (indicating that this variant could be a marker of resilience against the detrimental effects of stress).

The current 'boosting' effect of acute stress on the facilitation of negative information processing in S′/S′-allele carriers complies with recent findings that homozygous S′ allele are most susceptible to the negative affective (Firk and
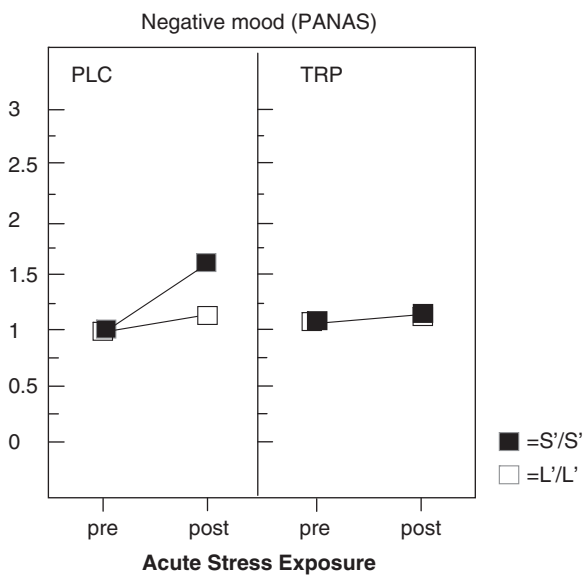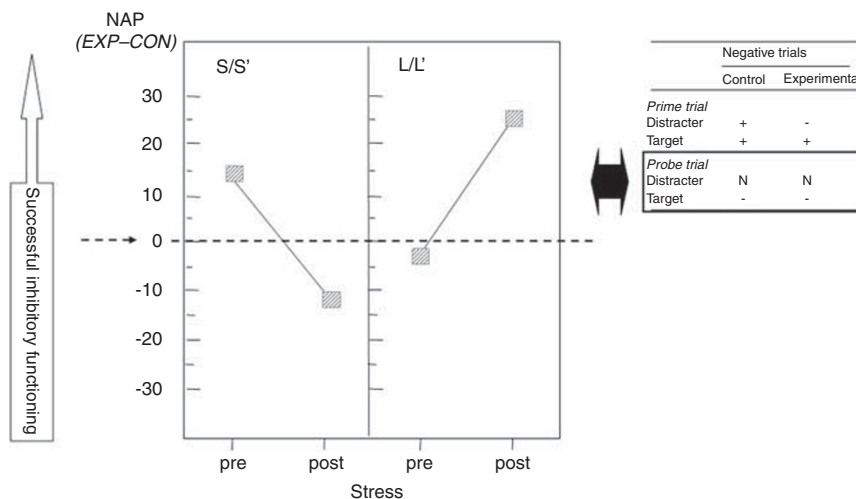
Figure 4 Acute stress exposure significantly impaired inhibition of negative emotional information (reduced NAP) only in S′/S′ genotypes ($p < 0.0001$), whereas in L′/L′ genotypes inhibition of negative information improved (higher NAP) by stress exposure ($p = 0.025$).

Markus, 2009) and hormonal (Gotlib *et al*, 2008) effects of stress exposure and to the depressogenic effects of life events (Caspi *et al*, 2003; Uher and McGuffin, 2008). In addition, the current study elaborates on previous findings by showing that the enhanced vulnerability in S′/S′-allele carriers for stress-related depression may be mediated by a stress-induced impairment in the ability to inhibit negative information. This also complies with findings from imaging studies showing that S-allele carriers exhibit enhanced amygdala responses to negative facial expressions (Pezawas *et al*, 2005; Dannlowski *et al*, 2008). This result has been attributed to a reduced amygdala-to-prefrontal-cortex connectivity (a reduced inhibition of the amygdala) that is also observed in depression (Siegle *et al*, 2007).

The current stress-induced increase in negative information processing in S′/S′-allele carriers can be explained by the mood-congruent hypothesis that negative affective interruption facilitates continued processing of negative information. In addition, the previously demonstrated depressogenic effects of life events on S/S-allele carriers (Caspi *et al*, 2003; Uher and McGuffin, 2008) may be moderated by a stress-induced increase in attentional bias allowing for maintained attention on negative material. Thus, in S′/S′-allele carriers, stress may reduce the successful inhibition of distracting negative stimuli. In fact, this stress-induced disturbed inhibitory functioning is thought to be a core factor that is related to the previously observed difficulties in depressed patients to disengage attention away from negative information (Leyman *et al*, 2007). In addition, comparable both with our current findings in healthy S′/S′-allele carriers and with the assumption that S′/S′-allele carriers are more prone to develop depression, depressed subjects have also been shown to exhibit a reduced ability to inhibit negative information during a NAP task (Goeleven *et al*, 2006; Leyman *et al*, 2010).

To reveal whether S′/S′ are more susceptible to the effects of stress due to brain 5-HT vulnerability, a TRP challenge was used in the current study. Based on previous studies reporting a 5-HT vulnerability to the mood-lowering effects of ATD in S′/S′-allele carriers (Neumeister *et al*, 2006; Neumeister *et al*, 2002; Roiser *et al*, 2006), it was hypothesized that TRP may reduce the negative stress effects. The current findings, however, did not reveal significant TRP effects on NAP and only marginally (reducing) effects on negative mood in S′/S′ carriers.

Limitations

One point that deserves special attention is the relatively small sample size of the S′/S′ and L/L groups. Even though the current findings reveal powerful genotype × stress interaction effects on NAP, the sample sizes might have been too small to also detect TRP × stress interactions on NAP. Another point of attention is that the current findings only included female subjects. It therefore remains necessary to conduct a comparable experiment in a comparable sample of male participants.

Conclusion

The current findings reveal that stress exposure in female S′/S′- not L′/L′-allele carriers reduces inhibitory functioning for negative information. Because S′/S′ carriers are found to be vulnerable to the negative depressogenic effects of stress, the current data is in line with the assumption that a stress-by-depression interaction in S′/S′-allele carriers may be promoted by a stress-induced facilitation of negative emotional information.

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