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Differential Effects of Tri-Allelic 5-HTTLPR Polymorphisms in Healthy Subjects on Mood and Stress Performance After Tryptophan Challenge

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Earlier data suggest that a polymorphism at the serotonin (5-HT) transporter gene (5-HTTLPR) may affect depression particularly in the face of stress due to interactions between 5-HT vulnerability and stress exposure. However, this interaction between 5-HT transporter-linked transcriptional promoter region (5-HTTLPR), 5-HT vulnerability and the affective effects of stress exposure has not yet been investigated. As participants with short-allele 5-HTTLPR genotypes may exhibit enhanced 5-HT vulnerability, this study examines the effects of tryptophan challenge on stress reactivity and performance in healthy participants with S'/S' *vs* L'/L' genotypes. Sixteen healthy subjects with homozygotic short alleles ($S'/S' = S/L_G$, L_G/L_G) and 14 subjects with homozygotic long alleles ($L'/L' = L_A/L_A$) of the 5-HTTLPR were tested in a double-blind placebo-controlled design under acute stress exposure following tryptophan challenge or placebo. Although there were no 5-HTTLPR-related differences in stress responses, significant beneficial effects of tryptophan challenge on mood and stress performance were exclusively found in participants with S'/S' genotypes. These findings suggest greater brain 5-HT vulnerability to tryptophan manipulations in participants with S'/S' as compared with L'/L' 5-HTTLPR genotypes. This apparent genetic 5-HT vulnerability may become a meaningful risk factor for depression when brain 5-HT falls below functional need in the face of real severe stressful life events.

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INTRODUCTION

Depression is still among the leading causes of disease burden throughout the world and is associated with severe medical consequences and mortality. Among the neurochemical processes involved, reduced brain serotonin (5-HT) function seem to be a relevant pathophysiological mechanism involved (Maes and Meltzer, 1995; Van Praag, 2004). Although there are still open questions about the exact involvement of 5-HT in the onset and course of depression, 5-HT dysfunction is commonly indicated in depressed patients by lower brain availability of tryptophan and 5-hydroxytryptophan (5-HTP) (Agren and Reibring, 1994; Maes *et al*, 1990); impaired 5-HT synthesis, release, reuptake, or metabolism (Maes and Meltzer, 1995; Malison *et al*, 1998; van Praag *et al*, 1970); or 5-HT receptor disturbances (Cowen *et al*, 1994; Sargent *et al*, 2000). In addition, antidepressant drugs mainly act by improving brain 5-HT function (Delgado *et al*, 1990; Delgado *et al*, 1993; Maes and Meltzer, 1995).

5-HT dysfunction in depression may be promoted by genetic vulnerabilities. A commonly recognized genotype involves a polymorphism in the length of the 5-HT transporter-linked transcriptional promoter region (5-HTTLPR). This region encodes the 5-HT transporter protein (5-HTT) that controls 5-HT reuptake and function and which is the main target mechanism for most SSRI antidepressant drugs (Lotrich and Pollock, 2004; Neumeister *et al*, 2002). The short-allele (S) variant 5-HTTLPR is associated with lower 5-HTT protein concentrations than the long-allele (L) variant and accompanied by a dramatic reduction of 5-HT reuptake that subsequently may promote 5-HT dysfunction as found in depression (Caspi *et al*, 2003; Van Praag, 2004).

Although the S-allele 5-HTTLPR has been associated with depression, the relationship is not clear and synchronized as well as inconsistent findings have been reported (Lotrich and Pollock, 2004). This may partly be explained by failing to take into account that the 5-HTTLPR is in fact functionally tri-allelic due to the presence of an A>G single-nucleotide polymorphism within the L allele, rendering an

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Lg variant (as opposed to La) as a functional equivalent of the S-allele (Hu *et al*, 2005; Wendland *et al*, 2006). However, genes may not affect depression directly but rather promote depression particularly in the face of stress. Depression is often preceded by stress (Van Praag, 2004) and previous work often suggest a positive association between S-allele 5-HTTLPR and self-reports of past depressive responses to stressful life events (Caspi *et al*, 2003; Jacobs *et al*, 2006; Wilhelm *et al*, 2006), although this interaction between 5-HTTLPR, stressful life events and risk for depression has not always been found (Risch *et al*, 2009). In addition, stress is thought to promote depression mainly in 5-HT vulnerable subjects (who are sensitive to alterations or dysregulations in the 5-HT system) due to mutual interactions between the brain 5-HT and hypothalamus–pituitary–adrenal stress system (Van Praag, 2004).

Although genetic 5-HT vulnerability for stress and depression is a major challenge in biological psychiatry, the interaction between 5-HTTLPR, 5-HT vulnerability, stress and depression has not yet been experimentally investigated. In a recent interesting stress study carried out by Gotlib *et al* (2008), young healthy children with S/S genotypes showed greater cortisol responses to backward-counting stress than did L-allele carriers. Although this study included the tri-allelic notion, it did not include measurements for mood or brain 5-HT vulnerability. Brain 5-HT vulnerability is often explored by acute tryptophan depletion (ATD). This intervention reduces brain 5-HT function by lowering plasma tryptophan, the precursor of 5-HT, which competes with other large neutral amino acids (TRP/LNAA ratio) for uptake into the brain (Biggio *et al*, 1974; Carpenter *et al*, 1998; Gotlib *et al*, 2008; Nishizawa *et al*, 1997). Several studies have shown enhanced vulnerability for mood deterioration after ATD in healthy s-carriers (Neumeister *et al*, 2006; Neumeister *et al*, 2002; Williams *et al*, 1999). However, mood effects of ATD are rather mild, if observable at all (Roiser *et al*, 2006). Moreover, as subjects with S/S genotypes may exhibit altered 5-HT sensitization as a compensatory response to lower 5HTT expression (David *et al*, 2005; Moore *et al*, 2000), tryptophan challenge may be an alternative method to reveal differences in 5-HT vulnerability for reduced stress responses and mood improvements associated with 5-HTTLPR after acute stress exposure. This is the first study investigating the interaction between 5-HTTLPR and brain 5-HT manipulation (augmentation) on cortisol stress and mood responses during exposure to acute stress.

This study investigated whether 5-HTTLPR mediates the cortisol responses and mood effects of tryptophan administration during stress exposure in healthy subjects. It is hypothesized that TRP challenge improves mood and reduces cortisol stress responses in healthy participants with S/S genotypes after stress exposure.

MATERIALS AND METHODS

Participants

Undergraduate Dutch students (all with European ancestry) at Maastricht University ($N = 200$) filled out a questionnaire screening package regarding 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 several questionnaires regarding relevant symptoms and psychopathology (Beck Depression Inventory, Inventory of College Students' recent life experiences, Inadequacy/neuroticism Scale of the Dutch Personality Inventory, and the Social Complaints List). Participants were excluded from further evaluation if they reported chronic and current 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 an initial buccal sample extraction session to genotype for 5-HTTLPR. Participants with the S/S, S/L_G, L_G/L_G genotype classified as S'/S' and participants with the L_A/L_A genotype classified as L'/L' were selected for the study and invited to the laboratory for a psychiatric structured interview (MINI, Mannelli *et al*, 2006) to double-check exclusion compliance, to rule out psychiatric diagnoses, and to receive information about the experiment.

Sixteen subjects with homozygotic short alleles (S'/S') and 14 subjects with homozygotic long alleles (L'/L') were included and completed the experiment. All of them revealed normal body mass indexes (BMI in $kg\,m^{-2}$ between 20 and 25; mean 22 ± 2), were non-smokers, and were requested not to use alcohol or any kind of drugs 24 h before and during the course of the study (all subjects believed that this was controlled by a first early morning salivary sample during each experimental session). Both groups did not differ with respect to sex, age, BMI and additional relevant symptoms related to life experiences, neuroticism, or social complaints (see Table 1). 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 principles of Helsinki Declaration of 1975 as revised in 1983. All subjects gave their written informed consent to participate in the experiment and were paid for participation.

Design and Procedure

A placebo-controlled, double-blind, crossover design was used. During two experimental sessions, subjects were

Table 1 Demographic and Clinical Characteristics for the Tri-Allelic S/S′ and L/L' 5-HTTLPR Genotype Participants

	S/S' (S/S, S/Lg, Lg/Lg)	L/L' (La/La)
Women	15	13
Men	1	1
Age (M ± SD)	19 ± 2	19 ± 2
BMI (M ± SD)	22 ± 2	21 ± 2
BDI (M ± SD)	2 ± 2	3 ± 2
IN (M ± SD)	8 ± 7	8 ± 4
ICSRLE (M ± SD)	70 ± 8	66 ± 11
SCL (M ± SD)	113 ± 21	110 ± 16

BMI, body-mass index, BDI, beck depression inventory; IN, inadequacy/neuroticicism; ICSRLE, inventory college students' recent life experiences; SLC, social complaints list.

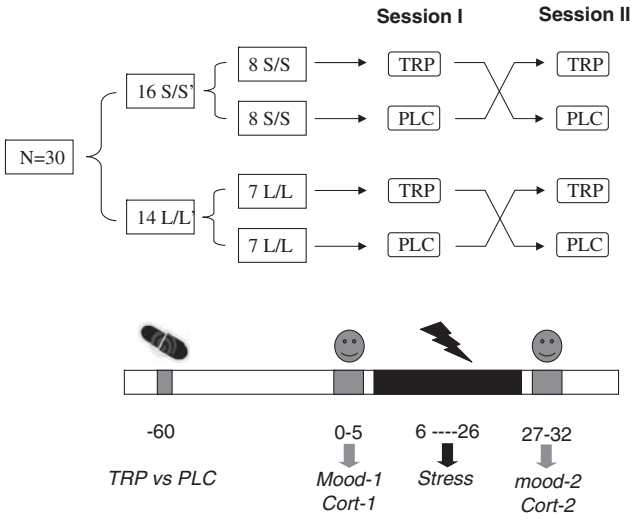

Figure 1 Schematic overview of the study design.

monitored (both occasions at the same time and day) for mood and stress coping following either intake of pure tryptophan (TRP) or placebo (PLC). The order of presentation of the TRP and PLC conditions was counterbalanced within groups; both experimental sessions were separated by at least 1 week. Female subjects were tested in the mid-late follicular phase of their menstrual cycle (days 4–10) or when actually taking oral contraceptives. An illustration of the design of the experiment is given in Figure 1.

All participants were instructed to fast overnight; only water or tea without sugar was permitted. On each experimental day, three participants arrived for testing at 0800, 1000, and 1200 hours, respectively. Capsules were consumed 1 h before arrival at the laboratory. (Capsules were collected 1–2 days before sessions and all subjects believed that intake was checked by salivary sample. All participants received a call-reminder the evening before the session; compliance was checked directly upon arrival.) After arrival, and a subsequent 15 min rest period, a first salivary sample was taken for measuring pre-stress cortisol and mood measurements were conducted with the Profile of Mood States (POMS) (Sheehan *et al*, 1994; Wald and Mellenbergh, 1990). Then, participants were exposed to a stress-inducing procedure including backward counting combined with frequent cold-pressor exposure at irregular unpredictable times for a total period of 20 min (see description below). After stress exposure, a second salivary cortisol sample was taken followed by a second POMS measurement. Both the experimenter and participant were blinded to the treatment condition.

Stress Exposure

To induce stress, subjects were placed in front of a camera (with the experimenter sitting behind) for 20 min while completing several 2-min serial subtraction sessions and frequent cold-pressor exposures. At different unpredictable occasions, they were signaled to start at a certain number (400, 425, 530, or 840) and count backwards by seven as quickly and accurately as possible. If they made a mistake, they were interrupted by the experimenter and had to start over. In addition, also at unpredictable intermittent 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, an in-house electronic software-controlled cooler device was developed. The device was composed of a copperplate surface (150 × 230 mm) that was thermoelectrically cooled by the way of 8 Peltier (TEC) device; it also included a powerful heatsink/fan combination and DC Volt controller. By this device, temperature was controlled at a constant level of 1.5 °C during exposures.

Backward counting was initiated at four different unpredictable 180 ± 30 s time intervals using four different numbers (400, 425, 530, or 840) that were counterbalanced within-subjects. Cold-pressor signals appeared at six different and unpredictable 120 ± 60 s time intervals either during (85%) or between (15%) counting sessions. In this way, subjects were unable to control the time course of exposure to backward counting and cold-plate exposure. During backward counting, the total amount of completed numbers and the total amount of mistakes were recorded. During cold-pressor exposure, the total time of endurance, pooled across exposures, was recorded. Before the start of the experiment, a pilot study was conducted in peer-students to obtain a sufficient level of temperature (maximum endurance of 60–90 s) and backward counting (maximum 50–60% completions within 2 min).

Tryptophan Challenge

To increase plasma tryptophan concentration for uptake into the brain, 2 × 0.4 g pure tryptophan (TRP) as compared with 2 × 0.4 g placebo (0.8 g lactose powder) capsules were used. Comparable intake of 0.8 g tryptophan has already been shown to increase plasma tryptophan availability (TRP/LNAA ratio) with 190% 60–90 min after intake (Wald and Mellenbergh, 1990). A sufficient quantity of TRP and PLC capsules were supplied by the Pharmacy of the Academic Hospital Maastricht (azM). These capsules were exactly identical in size and color and dispensed in containers distinguishable only by the label corresponding to the appropriate participant of the study. Subjects were instructed to swallow the capsules whole with water, being careful not to crush or break the capsule.

Profile of Mood States

Changes in mood were measured using the Dutch shortened version of the POMS questionnaire (Markus *et al*, 2008) including a visual-analogue scale ranging from 0 cm (not at all) to 10 cm (totally agree). The POMS comprises five different subscales for mood; ranging from anger, tension, depression, and fatigue that refer to a negative mood state, to vigor regarding a positive mood. This questionnaire enables researchers to measure mood changes either in diverse directions (including the five different POMS scales as multi-analysis measures) or as a generalized total mood disturbance (TMD) score (sum of anger, tension, depression, and fatigue subtracted from the vigor score).

Salivary Cortisol

Cortisol samples were obtained by using the Salivette sampling device (Sarstedt, Etten-Leur, Netherlands). With this procedure, saliva was collected on small cotton swabs and stored at −25 °C immediately upon collection until centrifugation. Saliva samples were centrifuged at 2650 g_{max} for 3 min at 20 °C. Salivary-free cortisol levels were determined in duplicate by direct radioimmunoassay (RIA; University of Liège, Belgium), including a competition reaction between ^{125}iodohistamine-cortisol and anti-cortisol serum raised against the 3-CMO–BSA conjugate. After overnight incubation of 100 µl of saliva at 4 °C, separation of free and antibody-bound ^{125}iodohistamine-cortisol was carried out by a conventional secondary antibody method. To reduce sources of variability, all samples were analyzed in the same assay. Mean intra- and inter-assay coefficients of variation were less than 4.5 and 8.5%, respectively.

Buccal Cells for Tri-Allelic 5-HTTLPR Polymorphism

Buccal cell samples for measuring tri-allelic variants of the 5-HTT-linked polymorphic region (5-HTTLPR) were obtained using sterile swabs (Omni Swabs, Whatman, 's Hertogenbosch, The Netherlands). Genomic DNA was isolated from buccal swabs using the QIamp DNA Mini Kits from Qiagen (Westburg, Leusden, The Netherlands) for determination of the 5-HTTLPR genotype. Genotyping was carried out using the polymerase chain reaction (PCR) protocol according to Glatz *et al* (2003). In compliance with previous work (Glatz *et al*, 2003; Neumeister *et al*, 2006; Walderhaug *et al*, 2007), 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

The main research questions were analyzed by means of repeated measures multivariate and univariate analyses of variance (MANOVA and ANOVA) by using the General Linear Model (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: pre-stress *vs* post-stress) on the several dependent measures (mood, performance, endurance, cortisol). Although we counterbalanced for order of treatment (TRP first followed by PLC, *vs* the opposite order), order of treatment was preliminary taken as a between-subjects factor. For measuring effects on the five levels of the POMS, multivariate analyses of variance were performed and only significant multivariate main or interaction effects were further interpreted by univariate results. Because order of diet did not contribute to any of the scores, final analyses were carried out 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

Salivary Cortisol

Repeated ANOVA with Treatment (TRP *vs* PLC) and stress (pre- *vs* post-stress) as within subjects factors and genotype (S'/S' *vs* L'/L') as between-subjects factor on cortisol concentrations revealed only a significant main effect of stress ($F(1,28) = 13.25$, $p = 0.001$). As shown in Figure 2, cortisol significantly declined post-stress (289 ± 160) compared with pre-stress (348 ± 189), regardless of treatment or genotype. No other effects were found.

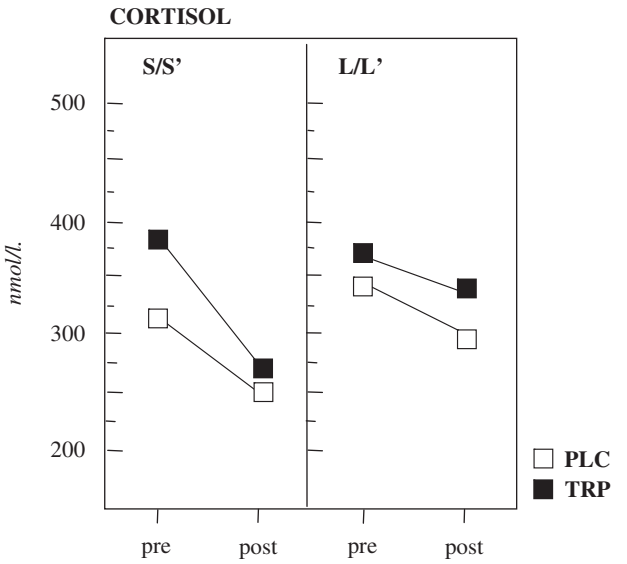

Figure 2 Salivary cortisol concentrations before (pre) and after (post) stress exposure in tri-allelic S'/S' and L'/L' 5-HTTLPR genotypes following TRP challenge (■) or PLC (□).

POMS

Repeated MANOVA with treatment (TRP *vs* PLC) and stress (pre- *vs* post-stress) as within-subjects factors and genotype (S'/S' *vs* L'/L') as between-subjects factors on the scores of the subscales of the POMS revealed a significant main MANOVA effect of stress ($F(5,24) = 5.35$, $p = 0.002$), meaning a significant change in mood during stress performance. Further univariate results revealed that this originated from changes in anger ($F(1,28) = 16.33$; $p < 0.001$), depression ($F(1,28) = 22.16$; $p < 0.001$), vigor ($F(1,28) = 13.0$; $p = 0.001$), and fatigue ($F(1,28) = 19.71$; $p < 0.001$). After stress, there was a significant increase in anger (from 8 ± 1 to 8.2 ± 1: $p < 0.001$) depression (from 7.7 ± 1 to 8.1 ± 1: $p < 0.001$), and fatigue (6.8 ± 1.1 to 7.4 ± 1.2: $p < 0.001$), as well as a reduction in vigor (from 3.0 ± 1 to 2.4 ± 1: $p = 0.001$).

Multivariate analyses of variance further revealed an interaction of treatment × genotype ($F(5,24) = 4.0$; $p = 0.009$), indicating that mood significantly differed between treatment conditions depending on 5-HTTLPR. Further univariate results revealed that this originated from changes in depression ($F(1,28) = 4.8$; $p = 0.038$), vigor ($F(1,28) = 14.8$; $p = 0.001$), and fatigue ($F(1,28) = 5.7$; $p < 0.024$). As shown in Figure 3, only in S/S' genotypes did TRP (as compared with PLC) result in a significant reduction in depression (from 8.1 ± 0.7 to 7.5 ± 1; $p = 0.03$) and fatigue (from 7.5 ± 0.8 to 6.8 ± 1; $p = 0.03$) and an increase in vigor (from 2.2 ± 0.9 to 3.1 ± 1; $p = 0.01$). No effects of treatment were found in L/L' ($p > 0.2$).

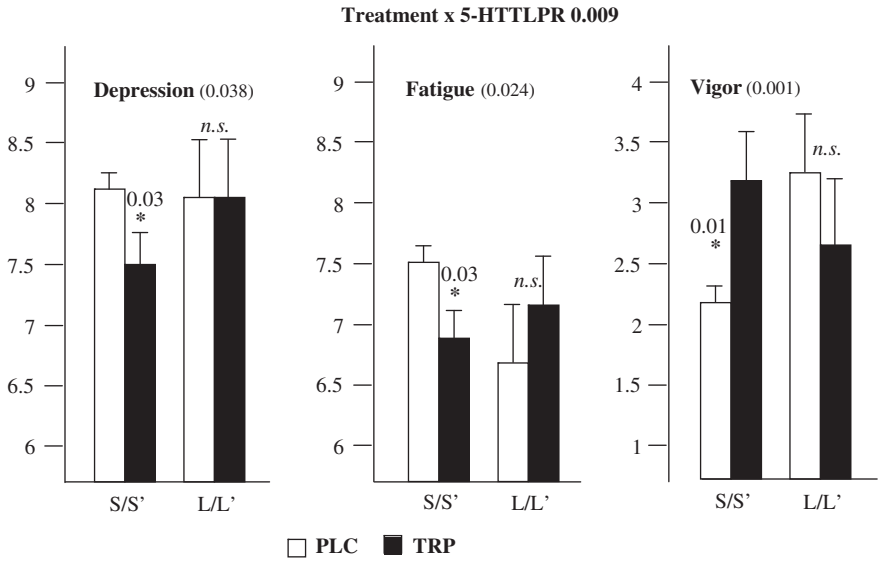

Figure 3 TRP challenge (■) compared with PLC (□) improved feelings of depression, fatigue, and vigor in tri-allelic S'/S' but not in L'/L' 5-HTTLPR genotypes regardless of stress (pooled for stress).

An additional ANOVA with treatment (TRP *vs* PLC) and stress (pre- *vs* post-stress) as within-subjects factors and genotype (S'/S' *vs* L'/L') as between-subjects factor was conducted on the TMD of the POMS. Analysis again revealed an effect of stress ($F(1,28) = 22.15$; $p < 0.0001$) and an interaction of treatment × genotype ($F(1,28) = 9.87$; $p < 0.004$). TMD became significantly higher after stress (30 ± 6) as compared with pre-stress (27 ± 5). As indicated in Figure 4, only in S'/S' genotypes did mood disturbance become significantly lower after TRP (27 ± 4) as compared with PLC (30 ± 3) ($t(15) = 2.8$; $p = 0.013$), whereas no differences between TRP and PLC was found in L'/L' genotypes ($p > 0.1$).

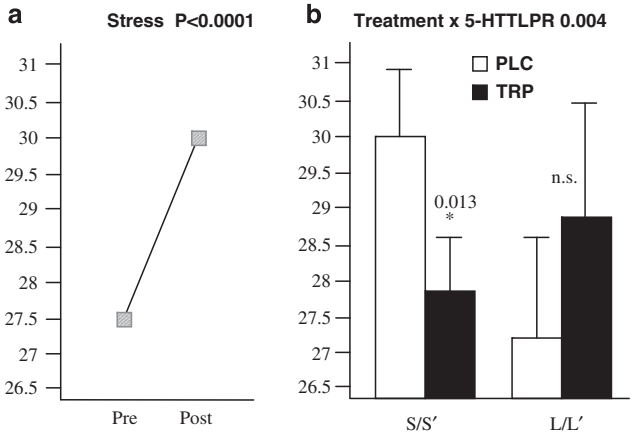

Figure 4 (a) Total mood disturbance scores significantly increased after stress (post) compared with before stress (pre). (b) TRP challenge (■), as compared with PLC (□), reduced total mood disturbance scores in tri-allelic S'/S' but not in L'/L' 5-HTTLPR genotypes regardless of stress (pooled for stress).

Cold-Plate Performance

Repeated measures ANOVA with treatment (TRP *vs* PLC) and stress (pre- *vs* post-stress) as within-subjects factor and genotype (S'/S' *vs* L'/L') as between-subjects factor on the total number of mistakes during backward counting revealed a significant treatment × genotype interaction ($F(1,28) = 7.11$; $p = 0.013$). This means that treatment influenced the total amount of mistakes during backward counting depending on 5-HTTLPR genotype. As indicated in Figure 5, S'/S' genotypes made significantly more mistakes during backward counting after PLC (5 ± 3) than after TRP (3 ± 2) ($p < 0.03$), whereas there were no treatment effects in L'/L' genotypes. Note that after PLC, S'/S' appeared to perform worse than L'/L' genotypes, which seem to be prevented in S'/S' after TRP treatment. This however did not reach significance ($p = 0.06$).

As improved backward counting in S'/S' genotypes after TRP might have been accompanied by reduced cold-pressor interference, an additional ANOVA with treatment (TRP *vs* PLC) as within-subjects factor and genotype (S'/S' *vs* L'/L') as between-subjects factor was conducted on the total time of cold-pressor endurance across exposures. Although only S'/S' seemed to withstand the cold-pressor longer after TRP (67 s) than after PLC (60 s), analysis did not reveal a significant treatment × genotype interaction ($p = 0.1$), or any other effect, on cold-pressor endurance.

DISCUSSION

The aim of this study was to assess 5-HT vulnerability of mood changes in healthy subjects with different tri-allelic 5-HTTLPR genotypes following TRP or PLC administration under stress exposure. Results revealed beneficial effects of TRP challenge on mood and backward counting exclusively in S'/S' genotypes.

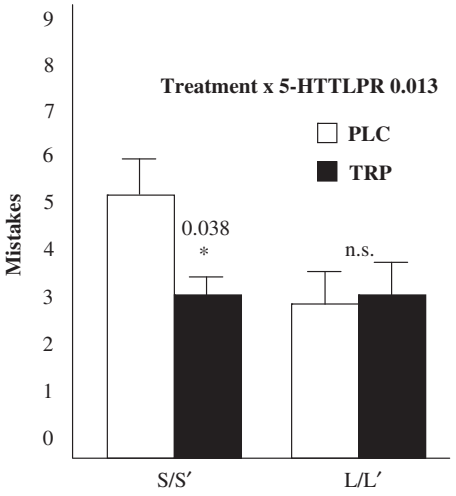

Figure 5 Backward-counting errors (mistakes) reduced after TRP challenge (■) compared with PLC (□) in tri-allelic S'/S' but not in L'/L' 5-HTTLPR genotypes regardless of stress (pooled for stress).

Effect of Tryptophan Challenge on 5-HT

The effectiveness of TRP as a method to increase plasma TRP/LNAA and brain 5-HT has consistently been demonstrated (Fernstrom, 1990; Young, 1986, 2007; Young and Gauthier, 1981; Zalsman *et al*, 2006). In addition, the same dose of TRP used in this study has previously been found to cause a large 190% increase in the plasma TRP/LNAA ratio (Markus *et al*, 2008). As even lower (50–70%) variations in this plasma TRP/LNAA ratio are sufficient to alter brain TRP and 5-HT content (Biggio *et al*, 1974; Carpenter *et al*, 1998; Fernstrom and Wurtman, 1971; Markus *et al*, 2008; Markus *et al*, 2000; Nishizawa *et al*, 1997; Williams *et al*, 1999) (Orosco *et al*, 2004), it seems safe to conclude that the current dose of TRP caused a sufficient increase in TRP/LNAA and brain 5-HT.

TRP, 5-HTTLPR, and Mood

As previous studies have reported mood-lowering effects of ATD in healthy S-allele genotypes (Neumeister *et al*, 2006; Neumeister *et al*, 2002; Wurtman *et al*, 2003), it was hypothesized that TRP challenge in these genotypes may cause mood improvement. Current findings indeed reveal that TRP administration improves mood only in S'/S' genotypes. More precisely, TMD scores were higher in S'/S' compared with L'/L' carriers after PLC; in S'/S' this was counteracted by TRP administration. This TRP-induced improvement in S'/S' originated mainly from changes in depression, fatigue, and vigor. Although effects of TRP intake have not been investigated before in different 5-HTTLPR genotypes, current findings comply with mood-improving effects of TRP in apparently (pre-clinical) 5-HT-vulnerable subjects with mild depression (Roiser *et al*, 2006) or chronic stress experiences (see for a review Young, 1986).

Assuming 5-HT vulnerability in S-allele genotypes and 5-HT involvement in stress, it was further predicted that 5-HTTLPR would mediate the effects of TRP administration on mood under stress. Hence, stress increases 5-HT neurotransmission for stress adaptation by mediating negative feedback control of cortisol on the HPA axis (Markus, 2008; Nuller and Ostroumova, 1980; Van Praag, 2004). Assuming that S-allele homozygotes are 5-HT-vulnerable, they might benefit from TRP challenge under stress. To induce stress, repeated backward counting was combined with frequent uncontrollable cold-pressor exposures. Single as well as repetitive cold-water exposure has already been shown to be sufficient to cause psychological and biological stress responses (Markus, 2007). In addition, a single backward-counting session followed by a competence interview caused a modest cortisol response in S'/S' but not L'/L' genotypes (Gotlib *et al*, 2008). Therefore, it was expected that frequent uncontrollable cold-pressor exposures in combination with repeated backward-counting sessions should increase stress severity. However, analyses did not reveal any interaction of stress with 5-HTTLPR. Even though mood became significantly worse after stress exposure, this did not depend on 5-HTTLPR. It might be that this stress procedure was still too mild for young adult university students. It is noteworthy that we did not find a cortisol stress response in S'/S' nor in L'/L' carriers. Gotlib *et al* (2008) reported a cortisol response already after a single 3 min backward-counting session (followed by a 12 min competence interview) exclusively in S'/S' young children (aged 9–14). This suggests that young S'/S' children are more stress vulnerable than young S'/S' or L'/L' adult university students. Regrettably, Gotlib *et al* (2008) did not include effective stress measures or 5-HT manipulations.

Besides mood improvement, backward counting also improved after TRP solely in S'/S' allele carriers. Only S'/S' genotypes made significantly (25%) fewer counting mistakes under cold-pressor exposures after TRP compared with PLC. This could not be attributed to differences in pain endurance and therefore may be best explained as a function of TRP-induced mood improvement; although a direct serotonergic effect on performance might still be possible (Gotlib *et al*, 2008).

Current findings raise the question about the underlying biochemical mechanism that explains the effects of TRP challenge in healthy S'/S' genotypes. These effects seem counterintuitive assuming that reduced 5-HTT expression causes lifelong increases in synaptic 5-HT and downregulation of 5-HT receptors (Roiser *et al*, 2006). For instance, healthy S'/S' compared with L'/L' genotypes showed depressive type responses after ATD, which was assumed to be caused by downregulation of 5-HT1a receptors and reduced 5-HT system adaptation to brief disruptions. In contrast, remitted depressive patients showed effects of ATD when carrying the L-allele, which was explained by more pronounced postsynaptic 5-HT1a desensitization combined with presynaptic 5-HT1a upregulation (resulting in a decreased threshold and less adequate 5-HT responses during ATD). This conception does not make it easy to explain the current beneficial effects of TRP in healthy S'/S' genotypes. Alternatively, although hypothetical and not yet clearly established, lower pre-synaptic 5-HTT expression in healthy S'/S' genotypes might still increase postsynaptic sensitization at specific receptor sites as a compensatory response. For instance, the post-synaptic 5-HT agonist meta-chlorphenylpiperazine is found to

enhance brain 5-HT responses in healthy S/S genotypes; although it must be taken into account that this was found in an African descent sample in whom the S-allele might behave differently (Neumeister *et al*, 2006).

CONCLUSION

5-HTTLPR differentially mediates the effect of TRP on mood and performance. Only in S'/S' genotypes, TRP improved mood and backward counting. This suggests pronounced 5-HT vulnerability to TRP challenge in healthy S'/S' as compared with L'/L' carriers. Effects of TRP administration in S'/S' genotypes were not influenced by stress exposure, probably because stress exposure was too mild to be of any relevance. Brain 5-HT manipulation might still influence mood under stress in S'/S' when including a more severe stressful event.

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DISCLOSURE

All the authors ensure the integrity of the work and none of them has any direct or indirect financial or personal interest, or conflict of interest, to the subject matter of the paper.

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