



Universiteit
Leiden
The Netherlands

Testing antidepressant compounds in a neuropsychological model of drug action

Cerit, H.

Citation

Cerit, H. (2015, March 12). *Testing antidepressant compounds in a neuropsychological model of drug action*. Retrieved from <https://hdl.handle.net/1887/32211>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/32211>

Note: To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/32211> holds various files of this Leiden University dissertation

Author: Cerit, Hilal

Title: Testing antidepressant compounds in a neuropsychological model of drug action

Issue Date: 2015-03-12



Chapter 4

The Effect of Tryptophan on the Cortisol Response to Social Stress Is Modulated by the 5-HTTLPR genotype

H Cerit, LAW Jans, AJW Van der Does

Abstract

Objective: The S'/S' (S/S, S/Lg and Lg/Lg) variant of the serotonin (5-HT) transporter gene linked polymorphic region (5-HTTLPR) is associated with less efficient neurotransmission and may be more reactive to 5-HT manipulations. We tested the effects of l-tryptophan supplements on the cortisol response induced by a social stressor in S'/S' and L'/L' (La/La) carriers.

Methods: In a double-blind parallel design, 25 S'/S' carriers and 21 L'/L' carriers were randomized to take l-tryptophan (2.8 g/d) or placebo supplements for six days. At day 7 participants were exposed to the Trier Social Stress Test. Salivary cortisol and subjective mood states were monitored before, during and after the stress procedure.

Results: S'/S' carriers who took l-tryptophan supplements had a significantly lower cortisol response to stress than S'/S' carriers who took placebo. Tryptophan had no effect on cortisol in L'/L' carriers and no effect on subjective mood states in either genotype group.

Conclusion: Tryptophan attenuates the cortisol response to acute social stress depending on 5-HTTLPR genotype. S'/S' carriers may indeed be more reactive to 5-HT manipulations.

Introduction

The serotonin (5-hydroxytryptamine; 5-HT) neurotransmission system and the hypothalamic-pituitary-adrenal axis (HPA axis) have complex interrelationships (Porter *et al.*, 2004). Both systems have important roles in the response to stress and are implicated in depression. In the present study, we examined the effect of the 5-HT precursor L-tryptophan (TRP) on reactivity of the HPA axis, specifically investigating whether this effect is modulated by genetic variation in the 5-HT system.

The serotonin transporter (5-HTT) membrane protein is essential in regulating the concentration of 5-HT in the synaptic cleft. The 5-HTT is encoded by the SLC6A4 gene, and transcriptional activity of this gene is regulated by the 5-HTT-linked polymorphic region (5-HTTLPR). Two major variants of the 5-HTTLPR exist, with functional significance in that the short (S) allele of the 5-HTTLPR is associated with less transcriptional efficiency and less 5-HTT expression than is the long (L) allele (Lesch *et al.*, 1996). A functional A/G polymorphism (rs25531) has been found within the L allele (Nakamura *et al.*, 2000) as well, indicating that the 5-HTTLPR is functionally triallelic (S / Lg / La). The Lg variant is associated with reduced 5-HTT transcriptional efficiency, comparable to the S allele (Hu *et al.*, 2006; Praschak-Rieder *et al.*, 2007; Reimold *et al.*, 2006). The reduced efficiency 5-HTT variants (S and Lg) are referred to as S', the La variant as L'. Biallelic classifications are indicated as S and L.

Several studies have found that differences in 5-HTTLPR genotype are associated with differing HPA axis reactivity in healthy individuals. Healthy girls (aged between 9 and 14 years) who were homozygous for the S allele showed an increase in cortisol response during and following exposure to a laboratory stress task, whereas girls carrying at least one copy of the L allele had a slight decrease in cortisol response (Gotlib *et al.*, 2008). This differential pattern of cortisol response was independent from history of depression in participants' mothers. Way and Taylor (2010) exposed healthy young adults to a similar social stress task and found that individuals homozygous for the S allele had a significantly stronger cortisol response 40 minutes after onset of the stressor. The individuals homozygous for the L allele had the least response, with the heterozygous (S/L) falling in between.

Wüst *et al.* (2009), however, did not observe an effect of 5-HTTLPR on the cortisol response to a social stress task. This study had a large sample size (N = 216) and grouped individuals based on triallelic classification. The non-replication may have been caused by the fact that all 126 female participants used oral contraceptives, which dampen the cortisol response to stress (Kirschbaum *et al.*, 1999). Another recent study reported no effect of 5-HTTLPR genotype or neuroticism on the cortisol response to social stress in 94 college students (Verschoor & Markus, 2011). Mueller *et al.* (2011) even reported a **larger** cortisol response to social stress in both younger (18-31 years) and older (54-68 years) adults who were homozygous for the L' allele than individuals carrying at least one S' allele. This effect was not observed in children (8-12 years). In the younger adults (N = 106), genotype interacted with early (during the first five years of life) stressful life events: L'/L' individuals had a much higher cortisol response to

stress than S' carriers in the absence of early stressful life events. This pattern was reversed for people who had experienced three or more early life events. In a study with new-borns, S'/S' individuals had a larger cortisol response to a pain stimulus (heel prick) (Mueller *et al.*, 2010). Finally, male S'/S' carriers with a history of stressful life events had a larger cortisol response 35 minutes after the onset of the social stressor than male S'/S' carriers without such history. The cortisol response in S'/S' carriers with a history of stressful life events was also higher than that of male S'/L' and L'/L' carriers (with and without life events) (Alexander *et al.*, 2009).

The central theme of these reports demonstrates that 5-HT function and 5-HTTLPR genotype affect HPA axis reactivity to social stress; however, the direction of this interaction has yet to be conclusively established. Age as well as both type and time of occurrence of stressful life events have been suggested to play a role in the effect of gene-environment interactions on cortisol response (Mueller *et al.*, 2011).

The relationship between the 5-HT system and HPA axis reactivity has also been investigated by manipulating tryptophan (TRP) levels in stress-vulnerable populations, as defined by family or personal history of depression or by questionnaire scores indicating vulnerability, e.g. high neuroticism. Experimental interventions that temporarily increase TRP include: carbohydrate rich/protein poor (CR/PP) meal (Markus *et al.*, 1998; Markus *et al.*, 2000b), tryptophan-rich egg protein hydrolysate (EPH) (Markus *et al.*, 2010), carbohydrate rich drink (Markus, 2007), whey protein α -lactalbumin (α -lac) (Markus *et al.*, 2000a; Merens *et al.*, 2005; Booij *et al.*, 2006; Nesic & Duka, 2008) and tryptophan-rich hydrolyzed protein (HP) (Firk & Markus, 2009). Stress responses were induced with the cold pressor test (CPT), arithmetic stress task (under noise stimulation) or public speaking. Stress-vulnerable populations were expected to benefit from increasing TRP availability, as expressed in improved mood and lower cortisol responses to stress. The above-mentioned interventions and stressors revealed no consistent pattern of effects. The lack of a consistent effect may have been due to methodological limitations, including: a relatively small impact of the interventions on tryptophan concentrations, less than optimal timing of the cortisol measurements, or the nature of the stressor. Non-social stressors (e.g. cold pressor) are less reliable in eliciting a cortisol response than are social stressors (e.g. public speaking) (Dickerson & Kemeny, 2004).

A recent study combined a TRP manipulation and stress exposure with measurement of 5-HTTLPR genotype (Markus & Firk, 2009). Sixteen healthy S'/S' and 14 L'/L' carriers were exposed to stress one hour after a single dose tryptophan (0.8 g) and after taking placebo in a cross-over design. The stress procedure consisted of a combination of backward counting tasks and cold-pressor exposures at unpredictable intervals. TRP improved mood and reduced backward counting errors in S'/S' participants but not in L'/L' participants, and no group differences were found on the cortisol response to stress. Remarkably, cortisol concentrations were lower at post-stress than at pre-stress regardless of intervention or genotype. The number of cortisol measurements was limited to single pre- and post-

stress samplings. The authors concluded that TRP challenge improves mood and stress performance in S'/S' participants (Markus & Firk, 2009). 'Stress performance' referred to the number of errors in the backward counting task that was part of the stressor. Under placebo conditions, S'/S' participants made more mistakes than L'/L' participants, which seems to have been corrected by TRP.

Given the methodological issues in previous TRP loading studies, we examined the effect of six days of TRP supplementation on reactivity of the HPA axis to a social stressor, specifically investigating whether its role is modulated by 5-HTTLPR genotype. We used a parallel design, exposing every participant to the stressor only once. Furthermore, we monitored physiological data for 95 minutes at six time points pre- and post-stress. Possible confounders of the stress response were avoided by excluding users of oral contraceptives and by testing female participants during the luteal phase of their menstrual cycle. We tested the following hypotheses: 1) S'/S' carriers have larger cortisol responses to social stress than L'/L' carriers; 2) the increased cortisol response to stress of S'/S' carriers will be reduced by tryptophan.

Methods and Materials

Participants

Participants were selected from a pool of 581 genotyped individuals who had been recruited at various sites at Leiden University and through local advertisements. Participants were non-smokers and were included if all four grandparents were West-European. For the present study, the age range was 18 to 35 years and Body Mass Index was between 19 and 29 kg/m². Exclusion criteria were a current diagnosis of depression or post-traumatic stress disorder, a lifetime history of psychosis, and use of medication, including oral contraceptives. The presence of anxiety disorders was also assessed, but this was not an exclusion criterion. The following two genotype groups S'/S' (S/S, S/Lg and Lg/Lg variants) and L'/L' (La/La variant) were invited to participate in the present study. Participants received a reward of € 40. The research was approved by the Ethics Committee of the Leiden University Medical Center in The Netherlands.

Genotyping

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 proteinase K and 0.5% w/v SDS) until further processing. Genomic DNA was isolated from the samples using the Chemagic buccal swab 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 mg of genomic DNA per sample.

Polymerase chain reaction amplification

The region of interest from the 5-HTT gene was amplified by triplex PCR using the following primers: a FAM-labeled primer HTTLPR-FWFAM 5'-TCCTCCGCTTTGGCGCCTCTTCC-3', and a reverse primer HTTLPR-RV 5'-TGGGGGTTGCAGGGGAGATCCTG-3'. Typical PCR reactions contained between 10 and 100 ng genomic DNA template, 10 pmol of forward and reverse primer. PCR was carried out in the presence of 5% DMSO with 0.5U 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 sec 96°C, 30 sec 61°C, 60 sec 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 HpaI in a total volume of 20 µl. Restriction enzyme mix was incubated with DNA for 3 hours 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 an AB 3100 genetic analyser set up for genotyping with 50 cm capillaries. Results were analysed using Genescan software version 3.7 (Applied Biosystems, Carlsbad, CA, USA), 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.

Instruments

Diagnosis. The Mini International Neuropsychiatric Interview (M.I.N.I.) was administered (Sheehan *et al.*, 1997; Van Vliet *et al.*, 2000) to assess psychiatric diagnoses.

Psychiatric symptoms and mood states. Three self-report questionnaires were used. Current symptoms of anxiety and depression were measured using the hospital anxiety and depression scale (HADS; Zigmond & Snaith, 1983; Spinhoven *et al.*, 1997). The HADS is a 14-item self-report scale which assesses past-week severity of anxiety and depressive symptoms. Positive and negative affect was assessed by means of the Positive and Negative Affectivity Scales (PANAS), a 20-item questionnaire (Watson *et al.*, 1988). The state version (today) was used. The mood states Sadness, Annoyance, Tension and Anxiety were assessed using single-item Mood States Scales (MSS) with scores ranging from 0 (not at all) to 10 (extremely).

Stress Induction

The Trier Social Stress Test (TSST; Kirschbaum *et al.*, 1993) is a combination of a public speaking task in front of an unresponsive audience and a mental arithmetic task at high speed and with public correction of every mistake. During an initial resting period of 50 minutes, baseline saliva cortisol samples were obtained at time points t_{15} and t_{50} . Subsequently, participants were informed that they were about to give a speech in front of an audience consisting of three persons, and that they would also complete another task that this audience would announce to them (i.e. the mental arithmetic task). The TSST protocol further prescribes that participants were informed that their speech would be videotaped and evaluated by skilled psychologists with regard to content and performance. During the verbal instructions the experimenter briefly showed the room with the audience waiting for him/her. Subsequently, participants were placed in a quiet room and given six minutes to prepare themselves. They were instructed that it was not allowed to keep any notes during their speech. At the end of this anticipation period, another saliva sample was taken and the participant entered the room in order to deliver the speech and complete the arithmetic task. At the end of the arithmetic task, the experimenter entered the room and took another saliva sample in front of the audience. Subsequently, the participant was guided to a quiet room and was allowed to rest and read magazines for 45 minutes. Saliva samples were collected at six points in time (with t_0 representing the time of arrival): rest 1 (t_{15}), rest 2 (t_{50}), after anticipation (t_{56}), after

speech and arithmetic task (t_{65}) and twice during the recovery period (t_{90} and t_{110}). During saliva sampling participants rated their mood states on the MSS.

Salivary cortisol assessment

Salivary cortisol was assessed using Salicaps (IBL International, Germany). Saliva samples were stored at -20°C until assayed at the laboratory of biopsychology at the University of Dresden, Germany. Free cortisol concentrations in saliva were measured using a commercially available “Luminescence Immunoassay for the in-vitro-diagnostic quantitative determination of cortisol in human saliva and serum” (IBL, Hamburg, Germany). The intra and interassay coefficients of variance for cortisol was below 8%.

Design and procedure

This study was a randomized double-blind placebo-controlled trial with stratification for genetic variation of the 5-HTTLPR genotype. Participants were randomly allocated to receive 7 capsules containing either TRP (total dose of 2.8 g/day) or placebo (cellulose microcrystalline) for a period of six days. The dosage and duration were based on previous studies that had shown social-behavioural effects of TRP administration (3 g/day) after a period of 15 days (Aan het Rot *et al.*, 2006) and cognitive effects after a single dose of 0.8g TRP (Markus & Firk, 2009). The experimental procedure included two visits to the laboratory, pre- and post-TRP.

First visit to laboratory.

Upon arrival at the laboratory, participants provided written informed consent for the study. Following the M.I.N.I. interview, participants performed experimental tasks and filled out questionnaires on a computer. At the end of the first visit, participants were provided with 42 capsules that contained 400 mg tryptophan or placebo (PLC). Oral and written instructions were provided to the participants regarding the timing of administration of capsules and lifestyle restrictions during the next six days and on the day of the second lab visit.

Tryptophan supplementation

Participants started to take the capsules the day after their first lab visit. They were instructed to take two capsules in the morning, two in the afternoon (before meals) and three in the evening (before 23.00h). Participants received a diary in which they were asked to write down the exact time of intake and number of capsules. Compliance was not measured through blood sample analyses, however participants were led to believe that compliance would be assessed at post-intervention through a saliva sample.

Lifestyle instructions included: no smoking, no use of dietary supplements and vitamins and consumption of alcohol limited to 3 units/day. Participants were also instructed to refrain from alcohol and caffeine-containing consumptions and avoid high carbohydrate meals on the day of their second visit. Further instructions for the day of the second visit included: no eating and drinking one hour before arriving at the laboratory (except water), and no physical exercise at least two hours before arrival. Female participants were tested in the luteal phase of their menstrual cycle. All test sessions started in the afternoon between noon and 5pm.

Second visit to laboratory

Upon arrival at the lab participants handed in their diary regarding the intake of capsules. In addition, they were asked to fill out a debriefing questionnaire regarding compliance to the instructions during the previous six days. They were also interviewed about their compliance to the instructions for the second lab visit. Next, participants were asked to perform a number of tests and to fill out questionnaires on a computer. Finally, participants performed the TSST. After completion of the TSST procedure participants were fully debriefed and paid.

Results

Sample characteristics

For the total sample of $n = 581$, genotype frequencies were as follows: SS, 16,9%; SLg, 4,8%; LgLg, 0,7%; LaLg, 8,6%; SLa, 43%; LaLa, 26%. Participants were divided on the basis of the triallelic classification (Lg alleles were collapsed with S variants into three genotype groups: S'/S' ($n = 130$); L'/S' ($n = 300$); L'/L' ($n = 151$). Genotype frequencies were consistent with Hardy-Weinberg Equilibrium ($\chi^2(1) = 0.67$; $p = 0.41$). We contacted 92 S'/S' carriers and 92 L'/L' carriers by email. Sixty-four S'/S' and 66 L'/L' carriers expressed interest in the study. After screening for in- and exclusion criteria we included 26 S'/S' and 22 L'/L' participants. In each group, one participant dropped out before day 2. Analyses were conducted on 25 S'/S' and 21 L'/L' carriers.

The first two participants (one S'/S' and one L'/L' carrier) received tryptophan single blind. Since the TSST panel was double-blind for these participants and no observer ratings were collected, we kept these participants in the analyses. The demographic details of both groups are shown in Table 1. Groups did not differ significantly on assessed demographic characteristics. One participant in the PLC group (S'/S' carrier) had a current diagnosis of panic disorder, and one participant in the TRP group (S'/S' carrier) had a specific phobia (needles). Both participants were not taking any medication.

Table 1. Demographic characteristics for both genotype groups.

	S'/S' (S/S, S/Lg, Lg/Lg)	L'/L' (La/La)
Female	11	11
Male	14	10
Age (M±SD)	20.4 ± 3.5	20.3 ± 2.5
BMI (M±SD)	19.4 ± 3.0	19.2 ± 1.7

Note: Mean ± Standard Deviation. Abbreviation: BMI, Body Mass Index.

Compliance

According to self-report, approximately 98% of the capsules were taken according to instructions. The minimum percentage of capsules taken by a participant was 69%. Three participants had taken two capsules in the morning before the second lab visit. All these participants were retained.

Effects of TRP on psychiatric symptoms

In order to analyse the effects of intervention and genotype on psychiatric symptoms, separate RM-GLMs for each of the questionnaire scores (HADS Anxiety, HADS Depression, PANAS-S Pos and PANAS-S Neg) were conducted with Time as within subject factor, and Intervention (TRP vs. PLC) and Genotype (S'/S' vs. L'/L') as between subject factors. No significant effects were found for intervention or genotype (Table 2).

Table 2. Symptom scores for each genotype and intervention group assessed pre- and post-intervention.

		Anxiety	Depression	Negative Affect	Positive Affect
S'/S' TRP	Pre	3.6 ± 2.0	1.9 ± 1.2	12.1 ± 4.3	30.1 ± 6.7
	Post	3.8 ± 2.4	2.8 ± 2.8	13.3 ± 3.3	32.1 ± 5.2
S'/S' PLC	Pre	4.8 ± 3.3	1.4 ± 1.6	12.8 ± 2.8	32.6 ± 4.0
	Post	3.7 ± 3.7	1.3 ± 1.5	12.3 ± 2.3	34.6 ± 4.9
L'/L' TRP	Pre	3.8 ± 2.4	1.4 ± 1.4	12.2 ± 2.7	30.3 ± 8.5
	Post	4.2 ± 3.3	1.5 ± 1.4	11.9 ± 2.1	28.5 ± 8.9
L'/L' PLC	Pre	3.9 ± 3.8	2.4 ± 2.6	13.1 ± 2.4	29.5 ± 5.9
	Post	3.7 ± 2.2	2.5 ± 2.3	15.4 ± 7.0	31.9 ± 8.2

Note: Mean ± Standard Deviation. Abbreviations: TRP, tryptophan; PLC, placebo.

Cortisol Response to TSST

The cortisol data were not normally distributed. Log¹⁰-transformations were successful in normalizing the distributions. Analyses of transformed data are reported, but the figures represent untransformed data. RM-GLMs were conducted in the S'/S' and L'/L' groups separately on cortisol concentrations, with Time (the six cortisol measurements) as within subjects factor and Intervention (TRP vs. PLC) as a between subjects factor. Gender was included as covariate. Greenhouse-Geisser statistics are reported. In the S'/S' group, the main effect of Time was significant ($F(1.50, 33.07) = 6.72, p = 0.007, \eta^2 = 0.234$) and the Time x Intervention interaction was borderline significant ($F(1.50, 33.07) = 3.55, p = 0.052, \eta^2 = 0.139$). In the L'/L' group, the main effect of Time was a trend ($F(1.76, 31.70) = 3.09, p = 0.065, \eta^2 = 0.146$) and the interaction was non-significant. Figure 1 displays the cortisol concentrations over time by intervention and genotype.

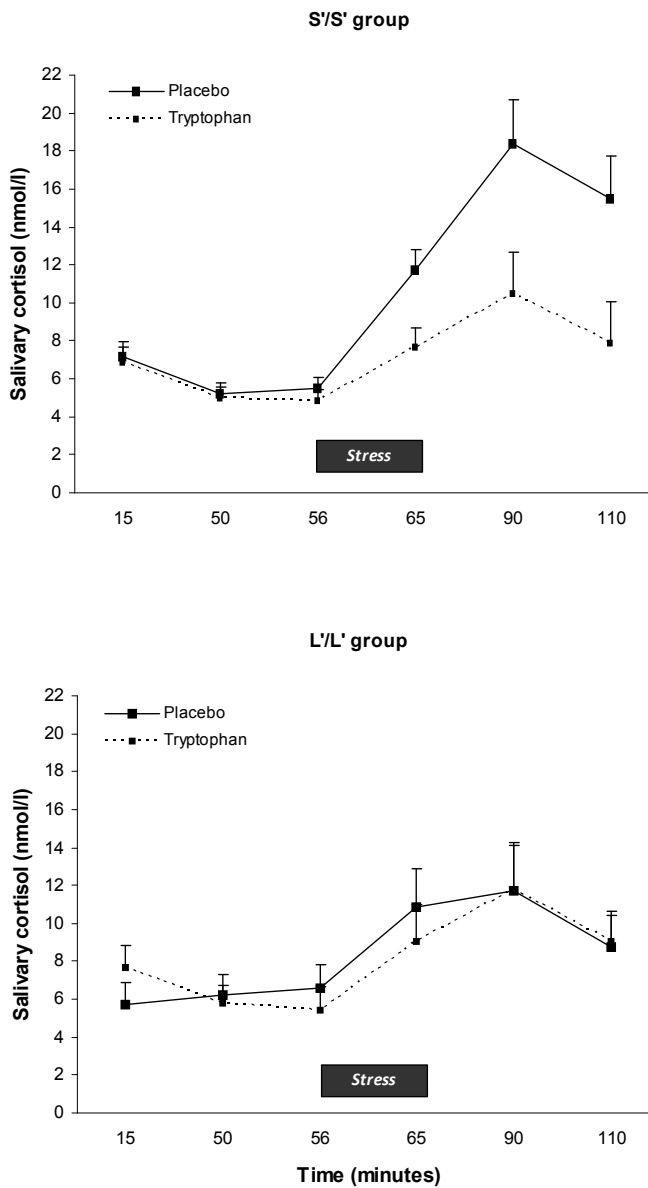


Figure 1. Salivary cortisol (nmol/l) before, during and after exposure to social stress in S'/S' group (Figure 1A) and in L'/L' group (Figure 1B).

To further probe the Time \times Intervention interaction effect within the S'/S' group, Independent-samples t-tests were conducted, comparing cortisol concentrations at each time point between TRP- and placebo-treated individuals. Significant differences were observed at t_{65} , t_{90} and t_{110} . Within the S'/S' group, the TRP-treated participants differed significantly from

PLC-treated participants at t_{65} [$t(19) = -2.73, p = 0.012, d = 1.11$]. Significant differences were also observed at t_{90} [$t(23) = -2.27, p = 0.033, d = 0.92$] and at t_{110} [$t(23) = -2.29, p = 0.031, d = 0.92$].

Subjective mood response to TSST

A separate analysis was conducted for each scale of the MSS questionnaire. RM-GLMs with Time as within subjects factor, Intervention and Genotype as between subjects factors and Gender as covariate revealed a significant main effect of Time for the Tension scale ($F(2.78, 113.95) = 32.76, p < 0.01, \eta^2 = 0.444$), Anxiety scale ($F(1.77, 72.59) = 11.23, p < 0.01, \eta^2 = 0.215$) and Annoyance scale ($F(2.37, 97.24) = 4.46, p = 0.01, \eta^2 = 0.098$). Tension, Anxiety and Annoyance scores varied between 0 and 5; after reaching a peak at t_{56} (after anticipation stress) and t_{65} (after speech and arithmetic task), the scores normalized to 0-1 at t_{90} and t_{110} (rest) in all intervention and genotype groups. The ratings of sadness remained between 0 and 1 at all time points ($F(2.16, 88.72) = 0.41, p = 0.68, \eta^2 = 0.010$) (Figure 2).

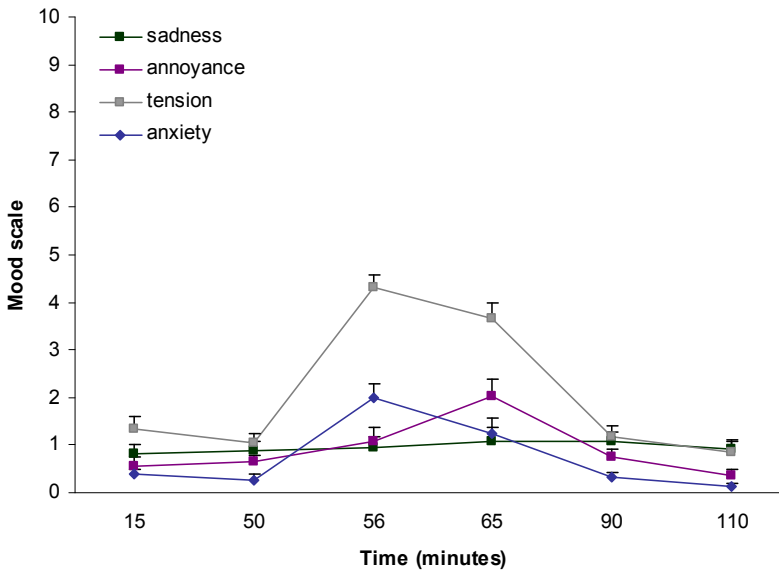


Figure 2. Mood state scores before, during and after exposure to social stress across genotype and intervention group.

Discussion

Six days of TRP supplements attenuated the cortisol response to stress in S'/S' carriers of the serotonin transporter gene. Following social stress, significantly lower cortisol concentrations were found in S'/S' carriers treated with TRP than in S'/S' carriers treated with placebo. TRP had no effect in L'/L' carriers, whose cortisol curves were comparable to TRP-treated S'/S' carriers. These effects of TRP on cortisol response to stress were observed in the absence of any effects on anxiety, depressive symptoms, or affect. Furthermore, neither genotype nor intervention had an effect on the subjective mood response to the TSST.

Previous studies have found differential HPA axis activity depending on 5-HTTLPR genotype (Gotlib *et al.*, 2008; Alexander *et al.*, 2009; Wüst *et al.*, 2009; Way & Taylor, 2010, Mueller *et al.*, 2010, Mueller *et al.*, 2011), but the effects of TRP supplements on this association had not been investigated. Only one study has investigated the effects of TRP (single dose, 0.8g) in S'/S' vs. L'/L' individuals (Markus & Firk, 2009), and found no effect on the cortisol response to stress in either genotype group. Notably, in this prior study the stressor failed to produce a rise in cortisol. While the lack of a cortisol response to stress in control conditions negated the opportunity to observe a dampening of this effect, a small improvement of mood was noted after 0.8 g TRP in S'/S' carriers but not in L'/L' carriers.

In the present study, we found no effect of TRP on anxiety and depression symptoms or mood states during exposure to public speaking stress in either L'/L' or S'/S' carriers. While the present findings seem to conflict with those of Markus & Firk (2009), the latter utilized pooled mood states (pre- and post-intervention) and also employed a crossover design. This means that their participants were exposed to the same stressor twice. Anticipation stress may have been different during the second administration. Earlier studies, in which genotype had not been assessed, found only small and rather inconsistent effects of tryptophan loading on mood and stress response (Markus *et al.*, 1998; Markus *et al.*, 2000a; Markus *et al.*, 2000b; Merens *et al.*, 2005; Markus, 2007; Nescic & Duka, 2008; Firk & Markus, 2009; Markus *et al.*, 2010). In contrast to the aforementioned studies, we pre-selected our participants based on genotype, used a longer intervention period of six days, and carefully selected participants to eliminate potential confounders as much as possible (e.g. use of contraceptives; smoking).

Although the complex relationship between HPA axis reactivity and 5-HTTLPR genotype remains to be further elucidated, several studies have indicated that neurobiological responses to negative or threatening stimuli are mediated by 5-HTTLPR genotype. Healthy S carriers had greater amygdala reactivity to negative facial expressions (e.g., fearful and angry) than L/L carriers in a face matching task (Hariri *et al.*, 2002; Hariri *et al.*, 2005). If 5-HTTLPR affects amygdala response to negative environmental stimuli in general, this may in turn also mediate HPA axis reactivity (Way and Taylor, 2009). At the molecular level the serotonin transporter promoter polymorphism alters SLC6A4 transcription efficiency and the level of

serotonin transporter function (Lesch *et al.*, 1996; Greenberg *et al.*, 1999). It remains unclear exactly how the 5-HTT polymorphism alters neurochemical processes between the pre- and post-synaptic cells. 5-HTTLPR genotype-related alterations found so far include: differential levels of extracellular 5-HT concentrations, difference in 5-HT clearance, changes in tissue 5-HT concentrations, and 5-HT synthesis and turnover in the brain (as reviewed by Murphy & Lesch, 2008). Since the short variant is associated with reduced uptake or clearance of 5-HT as compared with the long variant, it is also associated with higher concentrations of 5-HT in the synaptic cleft. Consequently, presynaptic 5HT1A autoreceptors may be overstimulated in S carriers by the increased 5-HT concentrations. The negative feedback mechanism of these autoreceptors causes a decrease in 5-HT synthesis in the presynaptic cell, reducing the amount of 5-HT in the synapse (Hoyer *et al.*, 1994). Subchronic administration of TRP might compensate this process and result in an attenuated cortisol response to social stress.

In summary, the 5-HTT polymorphism changes the dynamic between post- and pre-synaptic cell and this may also influence peripheral adrenomedullary and hypothalamo-pituitary responses (Murphy & Lesch 2008). As the precise neurochemical differences between S' and L' carriers remain unclear, the underlying mechanisms of our findings remain speculative.

Limitations and future directions

Limitations of our study include the relatively small sample size and the fact that we checked compliance only by self-report and not by measuring TRP concentrations in plasma before and after intervention. Self-reported compliance was excellent. To optimize compliance, participants kept a diary of the exact time of intake of capsules and had been led to believe that compliance would be checked through a saliva sample.

TRP supplementation of six days (2.8 g/day) may be used in further studies as an experimental intervention to increase TRP levels in 5-HT vulnerable populations (i.e. serotonergic genotypes associated with stress vulnerability). In this study we have shown that a group with a specific genotype seems to benefit more from TRP supplements, as TRP attenuated the cortisol response to stress. Future studies may investigate whether the same effects can also be reached with lower dosages of TRP supplementation. Another essential question that needs to be answered is the maximum duration of the intervention. The daily requirement of tryptophan for humans is between 3 and 5 mg/kg per day (World Health Organization, 2002). We administered 2800 mg/day, which is almost ten times this daily requirement. Although high tryptophan intake has no serious adverse effects in animals and humans (Garlick, 2004), the mechanism and safety of long-term and high-dose TRP supplementation needs further investigation (Le Floc'h *et al.*, 2010). For instance, cognitive dysfunctions have been observed after intravenous TRP infusion in healthy first-degree relatives of patients with bipolar disorder (Sobczak *et al.*, 2003). In 'quarrelsome' individuals, however, a high dose of tryptophan (3 g/d) for 15 days decreased quarrelsome behaviours and increased agreeableness (Aan het Rot *et al.*, 2006). Future studies may further investigate

how the effects of TRP supplements on the cognitive, physiological and behavioural levels depend on serotonergic genotypes. Research on TRP supplementation in individuals with serotonergic genotypes conferring vulnerability to stress might give new insights in personalized treatment of mood disorders.

References

- Aan het Rot, M., Moskowitz, D.S., Pinard, G., Young, S.N., 2006. Social behaviour and mood in everyday life: the effects of tryptophan in quarrelsome individuals. *J. Psychiatry Neurosci.* 31: 253-262.
- Alexander, N., Kuepper, Y., Schmitz, A., Osinsky, R., Kozyra, E., Hennig, J., 2009. Gene-environment interactions predict cortisol responses after acute stress: Implications for the etiology of depression. *Psychoneuroendocrinology* 34: 1294-1303.
- Booij, L., Merens, W., Markus, R., Van der Does, A.J.W., 2006. Diet rich in α -lactalbumin improves memory in unmedicated recovered depressed patients and matched controls. *J. of Psychopharm.* 20(4): p. 526-535.
- Dickerson, S.S., Kemeny, M.E., 2004. Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychol. Bull.* 130: 355-391.
- Firk, C., Markus, C.R., 2009. Mood and cortisol responses following tryptophan-rich hydrolyzed protein and acute stress in healthy subjects with high and low cognitive reactivity to depression. *Clin. Nutr.* 28: 266-271.
- Garlick, P.J., 2004. The nature of human hazards associated with excessive intake of amino acids. *J. Nutr.* 134: 1633S-1639S.
- Greenberg, B.D., Tolliver, T.J., Huang S.J., Li Q., Bengel D., Murphy, D.L., 1999. Genetic variation in the serotonin transporter promoter region affects serotonin uptake in human blood platelets. *Am J Med Genet* 88:83-87.
- Gotlib, I.H., Joormann, J., Minor, K.L., Hallmayer, J., 2008. HPA Axis Reactivity: A Mechanism Underlying the Associations Among 5-HTTLPR, Stress, and Depression. *Biol. Psychiatry* 63: 847-851.
- Hariri, A.R., Mattay, V.S., Tessitore A., Kolachana, B., Fera, F., Goldman, D., Egan, M.F., Weinberger, D.R., 2002. Serotonin Transporter Genetic Variation and the Response of the Human Amygdala. *Science* 297: 400-403.
- Hariri, A.R., Drabant, E.M., Munoz, K.E., Kolachana, B.S., Mattay, V.S., Egan, M.F., Weinberger, D.R., 2005. A Susceptibility Gene for Affective Disorders and the Response of the Human Amygdala. *Arch Gen Psychiatry* 62:146-152.
- Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R., Humphrey, P.P.A., 1994. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol Rev* 46: 157-203.
- Hu, X.Z., Lipsky, R.H., Zhu, G., Akhtar, L.A., Taubman, J., Greenberg, B.D., *et al.*, 2006. Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. *Am. J. Hum. Genet.* 78, 815-826.
- Kirschbaum, C., Pirke, K.M., Hellhammer, D.H., 1993. The "Trier Social Stress Test". *Neuropsychobiology* 28: 76-81.
- Kirschbaum, C., Kudielka, B.M., Gaab, J., Schommer, N.C., Hellhammer, D.H., 1999. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosom. Med.* 61: 154-162.
- Le Floc'h, N., Otten, W., Merlot, E., 2010. Tryptophan metabolism, from nutrition to potential therapeutic applications. *Amin. Ac.* 1-11.

- Lesch, K.P., Bengel, D., Heils, A., Sabol, S.Z., Greenberg, B.D., Petri, S., Benjamin, J., Müller, C.R., Hamer, D.H., Murphy, D.L., 1996. Association of Anxiety-Related Traits with a Polymorphism in the Serotonin Transporter Gene Regulatory Region. *Science* 274: 1527-1531.
- Markus, C.R., Panhuysen, G., Tuiten, A., Koppeschaar, H., Fekkes, D., Peters, M.L., 1998. Does carbohydrate-rich, protein-poor food prevent a deterioration of mood and cognitive performance of stress-prone subjects when subjected to a stressful task? *Appetite* 31: 49-65.
- Markus, C.R., Olivier, B., Panhuysen, G.E.M., Van der Gugten, J., Alles, M.S., Tuiten, A., Westenberg, H.G.M., Fekkes, D., Koppeschaar, H.F., De Haan, E.E.H.F., 2000a. The bovine protein α -lactalbumin increases the plasma ratio of tryptophan to the other large neutral amino acids, and in vulnerable subjects raises brain serotonin activity, reduces cortisol concentration, and improves mood under stress. *Am. J. Clin. Nutr.* 71: 1536-1544.
- Markus, C.R., Panhuysen, G., Tuiten, A., Koppeschaar, H., 2000b. Effects of food on cortisol and mood in vulnerable subjects under controllable and uncontrollable stress. *Physiol. Beh.* 70: 333-342.
- Markus, C.R., 2007. Effects of carbohydrates on brain tryptophan availability and stress performance. *Biol. Psychol.* 76: 83-90.
- Markus, C.R., Firk, C., 2009. Differential effects of tri-allelic 5-HTTLPR polymorphisms in healthy subjects on mood and stress performance after tryptophan challenge. *Neuropsychopharmacology* 34: 2667-2674.
- Markus, C.R., Verschoor, E., Firk, C., Kloek, J., Gerhardt, C.C., 2010. Effect of tryptophan-rich egg protein hydrolysate on brain tryptophan availability, stress and performance. *Clin. Nutr.* 29: 610-616.
- Merens, W., Booij, L., Markus, R., Zitman, F.G., Onkenhout, W., Van der Does, A.J.W., 2005. The effects of a diet enriched with α -lactalbumin on mood and cortisol response in unmedicated recovered depressed subjects and controls. *Br. J. Nutr.* 94: 415-422.
- Mueller, A., Brocke, B., Fries, E., Lesch, K.P., Kirschbaum, C., 2010. The role of the serotonin transporter polymorphism for the endocrine stress response in newborns. *Psychoneuroendocrinology* 35: 289-296.
- Mueller, A., Armbruster, D., Moser, D.A., Canli, T., Lesch, K.P., Brocke, B. and Kirschbaum, C., 2011. Interaction of serotonin transporter gene-linked polymorphic region and stressful life events predicts cortisol stress response. *Neuropsychopharmacology* 36: 1332-1339.
- Murphy, D.L., Lesch, K.P., 2008. Targeting the murine serotonin transporter: insights into human neurobiology. *Nat. Rev. Neuroscience* 9, 85-96.
- Nakamura, M., Ueno, S., Sano, A., Tanabe, H., 2000. The human serotonin transporter gene linked polymorphism (5-HTTLPR) shows ten novel allelic variants. *Mol. Psychiatry* 5, 32–38.
- Nesic, J., Duka, T., 2008. Effects of stress on emotional reactivity in hostile heavy social drinkers following dietary tryptophan enhancement. *Alcohol and Alcoholism* 43: 151-162.
- Porter, R., Gallagher, P., Watson, S., Young, A., 2004. Corticosteroid-serotonin interactions in depression: a review of the human evidence. *Psychopharmacol.* 173: 1-17.
- Praschak-Rieder, N., Kennedy, J., Wilson, A.A., Hussey, D., Boovariwala, A., Willeit, M., *et al.*, 2007. Novel 5-HTTLPR allele associates with higher serotonin transporter binding in putamen: a [(11)C] DASB positron emission tomography study. *Biol. Psychiatry* 62, 231–327.
- Reimold, M., Smolka, M.N., Schumann, G., Zimmer, A., Wrase, J., Mann, K., *et al.*, 2006. Midbrain serotonin transporter binding potential measured with [11C]DASB is affected by serotonin transporter genotype. *J. Neural. Transm.* 114, 635–639.

- Sheehan, D.V., Lecrubier, Y., Harnett Sheehan, K., Janavs, J., Weiller, E., Keskiner, A., Schinka, J., Knapp, E., Sheehan, M.F., Dunbar, G.C., 1997. The validity of the Mini International Neuropsychiatric Interview (MINI) according to the SCID-P and its reliability. *Eur. Psychiatry* 12: 232-241.
- Sobczak, S., Honig, A., Schmitt, J.A.J., Riedel, W.J., 2003. Pronounced cognitive deficits following an intravenous L-tryptophan challenge in first-degree relatives of bipolar patients compared to healthy controls. *Neuropsychopharmacology* 28: 711–719.
- Spinhoven, P., Ormel, J., Sloekers, P.P., Kempen, G.I., Speckens, A.E., Van Hemert, A.M., 1997. A validation study of the Hospital Anxiety and Depression Scale (HADS) in different groups of Dutch subjects. *Psychol. Med.* 27: 363–370.
- Van Vliet, I.M., Leroy, H., Van Megen, H.J.M., 2000. De MINI-Internationaal Neuropsychiatrisch Interview. Leiden, NL: Leiden University Medical Center.
- Verschoor, E., Markus, C.R., 2011. Effects of acute psychosocial stress exposure on endocrine and affective reactivity in college students differing in the 5-HTTLPR genotype and trait neuroticism. *Stress*. 14(4):407-19.
- Watson, D., Clark, L.A., Tellegen, A., 1988. Development and validation of brief measures of positive and negative affect: The PANAS scales. *J. Pers. Soc. Psychol.* 54: 1063-1070.
- Way, B.M., Taylor, S.E., 2010. The serotonin transporter promoter polymorphism is associated with cortisol response to psychosocial stress. *Biol. Psychiatry* 67: 487-492.
- World Health Organization (WHO), 2002. Protein and amino acid requirements in human nutrition: report of a joint FAO/WHO/UNU expert consultation (WHO technical report series; no. 935). Geneva: WHO.
- Wüst, S., Kumsta, R., Treutlein, J., Frank, J., Entinger, S., Schulze, T.G., Rietschel, M., 2009. Sex-specific association between the 5-HTT gene-linked polymorphic region and basal cortisol secretion. *Psychoneuroendocrinology* 34: 972-982.
- Zigmond, A.S., Snaith, R.P., 1983. The hospital anxiety and depression scale. *Acta Psychiatr. Scand.* 67: 361–370.