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Chapter 5

The Effect of 5-HTTLPR Genotype and Tryptophan Supplementation on the Response to Unfairness in Healthy Volunteers

H Cerit, RJ Schuur, AJW Van der Does
Abstract

Experimental manipulation of the availability of serotonin (5-HT) has been shown to increase or decrease the rejection rates of unfair offers in the Ultimatum Game (UG). The effect of 5-HT manipulations on UG performance may also be moderated by genotypic variation, in particular the 5HT-transporter-linked polymorphic region (5-HTTLPR). The aim of the present study was to investigate the effect of 5-HTTLPR genotype, tryptophan (TRP) supplementation and their interaction on performance on the UG in healthy individuals. The UG was completed by 26 S’/S’ and 21 L’/L’ carriers of the 5-HTTLPR, before and after a 6-day intervention of TRP (2.8 g/day) or placebo. We also measured impulsivity with a response inhibition (Go-Stop) task and with a questionnaire. 5-HTTLPR genotype did not affect the rejection rate to unfair offers. TRP supplementation also had no effect, but there was a non-significant tendency in the opposite direction as expected: the TRP-group had higher rejection rates of very unfair offers than the placebo group. Neither genotype nor intervention had an effect on impulsivity. A limitation of this study is that no blood samples were taken. The lack of effects cannot be explained by low statistical power and/or weakness of the intervention since the effect went in the opposite direction as expected. Our findings are not consistent with earlier studies with other 5-HT manipulations.
Introduction

Serotonin (5-HT) is involved in the modulation of many aspects of social cognition and behaviour. Genotypic variation, in particular regarding the serotonin transporter gene (SLC6A4) affects social cognition (Canli & Lesch, 2007), as well as 5-HT manipulations. For instance, experimental depletion of tryptophan (TRP), a precursor of 5-HT, leads to reduced cooperative behaviour in a Prisoners Dilemma paradigm (Wood et al., 2006). TRP depleted healthy volunteers judged couples as less intimate and less romantic (Bilderbeck et al., 2011). TRP depletion also increased reaction times for happy but not sad faces in an affective go/no go task (Murphy et al., 2002) and increased rapid-response impulsivity in healthy volunteers (Walderhaug et al., 2002). Serotonin also plays a role in the processing of facial expressions and social interactions. For example, acute tryptophan depletion (ATD) decreased the recognition of fearful facial expressions in healthy females (Harmer et al., 2003) and TRP supplementation for 15 days decreased quarrelsomeness in quarrelsome men and women and altered the perception of others in the quarrelsome males (Aan het Rot et al., 2006). These studies show that lowering serotonin availability is associated with disruptive social behaviour, whereas increasing serotonin availability is associated with pro-social perception and behaviour.

Serotonergic manipulations also affect performance in the Ultimatum Game (UG) (Crockett et al., 2008; Crockett et al., 2010). In the UG, the participant (responder) is exposed to offers to split a sum of money from other individuals. The responder can either accept the offer (in which the money is divided accordingly) or reject the offer (in which case both players receive nothing). Rationally, the responder should accept every offer regardless of its fairness to earn the most. However, very unfair offers (20% of the total) have a 50% chance of being rejected (Güth, Schmittberger & Schwartze 1982; Bolton & Zwick, 1995), which indicates that emotion plays an important role in making those decisions. Acute tryptophan depletion was associated with a higher rejection rate (approximately 81%) of very unfair offers (18-22% of the stake) than PLC (appr. 65%) in healthy individuals (Crockett et al., 2008). This effect was independent of the size of the offer and ATD had no effect on self-reported mood or on response inhibition. Conversely, a single dose of a selective serotonin reuptake inhibitor (SSRI), citalopram (30 mg) was associated with a lower rejection rate (appr. 34%) of unfair offers than PLC (appr. 48%) and atomoxetine (60 mg) a selective selective nor epinephrine reuptake inhibitor (NRI), (appr. 50%) in 30 healthy participants (Crockett et al., 2010). This time the effect was restricted to moderately unfair offers (27-33% of the stake). Citalopram did not alter self-reported mood. In another study, healthy students who rejected an unfair offer had lower platelet serotonin content than participants who accepted the offer (Emanuele et al., 2008). Finally, a PET study in 20 healthy males showed that individuals with low levels of 5-HT transporter binding in the dorsal raphe nucleus were more likely to reject unfair offers (Takahashi et al., 2012). In summary, UG behaviour seems to be under serotonergic influence and the effects are consistent across studies. The direction of the effect on the UG
is consistent with the direction of the 5-HT manipulation (Crocket et al., 2008; Crocket et al., 2010).

Although 5-HTTLPR genotype is linked to social cognition (Canli & Lesch, 2007; Antypa et al., 2011), no studies have been conducted in order to investigate whether 5-HTTLPR moderates the effect of 5-HT manipulations on UG performance. In the present study we investigated whether performance on the UG is affected by 5-HTTLPR genotype and by an increase of 5-HT availability through tryptophan (TRP) supplementation. Specifically, short and long allele carriers of the 5-HTTLPR completed the UG prior to and after a 6-day intervention of TRP (2.8 g/day) or placebo. In a previous report on this study, we have shown that TRP normalized the cortisol response to social stress in S′/S′ carriers only (Cerit et al., 2013). Based on the less efficient serotonin neurotransmission in S′/S′ carriers, the regulation of the reaction to unfairness was expected to be diminished in this group. We hypothesized that we would find higher rejection rates in S′/S′ carriers than in L′/L′ carriers and that this difference would be reduced after 6-day TRP supplementation. Response inhibition (Go-Stop task) and self-reported impulsivity (BIS-II) were measured as secondary outcomes. Based on Crockett et al. (2008) we expected a selective effect on the ultimatum game.


Methods and Materials

Participant pool
The participants of this study are the same as reported in Cerit et al. (2013). Participants were selected from a pool of 581 genotyped individuals who were non-smokers and whose grandparents were all West-European. 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. 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 invited only participants with two low-expressing alleles (S'/S': S/S, S/Lg and Lg/Lg variants) or two high-expressing alleles (L'/L': La/La). Written informed consent was obtained before data collection. The research was approved by the Medical Ethics Committee of Leiden University Medical Centre in The Netherlands. Participants received € 40 upon completing the study.

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 µg 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 HpaII 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.

Ultimatum Game. The ultimatum game consists of three different conditions in which participants are exposed to fair (45% of stake), unfair (32% of stake) and most (i.e. very) unfair (21% of stake) offers from a “proposer”, who had split a sum of money given by the experimenter. If the participant accepts the offer, both the proposer and participant will receive the money as promised in the offer. In case of a rejection by the participant neither one will receive money. An example of a highly unfair offer could be € 2 for the participant and € 8 for the proposer. Obviously, the most beneficial strategy economically for the participant is to accept each offer regardless of its fairness level. Next to social reward (fairness), monetary reward (offer size) was also manipulated as described in Crockett et al. (2008). The value of the stake was either low (between 1 and 7 euro) or high (between 8 and 33 euro).

After 10 practice trials, participants were asked to respond to 48 offers (16 per fairness level). With each offer a photograph of a new proposer, the amount of the stake, and the amount of the offer was shown. The 48 photographs were counterbalanced for gender (24 male and 24 female proposers). All 48 offers were presented in a random order at both sessions. The participants were told that they would receive a percentage of the total amount that they had gained after having completed both sessions. In reality, there were no actual proposers and all participants received the same propositions. To increase credibility, the participants were first asked to split 24 sums of money (on paper) and had their photograph taken to be used in future experiments.

Impulsivity. Self-reported impulsivity was assessed with the 30-item Barratt Impulsiveness Scale (BIS-II, state version; Patton et al., 1995). The Go-Stop test is a stop-signal task and
measures response inhibition aspects of impulsivity (Dougherty 2005; Dougherty et al., 2010). In this task a series of 5-digit numbers are displayed for 500 msec with a 1,500 msec inter-stimulus interval. The 5-digit numbers appear in series, and some of these numbers are identical to the immediately preceding 5-digit number. Participants are instructed to respond to these matching numbers (Go Signal). Some of these matching numbers are first presented in black and then suddenly turn red. This is a Stop Signal cue, and the participants are instructed to withhold responding to any matching numbers that turn red. The timing of these stop signals varied across the testing session (e.g. 50, 150, 250 and 350 msec). The two dependent measures of interest were: 1) correct responses and 2) response inhibition failures. The primary dependent measure is the Go-Stop Ratio, which is the ratio of these two measures. The Go-Stop Ratio has been validated as a measure of the ability to inhibit an already initiated response, and data from the 150 msec stop delay typically provides the best group discrimination (Dougherty et al., 2010).

**Design and procedure**

This study was a randomized double-blind placebo-controlled experimental study with stratification for genetic variation of the 5-HTTLPR genotype. Participants were randomly allocated to receive 7 capsules containing either 400 mg 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 on the days before and after the intervention (Day 0 and 7).

**First visit to laboratory (Day 0)**

Upon arrival at the laboratory, participants provided written informed consent. Following the M.I.N.I. interview, participants filled out the BIS-II, and completed the ultimatum game and Go-Stop test, respectively (as part of a larger test battery). 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 three 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 (Day 7)

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 complete the BIS-II questionnaire, the Ultimatum Game and Go-Stop test in fixed order.
Results

Sample characteristics

We contacted 92 S'/S' carriers and 92 L'/L' carriers from our participant pool (N=581) 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 L'/L' group, one participant dropped out before the second visit to laboratory. Analyses were conducted on 26 S'/S' and 21 L'/L' carriers.

The first two participants (one S'/S' and one L'/L' carrier) who received tryptophan single blind were kept in the analysis. The demographic details of both groups are shown in Table 1. Groups did not differ significantly on 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 (for needles). Both participants were not taking any medication.

Table 1. Demographic Characteristics of the Tri-Allelic S'/S' and L'/L' 5-HTTLPR Genotype groups

<table>
<thead>
<tr>
<th></th>
<th>S'/S' (N = 26)</th>
<th>L'/L' (N = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (M±SD)</td>
<td>20.4 ± 3.4</td>
<td>20.3 ± 2.5</td>
</tr>
<tr>
<td>Females/males</td>
<td>12/14</td>
<td>11/10</td>
</tr>
</tbody>
</table>

*Note: S'/S' includes: S/S, S/Lg, Lg/Lg and L'/L' includes: La/La; M, Mean; SD, Standard Deviation.

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 of the second lab visit. All these participants were retained.

Ultimatum Game

Genotype Effect on ultimatum game on Day 0. A Repeated Measures ANOVA (RM-ANOVA) with Offer size (low and high) and Fairness level (fair, unfair and most unfair) as within subjects factors and Genotype (S'/S' and L'/L') as between subjects factor on the rejection rates on Day 0 (pre-intervention) was conducted. This analysis revealed the expected main effects of Offer size (F (1.00, 45.00) = 5.66, p = 0.022, \( \eta^2 = 0.112 \)) and Fairness level (F (1.66, 74.60) =
100.49, \( p < 0.001, \eta^2 = 0.691 \)). The interaction of Offer size and Fairness was also significant (\( F(1.833, 82.49) = , p = 0.039, \eta^2 = 0.072 \)). No main or interaction effects involving genotype were found.

**Genotype and intervention effects on ultimatum game on Day 0 and 7.** A 2x2x3 RM-ANOVA with Time (pre/post intervention), Offer size and Fairness level as within subjects factors and Genotype and Intervention (TRP/PLC) as between subjects factors again revealed the expected main effects of Offer size and Fairness level (\( F(1.54, 66.26) = 112.44, p < 0.001, \eta^2 = 0.723 \)), but no main effects of Genotype, Time or Intervention. However, three-way interactions among Time x Offer size x Genotype (\( F(1.00, 43.00) = 6.22, p = 0.017, \eta^2 = 0.126 \)) and Time x Offer Size x Intervention (\( F(1.00, 43.00) = 8.12, p = 0.007, \eta^2 = 0.159 \)) were found.

Separate RM-ANOVAs with Time as within subject factor and Intervention as between subject factor were conducted on the rejection rates of high unfair, high most unfair, low unfair and low most unfair offers. None of these analyses revealed a main effect of time or intervention. However, in the analysis of the High Most Unfair offers, a significant Time x Intervention was found (\( F(1.00, 45.00) = 4.249, p = 0.045, \eta^2 = 0.086 \) (Figure 1). The interaction was borderline significant (\( p = 0.057 \)) when genotype was included as a between subjects factor. We conducted post-hoc Independent-Sample T-Test on day 7, and Paired-Sample T-Tests within the TRP and PLC groups separately. None of these analyses revealed a significant difference (all \( p \)-values around 0.15).

**Figure 1. Rejection of Most Unfair Offers with High offer size following TRP +**

![Rejection of High Most Unfair Offers following TRP+](image)

Note: Rejection rates of “most unfair offers” with high offer sizes pre and post intervention (TRP; Tryptophan, PLC; Placebo). Error bars represent Standard Error (SE)
**Self-reported Impulsivity**

**Genotype Effect on impulsivity on Day 0.** In order to assess effects of 5-HTTLPR genotype on impulsivity a RM-ANOVA with the Attentional, Motor and Non-planning scales of the BIS-II measured at Day 0 (pre-intervention), as within subjects factors and Genotype (S'/S' and L'/L') as between subjects factor on the scores of the three scales revealed a main effect of scale (F (1.96, 88.09) = 144.54, p < 0.001, ηp²= 0.763). Impulsivity did not interact with Genotype (F (1.96, 88.09) = 0.445, p = 0.638, ηp²= 0.010).

**Genotype or intervention effects on impulsivity on Day 0 and 7.** Effect of 5-HTTLPR genotype and intervention were assessed by conducting a RM-ANOVA with Time (pre/post intervention), and the three BIS-II scales as within subjects factors. Genotype and Intervention (TRP/PLC) were between subjects factors. No main effect of Time (F (1.00, 43.00) = 0.166, p = 0.686, ηp²= 0.004) was found. A main effect of Impulsivity (F (1.98, 84.92) = 183.34, p < 0.001, ηp²= 0.810) and Time x Impulsivity was found (F (1.68, 72.17) = 4.829, p = 0.015, ηp²= 0.101). Time x Impulsivity x Genotype was not significant (F (1.68, 72.17) = 2.483, p = 0.100, ηp²= 0.055). Time x Impulsivity x Intervention was also not found (F (1.68, 72.17) = 1.688, p = 0.196, ηp²= 0.038). The scores on Attentional, Motor and Non-planning scales of the BIS-II are shown in Table 2.

**Go-Stop Task**

**Genotype Effect on Response Inhibition on Day 0.** RM-ANOVA with two impulsivity outcomes of the Go-Stop paradigm (Response Inhibition Failure and Correct Detections) as within subjects factors and Genotype (S'/S' and L'/L') as between subject factor on Day 0 (pre-intervention), revealed a main effect of impulsivity outcome (F (1.00, 43.00) = 1200.20, p < 0.001, ηp²= 0.96) interaction, but no effect of Genotype on the two impulsivity outcomes were found.

A separate One-Way ANOVA was conducted for the Go-Stop Ratio outcome with Go-Stop Ratio on Day 0 as dependent variable and Genotype as factor. The genotype groups did not differ on the Go-Stop Ratio outcome (F (1, 45) = 1.415, p = 0.241).

**Genotype and intervention effects on Response Inhibition on Day 0 and 7.** RM-ANOVA with Time (pre/post intervention), and the two impulsivity outcomes of the Go-Stop paradigm (Response Inhibition Failure and Correct Detections) as within subjects factors, and Genotype and Intervention (TRP/PLC) as between subjects factors revealed a significant main effect of Impulsivity outcome, but no main effect on Time (F (1.00, 43.00) = 0.037, p = 0.848, ηp²= 0.001). No effect of genotype or Intervention on impulsivity outcomes on Day 0 and Day 7 were found (Table 3).
A separate RM-ANOVA with Time (pre/post intervention), and the Go-Stop Ratio outcome as within subjects factors, and Genotype and Intervention (TRP/PLC) as between subjects factors revealed a main effect of Time ($F(1.00, 43.00) = 11.89, p = 0.001$, $\eta^2_p = 0.217$), but no interaction effects.

**Table 2.** Self-reported Impulsivity. BIS-II scores on the 2nd order factors Attentional, Motor and Non-Planning with Intervention (TRP and PLC) and Genotype (S'/S' and L'/L')*, (Mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Pre (M ± SD)</th>
<th>Post (M ± SD)</th>
</tr>
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<tbody>
<tr>
<td><strong>Attentional</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S'/S'$</td>
<td>16.3 ± 2.4</td>
<td>15.9 ± 2.6</td>
</tr>
<tr>
<td>$L'/L'$</td>
<td>14.4 ± 2.5</td>
<td>14.6 ± 2.6</td>
</tr>
<tr>
<td>PLC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S'/S'$</td>
<td>15.3 ± 4.0</td>
<td>13.9 ± 3.5</td>
</tr>
<tr>
<td>$L'/L'$</td>
<td>15.1 ± 3.9</td>
<td>14.7 ± 3.2</td>
</tr>
<tr>
<td><strong>Motor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S'/S'$</td>
<td>22.1 ± 3.8</td>
<td>23.1 ± 3.3</td>
</tr>
<tr>
<td>$L'/L'$</td>
<td>20.8 ± 2.4</td>
<td>21.1 ± 2.3</td>
</tr>
<tr>
<td>PLC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S'/S'$</td>
<td>20.0 ± 2.6</td>
<td>21.5 ± 3.4</td>
</tr>
<tr>
<td>$L'/L'$</td>
<td>21.0 ± 3.7</td>
<td>21.5 ± 4.1</td>
</tr>
<tr>
<td><strong>Non-planning</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S'/S'$</td>
<td>27.0 ± 3.9</td>
<td>25.3 ± 3.6</td>
</tr>
<tr>
<td>$L'/L'$</td>
<td>26.2 ± 3.3</td>
<td>25.6 ± 3.4</td>
</tr>
<tr>
<td>PLC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$S'/S'$</td>
<td>25.0 ± 3.4</td>
<td>24.2 ± 3.7</td>
</tr>
<tr>
<td>$L'/L'$</td>
<td>23.3 ± 4.0</td>
<td>23.9 ± 4.7</td>
</tr>
</tbody>
</table>

(M, Mean; SD, Standard Deviation; TRP, Tryptophan; PLC, Placebo)

*Note: S'/S' includes: S/S, S/Lg, Lg/Lg and L'/L' includes: La/La
Table 3. Go-Stop Impulsivity Measures at 150 ms, (Mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Pre (M ± SD)</th>
<th>Post (M ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TRP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correct detections</td>
<td>67.28 (12.03)</td>
<td>63.36 (23.01)</td>
</tr>
<tr>
<td>Response Inhibition Failure</td>
<td>0.37 (0.20)</td>
<td>0.33 (0.20)</td>
</tr>
<tr>
<td>Go-Stop Ratio</td>
<td>0.48 (0.25)</td>
<td>0.36 (0.21)</td>
</tr>
<tr>
<td><strong>PLC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correct detections</td>
<td>63.18 (13.62)</td>
<td>65.91 (14.51)</td>
</tr>
<tr>
<td>Response Inhibition Failure</td>
<td>0.42 (0.20)</td>
<td>0.36 (0.23)</td>
</tr>
<tr>
<td>Go-Stop Ratio</td>
<td>0.53 (0.23)</td>
<td>0.40 (0.28)</td>
</tr>
</tbody>
</table>
Discussion

Genotypic variation of the 5-HTTLPR did not influence the response of healthy participants to unfair offers. Six days of tryptophan supplementation seemed to influence response to very unfair offers, however, this effect did not reach statistical significance. We also found no effects of genotype, intervention or their interactions on impulsivity as measured by the Go-Stop Task or by self-report.

Our findings are not consistent with other studies in which the ultimatum game was performed by healthy participants following a serotonergic manipulation. A single dose of citalopram reduced the rejection rate of unfair offers compared to placebo condition in healthy volunteers (Crockett et al., 2010), whereas tryptophan depletion had an opposite effect (Crocket et al., 2008). In another study, low platelet serotonin was found to be associated with heightened rejection of unfair offers (Emanuele et al., 2008). Our non-significant findings were in the opposite direction as these three studies, which suggest that our findings were not due to low statistical power. Insufficient strength of our intervention is also unlikely, since we did observe an attenuated cortisol response to social stress in S'/S' carriers, as reported previously (Cerit et al., 2013).

It is conceivable that the ultimatum game may be less sensitive when administered more than once. This cannot explain the lack of effect of genotypic variation, but might explain the findings regarding tryptophan supplements. Participants may have applied different strategies during the two experimental sessions. However, others (e.g., Crockett et al., 2008) have also administered the UG more than once.

Our participants did not take TRP on the day of testing. Therefore, one might argue that the opposite trend in our findings is due to the acute withdrawal of tryptophan that caused a relative depletion. Since we did not take blood samples, we cannot be sure about the tryptophan concentrations at the time of testing. This relative depletion hypothesis seems quite unlikely, considering that we found a reduced cortisol response to social stress in the same participants, which is theoretically consistent with supplementation (Cerit et al., 2013).

We have no information on the diet of the participants during the 6-day lasting TRP intervention, neither do we have information on the type of meal that participants have consumed on day 7. We did not take blood samples to measure peripheral parameters (e.g. TRP/LNAA ratios) which could provide us with 1) an indication of a possible interaction of nutrients with the intervention during the study and/or 2) an indication of the effect of the intervention on central serotonin levels. However, the aim of the current study was not to measure the acute effect of TRP loading on central serotonin levels and its consequent effect on behaviour. The perspective of the current study is rather based on the idea that increasing serotonin availability leads to a shift from negative towards positive information.
processing (Harmer et al., 2009) and alters social behaviour and perception of others in a positive manner (Aan het Rot et al., 2006).

The effects of 5-HT manipulations on UG behaviour may be mediated by more complex underlying processes than we had presumed. For example, the personality trait “agreeableness” was found to be a mediating factor between 5-HTT binding and UG behaviour in healthy men: higher scores on “straightforwardness” and “trust” (two subscales of “agreeableness”) were correlated with lower 5-HTT binding and higher rejection rates of unfair offers (Takahashi et al., 2012). Further studies are required to unravel the underlying processes between 5-HT neurotransmission and behavioural responses to unfairness.

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References


