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ORIGINAL PAPER

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Effects of experimental acute tryptophan depletion on acoustic startle response in females

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Abstract Previous studies suggest an important role for serotonergic (5-HT) modulation of the acoustic startle reflex (ASR) and prepulse inhibition (PPI). Acute challenge of brain serotonin by means of tryptophan depletion test (TDT) represents an established human challenge tool for temporary reduction of tryptophan (−TRP) levels and central nervous serotonin. Under these experimental conditions, PPI was found attenuated in males, but greater biochemical effects of TDT in the central nervous system of females are known. Therefore, in order to explore influence of 5-HT on various standard startle parameters in females, 16 young healthy females participated in a double-blind, cross-over TDT study. Acoustic stimuli were presented in 15 pulse-alone trials (100 dB, 40 ms) randomly followed by 25 pulse-alone or prepulse (70 dB, 30 ms; 120 ms interval) trials alongside electromyographic eyeblink recordings and mood state assessments. During 81% depletion of free plasma TRP, mean ASR magnitudes were significantly reduced compared to control (+TRP) condition while there were no differences in habituation or PPI nor did startle parameters correlate with mood states. Changes of plasma TRP and mood states correlated in tendency negatively in (−TRP) for depression and positively in (+TRP) for fatigue. In conclusion, this first study of startle parameters after TDT in a homogenous female population demonstrates that depletion of brain 5-HT in women only influences ASR.

Key words acoustic startle response · mood states · prepulse inhibition · serotonin · tryptophan depletion

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Introduction

The startle reflex, used in acoustic response paradigms (ASR), is the contraction of facial and skeletal muscles following a sudden adverse loud stimulus [10]. Prepulse inhibition (PPI)—i.e., the suppression of the startle reflex in response to an earlier and weaker non-startling prepulse [19]—provides an operational measure of "sensorimotor gating", which is the ability to suppress irrelevant sensory stimuli [6]. While the ASR can be traced back to a three-synapse bulbopontine circuit with projections to the craniofacial motor nuclei and spinal cord, PPI modulation by the neural circuitry connecting the limbic system and the basal ganglia is more complex [34]. In humans, especially the PPI has gained importance due to disturbance in patients with schizophrenia [6, 44] and others like obsessive compulsive disorder [63], Huntington's disease [65] and Tourette syndrome [9], whereas the ASR is also used as a measure of emotional valence, e.g. in schizophrenia [60].

Although—as in schizophrenia research—most animal studies concentrated on dopaminergic pathways, glutamate antagonists, GABA and acetylcholine, serotonergic influences on startle also were brought into a major focus [13, 17, 34, 40, 47, 52]. Particularly, manipulation of the serotonergic (5-hydroxytrypta-

mine, 5-HT) system with 5-HT-releasing agents, like selective 5-HT receptor stimulation and local administration of 5-HT_{1a} and 5-HT_2 agonists in the raphe nuclei, i.e., 5-HT depletion, induced a clear disruption of PPI [15, 41, 54, 58, 61]. Further support for the hypothesis of a decreased 5-HT neurotransmission was lent by the fact that PPI-disruptive effects were prevented by 5-HT overactivation [33, 42].

However, a direct conclusion from animal data to humans is not possible, and in humans knowledge of serotonergic mechanisms in startle modulation is fragmentary: 3,4-methylene-dioxy-*N*-methamphetamine (MDMA), a 5-HT-releaser, increased the startle magnitude and PPI, while pretreatment with SSRI (citalopram) enhanced baseline amplitude of ASR [37, 69]. Conversely, single dosages of SSRI (fluvoxamine) did not alter ASR or PPI at all [50]. 5-HT_2 antagonists clozapine, ketanserin and quetiapine reduced ASR but only ketanserin reduced electromyographic PPI [20–22]. A clinical study of SSRI (sertraline) treatment in patients with major depression showed an increased ASR and attenuated habituation without any alteration of PPI [56]. Changes of startle parameters were also seen in patients suspected of having a 5-HT imbalance, such as post-traumatic stress disorder [62], obsessive-compulsive [36] or panic disorder [38], though not confirmed in borderline personality disorder [27–29].

Still, first experimental human 5-HT challenge with acute tryptophan depletion test (TDT) on ASR produced no alterations in magnitude but did suppress PPI in healthy males [51]. In contrast, there were elevated startle amplitudes in the only available animal model with TDT [70]. TDT is an established human serotonergic research tool [11]: The ingestion of a concentrated tryptophan (TRP)-free amino acid drink induces a transient decrease first in plasma TRP [3, 72], then in cerebrospinal fluid (CSF) TRP and its major metabolite 5-hydroxyindolacetic acid (5-HIAA) [8, 71]. This is also supported in animal studies measuring CNS levels of 5-HT and 5-HIAA [59, 73]. Clinically, TDT was followed by mood changes with transient return of depressive symptoms in vulnerable or previously depressed subjects, reversal of antidepressive treatments effects [5, 7, 48, 72] and other main predictors, i.e. suicidality, recurrence or chronicity of depressive episodes, and especially female gender [5]. Imaging studies (single-photon emission computer tomography, SPECT, and on positron emission tomography, PET) in remitted patients with major depression revealed not only different patterns of reduced 5-HT brain metabolism after TDT-induced relapse, but with a greater vulnerability and activity decrease in females [7, 48, 49]. Gender differences with inconsistent findings of higher ASR and lower PPI in females also seem to apply for humans [1, 4, 35, 39, 64] and are currently not fully understood, especially in the field of 5-HT research. Furthermore, the impact of 5-HT challenge with TDT on startle characteristics in females has not been addressed so far.

While 5-HT modulation of startle responses in previous pharmacological studies was examined with either male or smaller mixed-gender populations of various ages, the purpose of this explorative study was to investigate how acute experimental TDT would affect different startle parameters (ASR, PPI, and habituation) and different mood states (e.g. depression) in a homogeneous group of healthy females with a narrow age range. Results of this kind of research could give new insights into neuronal mechanisms of functional information processing after serotonin challenge in the particularly vulnerable group of females with future clinical relevance.

Methods

Participants

A total of 16 healthy females (25.0 years, SD 2.0) volunteered twice to ingest a highly concentrated amino acid mixture with TRP (control) or without TRP (depletion). They were recruited from hospital staff, trainees or university students. None of them had taken oral contraceptives during the last 6 months. No regular medication was allowed during the preceding 6 months. Further exclusion criteria were: pregnancy or lactation; BMI <20 or >24; severe previous somatic illness; any previous neurological or psychiatric disorder; current or previous substance abuse; as well as any cognitive or mental disorder due to a medical condition. None of the participants had a family history of neuropsychiatric disorders.

All participants were individually interviewed, including a semi-structured DSM-IV (SKID II) diagnostic interview [2], and their affective status assessed using the Hamilton Rating Scale for Depression (21 Item Version, HAM-D; Hamilton 1960). Initial startle response tests were run to exclude non-responders from the extensive study protocol (n = 1). One of the 16 females who were then recruited for the study had to be excluded from analysis because she started vomiting during the second session after drinking the amino acid mixture too quickly.

All subjects gave written informed consent after being informed in detail. The study was approved by the ethics committee of the Aachen Medical Faculty and was in line with the Helsinki declaration of the World Medical Assembly.

Design

This was a double-blind, cross-over study, with volunteers participating twice randomly either ingesting the experimental (−TRP) or the control (+TRP) amino acid mixture. They were tested approximately 4 weeks apart, each during their first half of the menstrual cycle (follicular phase), when estrogen levels were low, to exclude premenstrual symptoms [45] and to control for the influence of menstrual cycle when plasma 5-HT is lowest [30].

Amino Acid Mixture

The amino acid mixture—administered in 300 ml of water and to be ingested within 2 h starting at 8:00 a.m.—contained approximately 75 g (−TRP, verum) resp. 78 g (+TRP, control) in the same proportions as the original 100 g amino acid mixture [72]. It therefore consisted of 4.125 g L-alanine, 2.4 g glycine, 2.4 g L-histidine, 6.0 g L-isoleucine, 10.125 g L-leucine, 6.675 g L-lysine, 4.275 g L-phenylalanine, 9.15 g L-proline, 5.175 g L-serine, 4.875 g L-threonine, 5.175 g L-tyrosine, 6.675 g L-valine, 3.675 g L-arginine, 2.025 g L-cysteine and 2.25 g L-methionine, plus 3.0 g L-tryptophan in the control drink. This was the same mixture (Flex Pharma, Roermond, Netherlands) as that used by Riedel et al. [57].

Protocol

Subjects arrived at 7:30 a.m., having fasted since 10:00 p.m. the previous night. Before receiving a low-protein breakfast, a venous blood sample was taken for TRP sampling (t0). Further blood samples were taken at 1:00 p.m. (t1), 3:00 p.m. (t2), 5:00 p.m. (t4) and 8:00 a.m. the next day (t5). The 10 ml samples were immediately centrifuged and refrigerated, and the concentration of free plasma TRP determined later using high-pressure liquid chromatography (Labor Eberhard & Partner, Dortmund, Germany). Administration of amino acid mixture began at 8:00 a.m. and had to be completed within 2 h. For lunch (12:00 a.m.) and any time thereafter, participants were allowed to take low-protein meals and drinks (low-protein bread, tomatoes, mints, etc.). At the end of the study day, they received a high-protein dinner.

Stimuli and session

Following standard procedure [16, 23], the acoustic startle was generated by a function generator in a commercial startle system and delivered binaurally through headphones. A continuous 52 dB background noise level was used to mask extraneous sounds. Startle probes consisted of a burst of white noise whose intensity was calibrated by an artificial ear. Two types of pulse stimuli were presented: first a block of 15 pulse-alone (PA) trials (100 dB, 40 ms), followed by a second and a third block of six pulse-alone trials (100 dB, 40 ms) and six prepulse-pulse (ppP) trials (70 dB, 30 ms resp. 100 dB, 40 ms) applied in randomized order. The end of the prepulse was separated from the onset of the pulse by a 120 ms interval [67]. The duration of interstimulus intervals was randomly selected from a range of 22–28 s.

The startle reflex was recorded with silver/silver chloride disc electrodes (Beckan Ag/AgCl) and directed by a preamplifier (SYNAMPS; model 5083, NeuroScan) to a commercial computerised startle monitoring system (San Diego Instruments, San Diego, CA). In order to measure the eye blink component of the startle response, two miniature electrodes were placed approximately 0.5 cm under the left eye and the outer canthus. The ground electrode was placed over the right mastoid. Impedance was kept at <5 kΩ. The time window after startle probe onset for recording EMG activity of the orbicularis oculi muscle was set at 20–150 ms. Participants were asked to sit in an armchair, relaxed but awake, the eyes open and the gaze fixed on a point straight ahead. EMG activity was continuously recorded for 250 ms (sampling rate 1 ms) starting with the onset of stimuli. EMG activity was amplified by a factor of 10,000 with a 100–1,000 Hz band-pass filter, digitized at 1 kHz in a 10–150 ms time window from the start of the acoustic stimuli, then rectified and stored for offline analysis.

Psychometric assessments

Participants assessed their mood and somatic status whenever venous blood sample were drawn (see above). They completed self-ratings, i.e. of Profile of Mood States Scale (POMS) [43] which comprised a bipolar set of adjectives in four different mood scales (see Results) ranging from 0–35. Using a visual analogous scale with 10 items (adopted from [57]), they also determined the severity of possible side effects (headache, chills, hot flushes, sweating, dizziness, blurred vision, dry mouth, nausea, abdominal pain, palpitations).

Data processing

The startle response values were stored in arbitrary analogue–digital units. A minimum of 10 units above baseline level was required for the EMG activity to be considered a startle response. The criterion for startle non-responders was defined as <25 units for the mean of response amplitude. Latency to onset of stimulus response was determined as a shift of 6 digital units above baseline occurring within 18–100 ms after the stimulus. The latency to the response peak was defined as the maximum magnitude occurring within 150 ms from the onset of stimulus.

In the 40-trials design—including the 12 prepulse-pulse trials—the first 15 pulse-alone trials (i.e. the first PA-block plus the first trial of the second ppP-block) were applied to determine mean magnitudes and habituation of EMG responses, while prepulse inhibition was analysed in the second and third block. Habituation was defined as the difference of amplitude between the first five (1–5) and last five PA trials (11–15) relative to the first five PA trials (1–5): [amplitude ((mean block 1A−mean block 1C)/mean block 1A) × 100]. The percentage PPI was calculated as defined by the percent decrease in the startle magnitude if a prepulse was given: [100−(100 × amplitude on prepulse trial/amplitude on PA trial)].

Statistical analysis

To test the effects of TDT, order of treatment and sequence of ASR blocks on acoustic startle responses, we calculated two-way analyses of variance (ANOVA) with the factors group (−TRP, +TRP) and order of treatment as between-subject factors and repeated measurement of sequence of ASR blocks as within subject factors. After that, for specific experimental parameters of electrophysiology and psychometry two-tailed paired *t*-tests for dependent samples were used in an explorative manner. Correlation analyses between EMG parameters (amplitudes, latencies of ASR, PPI, and habituation) and psychometric tested mood states (Depression/Dejection, Fatigue/Inertia, Vigour/Activity, Anger/Hostility) were calculated with Pearson correlation. Results were considered significant at $p < 0.05$. SAS package 9.1 (SAS/STAT 2004) was used for all statistical analyses.

Results

Biochemical measurements

Only 0.6% of blood samples had to be recorded as missing samples. Baseline levels of free plasma tryptophan did not differ between the experimental (−TRP) and the control (+TRP) group ($T = 1.46$, $p < 0.21$). In the experimental (−TRP) group, peak free plasma TRP concentrations showed a mean decrease of 81.3% (±5.3%), or a peak decrease to 18.7% relative to the individual basic values (Fig. 1). The control (+TRP) group showed an increase of 328.4% (±41.61%) of free plasma TRP. Regarding absolute group means, participants showed in the experimental (−TRP) condition a significant decrease from 13.71 (±0.71) μg/ml to 2.47 (±0.92) μg/ml ($T = -16.91$, $p < 0.0001$), and in the control (+TRP) condition a significant increase from 12.84 (±2.55) μg/ml to 41.65 (±0.42) μg/ml ($T = -12.06$, $p < 0.0001$).

EMG startle responses

In the experimental (−TRP) condition, mean startle magnitudes of the pulse-alone trials were reduced as opposed to control (+TRP) condition (Table 1, Fig. 2), and group differences could be found for the mean magnitude ($T = 3.82$, $p < 0.002$). In detailed comparison, all three individual blocks of pulse-alone trial showed remarkable different peak amplitudes (1st: $T = 3.85$, $p < 0.002$; 2nd: $T = 4.16$, $p < 0.01$; 3rd:

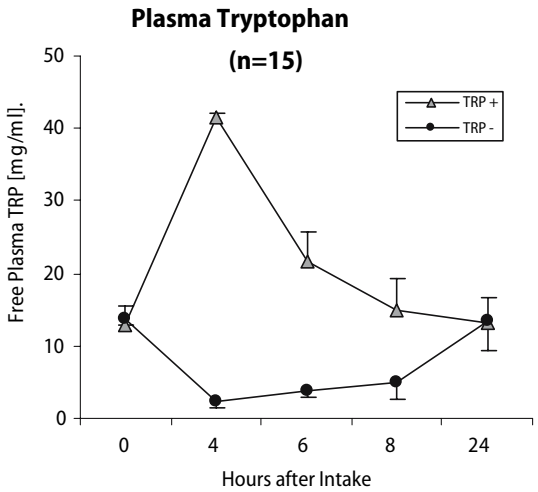

Fig. 1 Course of venous free plasma tryptophan levels, group means and standard deviations (SD) of participants in experimental (tryptophan depletion, −TRP) and control (+TRP) condition showing the depletion or stimulation of TRP levels and return to baseline over 24 h

$T = 2.27$, $p < 0.04$) between the experimental (−TRP) and in the control (+TRP) condition. Nevertheless, ANOVÁs showed no significant group effects (−TRP vs. +TRP) ($F = 2.38$, $p > 0.14$) but repeated measurement analysis demonstrated significant differences between the sequence of ASR blocks ($F = 38.11$, $p < 0.0001$). Interaction effects between the groups (−TRP vs. +TRP) and the sequence of ASR-blocks as well as the order of treatment on ASR amplitudes were not significant. ANOVA's also failed to show any significant results concerning habituation, time course of startle reactivity and PPI.

Nevertheless, habituation effects were observed in both conditions, with the experimental (−TRP) condition showing more of a decrease (Table 1). However, there were no group differences for habituation ($T = -0.55$, $p < 0.59$). Interestingly, the range of standard deviation showed a decrease from the first to the third block under depletion (−TRP), whereas controls (+TRP) showed an increase, pointing to some instability of the magnitudes.

Regarding the time course of startle reactivity, the onset latency (i.e., the time from stimulus onset to reflex onset) of the pulse-alone trials did not differ between the two conditions (Table 1), but participants in the experimental (−TRP) condition showed slightly increased onset latencies compared to the control (+TRP) group ($T = -1.70$, $p < 0.11$). Differences in results for individual blocks were most prominent in the first of the PA-blocks, although not significant (1st: $T = -1.86$ $p < 0.08$; 2nd: $T = -1.29$, $p < 0.22$; 3rd: $T = -0.51$, $p < 0.61$). Results of peak startle latency (i.e. the time from stimulus onset to peak reflex amplitude) of startle PA trials were comparable without any differences, neither in the mean peak latency nor between the three blocks (Table 1).

Analysis of prepulse inhibition did not reveal any changes between the two conditions ($T = -0.34$, $p < 0.74$). Prepulse amplitudes were reduced for depletion (−TRP) as opposed to control (+TRP) group, albeit not significantly ($T = 1.47$, $p < 0.16$). Comparison of prepulse onset latencies revealed no differences, nor did the peak latencies for prepulse reactivity differ. However, startle magnitude of the pulse-alone amplitude in the ppP/PA block also showed clear difference between treatment conditions ($T = 3.40$, $p < 0.004$) with reductions under (−TRP) depletion.

Mood assessment

Comparison of POMS dimensions at baseline and after treatment showed differences for both conditions which were either significant or indicated a trend (Table 2) for decrease of depression/dejection (−TRP: $T = -1.70$, $p < 0.11$; +TRP: $T = -2.74$,

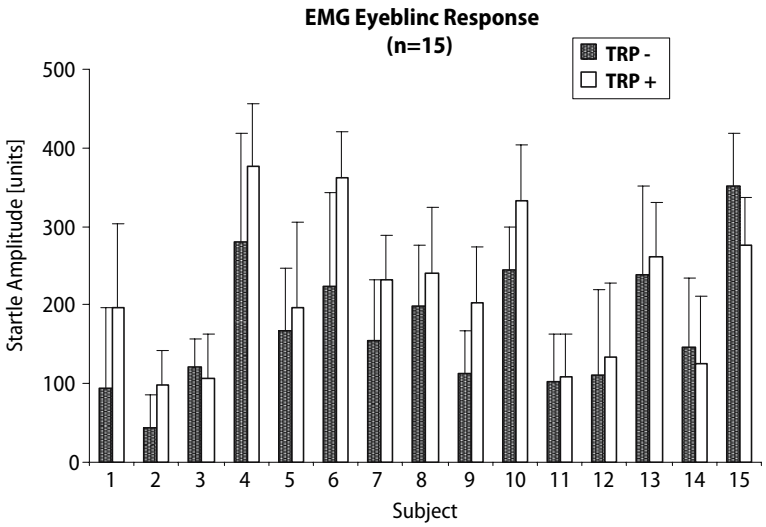

Fig. 2 Overview of amplitude of the EMG eye blink response of participants in experimental (−TRP) and control (+TRP) condition: means and standard deviations (SD) of individual results of each participant

Table 1 Comparison of the effects of tryptophan depletion on the EMG eyeblink response (startle), i.e., different parameters of the acoustic startle response and PPI in healthy females: group means and standard deviations (SD) of participants in experimental (−TRP) and control (+TRP) condition as well as the difference of the two conditions (−TRP)−(+TRP); *t*-test, $p < 0.05$

Startle	−TRP	+TRP	(−TRP)−(+TRP)	*p*
Amplitude				
Magnitude[a]	163.31 (88.90)	215.73 (92.19)	−52.42 (53.12)	0.002
Block 1[b]	214.38 (103.83)	262.10 (84.17)	−47.72 (47.96)	0.002
Block 2[c]	151.25 (93.76)	206.85 (92.72)	−55.59 (51.72)	0.01
Block 3[d]	132.40 (88.33)	185.88 (108.42)	−53.48 (91.12)	0.04
Habituation[e]	37.91 (30.52)	32.71 (21.89)	5.20 (36.42)	–
Onset latency	33.94 (6.45)	32.6 (5.35)	1.35 (3.07)	(0.11)
Block 1[b]	32.65 (6.13)	31.2 (4.59)	1.45 (3.03)	(0.08)
Block 2[c]	34.81 (6.79)	33.04 (6.40)	1.77 (5.35)	–
Block 3[d]	34.29 (8.03)	33.53 (5.81)	0.75 (5.73)	–
Peak latency	61.23 (5.90)	60.77 (5.47)	0.46 (4.19)	–
Block 1[b]	63.67 (7.45)	61.39 (7.74)	2.28 (8.67)	–
Block 2[c]	60.44 (6.34)	60.19 (5.46)	0.25 (6.41)	–
Block 3[d]	59.48 (8.49)	60.69 (6.68)	−1.21 (5.83)	–
Prepulse				
Prepulse amplitude	42.7 (45.70)	61.05 (48.86)	−18.35 (48.27)	(0.16)
Pulse amplitude	107.14 (71.92)	159.21 (88.32)	−52.07 (59.29)	0.004
Prepulse inhibition[f]	57.15 (25.62)	53.14 (49.06)	4.02 (45.90)	–
Prepulse onset latency	33.45 (6.41)	33.43 (5.86)	0.02 (7.66)	–
Prepulse peak latency	49.69 (5.46)	51.18 (7.45)	−1.48 (6.97)	–

[a]All pulse-alone trials 1–15, [b]Pulse-alone trials 1–5, [c]Pulse-alone trials 6–10, [d]Pulse-alone trials 11–15, [e]Habituation: [amplitude ((mean block 1 − mean block 3)/mean block 1) × 100]
[f]Prepulse inhibition: [amplitude ((mean pulse-alone − mean prepulse)/mean pulse-alone) × 100]

$p < 0.02$), vigour/activity (−TRP: $T = -2.39$, $p < 0.03$; +TRP: $T = -2.01$, $p < 0.06$) and anger/hostility (−TRP: $T = -1.65$, $p < 0.12$; +TRP: $T = -2.59$, $p < 0.02$) while there was no change for fatigue/inertia (−TRP: $T = -0.69$, $p < 0.50$; +TRP: $T = -0.18$, $p < 0.86$). The results of somatic analogous scales did not differ between scores of treatment conditions or differences of peak and baseline values (data not shown).

Correlation of parameters

Considering startle variables and changes in plasma TRP levels (t_1-t_0) in the control (+TRP) group there were only non-significant trends regarding the mean difference in onset latency ($r = -0.49$, $p < 0.06$), but none in the experimental (−TRP) condition ($r = -0.33$, $p < 0.24$). We could not find any correlations of differences in startle variables in the two conditions (+TRP, −TRP) and changes in plasma TRP levels (t_1-t_0). Further analyses of startle parameters and mood states (POMS) at peak maximum or minimum plasma TRP levels (t_1) revealed no correlations for the two conditions. Changes (t_1-t_0) in both TRP plasma levels and mood states (POMS) correlated negatively in the experimental (−TRP) group, with a tendency for depression/dejection ($r = -0.48$, $p < 0.067$), and in the control (+TRP) group for fatigue/inertia ($r = -0.50$, $p < 0.061$).

Discussion

Results from this first study of TDT in an all-female study group point to a 5-HT modulation of specific parameters of the human startle response, indicating early information processing. The major finding is a reduction of mean ASR after TDT in females. Interestingly, this effect was not seen in healthy males following TDT challenge [51]. Earlier observations in women of a higher probability of startle reflex (towards tactile stimuli) by Blumenthal and Gescheider [4] as well as a higher acoustic ASR [35] may also underline different influences of gender in the two TDT studies. Moreover, while higher age has been identified to affect ASR [39] our female group was of almost uniformly young age as opposed to a wider age range (21–52 years) in the TDT study by Philips et al. [51].

The interesting finding from an early TRP depletion study on incubated rats showed an enhanced

Table 2 Profile of Mood States (POMS) scores at baseline and 5 h after amino acid drinks of participants in experimental (−TRP) and control (+TRP) condition: group means and standard deviations (SD); *t*-test, $p < 0.05$

POMS	−TRP *Baseline* (t_0)	−TRP + 5 *h* (t_1)	*p*	+TRP *Baseline* (t_0)	+TRP + 5 *h* (t_1)	*p*
Depression/ Dejection	17.50 (4.21)	16.27 (3.54)	$p < 0.11$	17.36 (3.71)	15.53 (2.33)	$p < 0.02$
Fatigue/ Inertia	15.63 (6.27)	14.27 (4.38)	$p < 0.50$	16.86 (6.85)	16.00 (6.36)	$p < 0.86$
Vigour/ Activity	35.22 (5.90)	31.80 (7.35)	$p < 0.03$	34.07 (6.60)	31.73 (7.07)	$p < 0.06$
Anger/ Hostility	11.21 (5.54)	9.27 (4.11)	$p < 0.12$	11.21 (4.15)	9.40 (4.50)	$p < 0.02$

startle magnitude after being fed an L-tryptophan free diet for 13 days, and their startle magnitude returned to control levels after L-tryptophan was again added to the diet [70]. However, heterogeneity of species-specific effects on startle—for example after treatment with MDMA with opposite findings of either enhanced or reduced startle magnitude in mice and rats, but enhanced ASR in humans—remains an unsolved issue [13, 69].

In this study, habituation of ASR—i.e. reduction after repeated presentation of startle without any influence by muscle fatigue or blunting of sensory receptor responsiveness [24]—tended to decrease more in the depletion (−TRP) condition even though group differences were not significant. In parallel, the range of standard deviation decreased from the first to the third pulse alone block under (−TRP) whereas it increased in (+TRP), pointing to some instability of the magnitudes by the strong manipulation of metabolism by amino acids including TRP. Otherwise, severity of MDMA abuse seemed to produce some effects with enhanced sensitisation in the heavy users but habituation of ASR was not affected by MDMA [25, 37, 55, 69]. No specific habituation effects of acute administration of 5-HT agonists in healthy humans have been reported [26, 37, 50], whereas after long-term SSRI treatment depressed patients showed a strong disruption of their initial habituation [56]. These findings need corroboration by further studies.

In our study, we observed a lack of any significant alteration of PPI (except for a trend towards a percentage PPI more marked in TDT). Contrary results of significantly attenuated PPI in males were found by Phillips et al. [51]. Previously, a decreased PPI response has also been observed in females compared to males [64]. In fact, the more recent findings from human studies of an unchanged PPI after stimulation with SSRI [50, 56] weaken the assumed predictive value of the 5-HT influence on PPI disruption based on animal studies [17]. For example, an enhanced PPI in humans on MDMA [69]—or psilocybin, a hallucinogenic 5-$HT_{1A/2}$ agonist [18]—was also observed after long-term use of MDMA but rather believed to indicate alterations of neurotransmission other than lowered 5-HT or a chronic 5-HT deficiency condition [55]. On the other hand, reduction of PPI in humans by some atypical antipsychiotics with antiserotonergic properties (clozapine, ketanserin) was rather related to their sedative properties [20, 21], similar to the same effect of the tricyclic antidepressant amitriptyline [50]. Hence, the non-significant reduction of PPI in our female cohort may also result from some sedation of +TRP mixture as this group showed some interrelation in tendency between control (+TRP) treatment and increased fatigue. Thus, we believe that the so-called "control" or "placebo" situation of the (+TRP) condition must be questioned regarding alterations of brain metabolism.

With respect to methodological issues, this study focused on healthy females without a family history of major affective disorder. However, we found some weak correlations failing significance of changes of plasma TRP under (−TRP) depletion, with a negative trend towards subtler depressive feelings of sadness. At the same time, interactions of mood states with startle parameters remained unaffected presumably because these mood changes were too low compared to clinical populations like depressed patients. Thus, the psychometrically tested non-clinical changes of mood states may indicate an endogenous emotional vulnerability with a low temporal stability in females for this challenge test [5, 14].

One limitation in the laboratory analysis was that plasma ratios for large amino acids other than TRP could not be determined, nor was it possible to control for cortisol or other hormone levels during the study. The time point of the menstrual cycle, however, was carefully controlled—our results exclusively apply to women in the first half of menstrual cycle—because of interactions of estrogens with the monoaminergic system and the assumed influences of altered dopamine receptor sensitivity during the luteal phase on PPI [31, 66]. The impact of lower inhibitory gating in women under experimental and clinical conditions—e.g., cognitive alterations or depressive disorder—can only be speculation.

The main advantage of the TDT paradigm is inducing a transient 70–90% decrease in plasma TRP [3, 72]. However, although levels of TRP in plasma and CSF correlated closely in humans, the factual CSF nadirs of TRP and metabolites did not dip until several hours after the plasma values did, i.e. 8–12 h after taking the TRP free amino acid drink with only moderate decrease (31%) of 5-HIAA in CSF from baseline [8]. Thus, the ideal time-point to estimate the 5-HIAA nadir following depletion remains unclear [46], and it is possible that our results might have been clearer at a later point in time. Still, the study began about 6 h after the amino acid drink was started, which was even after the established time window of 4–6 h following TDT.

From our serial plasma measurements it also became obvious, that 6 h after start of study procedure the peripheral TRP turnover began to show an increasing interindividual variability with progression over time, pointing to higher variances in 5-HT brain depletion which may account for diverse results in different study populations and time points, too. Also, it has be kept in mind that due to factors influencing transport and catabolism of 5-HIAA, its levels were assumed to be a poor index of dynamic changes in 5-HT synthesis in brain tissue [49], and effects were even shown to be unrelated to neuronal activity [59]. These observations may go in line with the unexpected lack of effect of TDT on ^{18}F-MPPF PET in healthy subjects or remitted patients with major depressive disorder despite some relapses [53, 68]. In

our study, the dosage of amino acids of 75 g did not induce any severe somatic symptoms, but it did cause a decrease >81% in the (−TRP) condition, which is considered an extensive and sufficient decline in peripheral TRP. A larger study cohort would be instructive despite the extensive TDT protocol.

Comparing our results to current findings from other human ASR studies, it becomes clear that several aspects must be taken into account, including age, gender, hormones and menstrual cycle, as well as their potential interaction with interindividual differences in affective response. Still, it remains unclear if suspected gender differences may exist considering methodological differences between previous studies (e.g. range of age, amino acid composure or medication and electrophysiological study design). Furthermore, ASR appears to depend on various factors and does not show a normal distribution, as is generally assumed. In view of the inconsistent data from clinical startle studies, research would need to focus more on these factors as well as on symptomatology [32] and eventually consistency of treatment. Accordingly, a higher baseline ASR in patients with major depression which was related to a better improvement under sertraline medication [56] suggests that the ASR might serve as a neurobiological predictor for therapy response. Previous animal studies on ASR and PPI must be surveyed regarding the complex neuroanatomy as well as the pharmacology of multiple 5-HT receptor subtypes and their mechanisms in the modulation of startle parameters [61] in contrast to a more general manipulation of the human 5-HT system by TDT, as evident from different responses to various antidepressant treatments [12].

In conclusion, this first study of startle parameters after acute TDT in a homogenous female population demonstrates that depletion of brain 5-HT in women only influences ASR but does not alter PPI or habituation. More research is needed on the relevance of human 5-HT modulation on ASR and PPI as a psychobiological marker for early attentional sequencing and a protective buffer for information processing, particularly in the clinical context.

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References

1. Aasen I, Kolli L, Kumari V (2005) Sex effects in prepulse inhibition and facilitation of the acoustic startle response: implications for pharmacological and treatment studies. J Psychopharmacol 19:9–45
2. APA (1994) Diagnostic and statistical manual of mental disorders. 4th ed., American Psychiatric Association, Washington, DC
3. Benkelfat C, Ellenbogen MA, Dean P, Palmour RM, Young SN (1994) Mood-lowering effect of tryptophan depletion. Enhanced susceptibility in young men at genetic risk for major affective disorders. Arch Gen Psychiatry 51:687–697
4. Blumenthal TD, Gescheider GA (1987) Modification of the acoustic startle response by a tactile prepulse: effects of stimulus onset asynchrony and prepulse intensity. Psychophysiology 24:320–327
5. Booij L, Van der Does W, Benkelfat C, Bremner JD, Cowen PJ, Fava M, Gillin C, Leyton M, Moore P, Smith KA, Van der Kloot WA (2002) Predictors of mood response to acute tryptophan depletion. A reanalysis. Neuropsychopharmacology 27:852–861
6. Braff D, Stone C, Callaway E, Geyer M, Glick I, Bali L (1978) Prestimulus effects on human startle reflex in normals and schizophrenics. Psychophysiology 15:339–343
7. Bremner JD, Innis RB, Salomon RM, Staib LH, Ng CK, Miller HL Bronen RA, Krystal JH, Duncan J, Rich D, Price LH, Malison R, Dey H, Soufer R, Charney DS (1997) Positron emission tomography measurement of cerebral metabolic correlates of tryptophan depletion-induced depressive relapse. Arch Gen Psychiatry 54:364–374
8. Carpenter LL, Anderson GM, Pelton GH, Gudin JA, Kirwin PD, Price LH, Heninger GR, McDougle CJ (1998) Tryptophan depletion during continuous CSF sampling in healthy human subjects. Neuropsychopharmacology 19:26–35
9. Castellanos FX, Fine EJ, Kaysen D, Marsh WL (1996) Sensorimotor gating on boys with Tourettés Syndrome and ADHD: preliminary results. Biol Psychiatry 39:33–41
10. Davis M (1984) The mammalian startle response. In: Eaton RC (ed) Neural mechanisms of startle behaviour. Plenum, New York
11. Delgado PL, Charney DS, Price LH, Aghajanian GK, Landis H, Heninger GR (1990) Serotonin Function and the mechanism of antidepressant action: reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan. Arch Gen Psychiatry 47:411–418
12. Delgado PL, Miller HL, Salomon RM, Licinio J, Krystal JH, Moreno FA Delgado PL, Miller HL, Salomon RM, Licinio J, Krystal JH, Moreno FA, Heninger GR, Charney DS (1999) Tryptophan-depletion challenge in depressed patients treated with desipramine or fluoxetine: implications for the role of serotonin in the mechanism of antidepressant action. Biol Psychiatry 46:212–220
13. Dulawa SC, Hen R, Scearce-Levie K, Geyer MA (1997) Serotonin 1 B receptor modulation of startle reactivity, habituation, and prepulse inhibition in wild-type and serotonin 1B receptor knockout mice. Psychopharmacology 132:125–134
14. Ellenbogen MA, Young SN, Dean P, Palmour RM, Benkelfat C (1996) Mood response to acute tryptophan depletion in healthy volunteers: sex differences and temporal stability. Neuropsychopharmacology 15:465–474
15. Fletcher PJ, Selhi ZF, Azampanah A, Sills TL (2001) Reduced brain serotonin activity disrupts prepulse inhibition of the acoustic startle reflex. Effects of 5,7-dihydroxytryptamine and p-chlorophenylalanine. Neuropsychopharmacology 24:399–409
16. Geyer MA, Braff DL (1982) Habituation of the blink reflex in normals and schizophrenic patients. Psychophysiology 19:1–6
17. Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR (2001) Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology 156:117–154
18. Gouzoulis-Mayfrank E, Heekeren K, Thelen B, Lindenblatt H, Kovar KA, Sass H, Geyer MA (1998) The hallucinogen psilocybin increases prepulse inhibition of the startle reflex in the healthy humans. Behav Pharmacol 9:561–566
19. Graham FK (1975) The more or less startling effects of a weak prestimulation. Psychophysiology 12:238–248

20. Graham SJ, Langely RW, Bradshaw CM, Szabadi E (2001) Effects of haloperidol and clozapine of the acoustic startle response and the N1/P2 auditory evoked potential in man. J Psychopharmacology 15:243–250
21. Graham SJ, Langley RW, Balboa VA, Bradshaw CM, Szabadi E (2002) Effects of ketanserin and haloperidol on prepulse inhibition of the acoustic startle (eyeblink) response and the N1/P2 auditory evoked response in man. J Psychopharmacology 16:15–22
22. Graham SJ, Scaife JC, Balboa Verduzco AM, Langley RW, Bradshaw CM, Szabadi E (2004) Effects of quetiapine and haloperidol on prepulse inhibition of the acoustic startle (eyeblinc) response and the N1/P2 auditory evoked response in man. J Psychopharmacology 18:173–180
23. Grillon C, Davis M (1997) Fear-potentiated startle conditioning in humans: explicit and contextual cue conditioning following paired versus unpaired training. Psychophysiology 34:451–458
24. Groves PM, Thompson RF (1970) Habituation: a dual-process theory. Psychol Rev 77:419–450
25. Heekeren K, Daumann J, Geyer MA, Gouzoulis-Mayfrank E (2004) Plasticity of the acoustic startle reflex in currently abstinent ecstasy (MDMA) users. Psychopharmacology (Berl) 173:418–424
26. Hellewell JS, Guimarães FS, Wang M, Deakin JF (1999) Comparison of buspirone with diazepam and fluvoxamine on aversive classical conditioning in humans. J Psychopharmacology 13:122–127
27. Herpertz SC, Koetting K (2005) Startle response in inpatients with borderline personality disorder vs. healthy controls. J Neural Transm 112:1097–2106
28. Herpertz S, Kunert HJ, Schwenger UB, Sass H (1999) Affective response in borderline personality disorder: a psychophysiological approach. Am J Psychiatry 156:1550–1556
29. Herpertz SC, Werth U, Lukas G, Qunaibi M, Schuerkens A, Kunert HJ, Freese R, Flesch M, Mueller-Isberner R, Osterheider M, Sass H (2001) Emotion in criminal offenders with psychopathy and borderline personality disorder. Arch Gen Psychiatry 58:737–745
30. Hindberg I, Naesh O (1992) Serotonin concentrations in plasma and variations during the menstrual cycle. Clin Chem 38:2087–2089
31. Jovanovic T, Szilagyi S, Chakravorty S, Fiallos AM, Lewison BJ, Parwani A, Schwartz MP, Gonzenbach S, Rotrosen JP, Duncan EJ (2004) Menstrual cycle phase effects on prepulse inhibition of acoustic startle. Psychophysiology 41:401–406
32. Kaviani H, Gray JA, Checkley SA, Raven PW, Wilson GD, Kumari V (2004) Affective modulation of the startle response in depression: influence of the severity of depression, anhedonia, and anxiety. J Affect Dis 83:21–31
33. Kehne JH, Padich RA, Mc Closkey TC, Taylor VL, Schmidt CJ (1996) 5-HT modulation of auditory and visual sensorimotor gating: I. Effects of 5-HT releasers on sound and light prepulse inhibition in Wistar rats. Psychopharmacology 124:95–106
34. Koch M (1999) The neurobiology of startle. Progr Neurobiol 59:107–128
35. Kofler M, Muller J, Reggiani L, Valls-Sole J (2001) Influence of gender on auditory startle responses. Brain Res 921:206–210
36. Kumari V, Kaviani H, Raven PW, Gray JA, Checkley SA (2001) Enhanced startle reactions to acoustic stimuli in patients with obsessive-compulsive disorder. Am J Psychiatry 158:134–136
37. Liechti ME, Geyer MA, Hell D, Vollenweider FX (2001) Effects of MDMA (Ecstasy) on prepulse inhibition and habituation of startle after pretreatment with citalopram, haloperidol, or ketanserin. Neuropsychopharmacology 24:240–252
38. Ludewig S, Ludewig K, Geyer MA, Hell D, Vollenweider FX (2002) Prepulse inhibition deficits in patients with panic disorder. Depress Anxiety 15:55–60
39. Ludewig K, Ludewig S, Seitz A, Obrist M, Geyer MA, Vollenweider FX (2003) The acoustic startle reflex and its modulation: effects of age and gender in humans. Biol Psychol 63:311–323
40. Mansbach RS, Geyer MA (1988) Dopaminergic stimulation disrupts sensorimotor gating in the rat. Psychopharmacology 94:507–514
41. Mansbach RS, Braff DL, Geyer MA (1989) Prepulse inhibition of the acoustic startle response is disrupted by N-ethyl-3,4-methylenedioxyamphetamine (MDEA) in the rat. Eur J Pharmacology 167:49–55
42. Martínez DL, Geyer MA (1997) Characterization of the disruptions of prepulse inhibition and habituation of startle induced by alpha-ethyltryptamine. Neuropsychopharmacology 16:246–255
43. McNair DM, Lorr M, Droppleman LF (1971) Manual for the profile of mood states. Educational and Industrial Testing Services, San Diego CA
44. Meincke U, Morth D, Voss T, Thelen B, Geyer MA, Gouzoulis-Mayfrank E (2004) Prepulse inhibition of the acoustically evoked startle reflex in patients with an acute schizophrenic psychosis—a longitudinal study. Eur Arch Psychiatry Clin Neurosci 254:415–421
45. Menkes DM, Coateds DC, Fawcett JP (1994) Acute tryptophan depletion aggravates premenstrual syndrome. J Affect Dis 32:377–387
46. Moreno FA, McGavin C, Malan TP, Gelenberg AJ, Heninger GR, Mathe AA, Delgado PL (2000) Tryptophan depletion selectively reduces CSF 5-HT metabolites in healthy young men: results from single lumbar puncture sampling technique. Int J Neuropsychopharmacology 3:277–283
47. Nancy KP, Tisson HA (1989) The role of 5HT1A receptors in the modulation of the acoustic startle reflex in rats. Psychopharmacology 97:507–531
48. Neumeister A, Nugent AC, Waldeck T, Geraci M, Schwarz M, Bonne O, Bain EE, Luckenbaugh DA, Herscovitch P, Charney DS, Drevets WC (2004) Neuronal and behavorial responses to tryptophan depletion in unmedicated patients with major depressive disorder and healthy controls. Arch Gen Psychiatry 61:765–773
49. Nishizawa S, Benkelfat C, Young SN, Leyton M, Mzengeza S, De Montigny C, Blier P, Diksic M (1997) Differences between males and females in rates of serotonin synthesis in human brain. Proc Natl Acad Sci USA 94:5308–5313
50. Phillips M, Langley RW, Bradshaw CM, Szabadi E (2000a) The effects of some antidepressant drugs on prepulse inhibition of the acoustic startle (eyeblink) response and the N1/P2 auditory evoked response in man. J Psychopharmacol 14:40–45
51. Phillips M, Oxtoby EK, Langley RW Bradshaw CM, Szabadi E (2000b) Effects of acute tryptophan depletion on prepulse inhibition of the acoustic startle (eyeblink) response and the N1/P2 auditory evoked response in man. J Psychopharmacol 14:258–265
52. Pouzet B, Paabol Andersen M, Hogg S (2005) Effects of acute treatment with antidepressant drugs on sensorimotor gating deficits in rats. Psychopharmacology 178:9–16
53. Praschak-Rieder N, Hussey D, Wilson AA, Carella A, Lee M, Dunn E, Willeit M, Bagby RM, Houle S, Meyer JH (2004). Tryptophan depletion and serotonin loss in selective serotonin reuptake inhibitor-treated depression: an [^{18}F]MPFF Positron Emission Tomography Study. Biol Psychiatry 56:587–591
54. Prinssen EPM, Assié M-B, Koek W, Kleven MS (2002) Depletion of 5-HT disrupts prepulse inhibition in rats: dependence on the magnitude of depletion, and reversal by a 5-HT precursor. Neuropsychopharmacology 26:340–347
55. Quednow BB, Kühn KU, Hoenig K, Maier W, Wagner M (2004a) Prepulse inhibition of acoustic startle response in male MDMA ('Ecstasy') users, cannabis users, and healthy controls. Neuropsychopharmacology 29:982–990
56. Quednow BB, Kühn K-U, Stelzenmueller R, Hoenig K, Maier W, Wagner M (2004b) Effects of serotonergic and noradrenergic antidepressants on auditory startle response in patients with major depression. Psychopharmacology 175:399–405
57. Riedel WJ, Klaassen T, Deutz NE, van Someren A, van Praag HM (1999) Tryptophan depletion in normal volunteers produces selective impairment in memory consolidation. Psychopharmacology (Berl) 141:362–369

58. Rigdon GC, Weatherspoon JK (1992) 5-Hydroxytryptamine 1a receptor agonists block prepulse inhibition of acoustic startle reflex. J Pharmacol Exp Ther 263:486–493
59. Schaechter JD, Wurtman RJ (1990). Serotonin release varies with brain tryptophan levels. Brain Res 532:203–210
60. Schlenker R, Cohen R, Hopmann G (1995) Affective modulation of the startle reflex in schizophrenic patients. Eur Arch Psychiatry Clin Neurosci 245:309–318
61. Sipes TA, Geyer MA (1994) Multiple serotonin subtypes modulate prepulse inhibition of the startle response in rats. Neuropharmacology 33:441–448
62. Shalev AY, Orr SP, Peri T, Schreiber S, Pitman RK (1992) Physiologic responses to loud tones in Israel patients with posttraumatic stress disorder. Arch Gen Psychiatry 49:870–875
63. Swerdlow NR, Benbow CH, Zisook S, Geyer MA, Braff DL (1993a) A preliminary assessment of sensorimotor gating in patients with obsessive compulsive disorder. Biol Psychiatry 33:298–301
64. Swerdlow NR, Monroe SM, Hartston HJ, Braff DL, Geyer MA, Auerbach PP (1993b) Men are more inhibited than women by weak impulses. Biol Psychiatry 33:298–301
65. Swerdlow NR, Paulsen J, Braff DL, Butters N (1995) Impaired prepulse inhibition of acoustic and tactile startle response in patients with Huntingtons disease. J Neurol Neurosurg Psychiatry 58:192–200
66. Swerdlow NR, Hartman PL, Auerbach PP (1997) Changes in sensorimotor inhibition across the menstrual cycle: implications for neuropsychiatric disorders. Biol Psychiatry 41:452–460
67. Turpin G, Schaefer F, Boucsein W (1999) Effects of stimulus intensity, risetime, and duration on autonomic and behavioural responding: implications for the differentiation of orienting, startle, and defense responses. Psychophysiology 36:453–463
68. Udo de Haes JI, Bosker FJ, Van Waarde A, Pruim J, Willemsen AT, Vaalburg W, Den Boer JA (2002) 5-HT(1A) receptor imaging in the human brain: effect of tryptophan depletion and infusion on [(18)F]MPPF binding. Synapse 46:108–115
69. Vollenweider FX, Remensberger S, Hell D, Geyer MA (1999) Opposite effects of 3,4-methylenedioxymethamphetamine (MDMA) on sensorimotor gating in rats versus healthy humans. Psychopharmacology 143:365–372
70. Walters JK, Davis M, Sheard MH (1979) Tryptophan-free diet: effects on the acoustic startle reflex in rats. Psychopharmacology 62:103–109
71. Williams WA, Shoaf SE, Hommer D, Rawlings R, Linnoila M (1999) Effects of acute tryptophan depletion on plasma and cerebrospinal fluid tryptophan and 5-hydroxyindoacetetc acid in normal volunteers. J Neurochem 72:1641–1647
72. Young SN, Smith SE, Pihl RO, Erwin FR (1985) Tryptophan depletion causes a rapid lowering mood in normal males. Psychopharmacology 87:173–177
73. Young SN, Ervin FR, Pihl RO, Finn P (1989) Biochemical aspects of tryptophan depletion in primates. Psychopharmacology 98:508–511