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Research report

Aggression—Interactions of serotonin and testosterone in healthy men and women

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ABSTRACT

Serotonin (5-HT) and testosterone (T) have both been implicated in the regulation of aggression. Findings in humans however are very inconclusive, with respect to main effects of either system. Animal models implicate T to modulate 5-HT system activity, and furthermore have shown behaviorally relevant interactions of T and 5-HT with respect to aggression.

We tested for associations between habitual T-level and 5-HT system activity, as well as behaviorally relevant interactions of T and 5-HT with respect to trait aggression in 48 healthy male and female subjects. 5-HT activity was measured by means of neuroendocrine challenge paradigm with S-citalopram. T-levels were measured in saliva samples. Trait aggression was assessed by self-report measures.

T-levels were not associated with indices of central 5-HT activity. Results showed significant interaction effects between 5-HT and T for trait aggression in men only ($p < 0.05$). Trait aggression was significantly higher in the *combinations* "high T + high cortisol responses" (indicating decreased 5-HT availability), and "low T + low cortisol responses" (indicating increased 5-HT availability), after S-citalopram.

Results support the notion of behaviorally relevant interactions between T and 5-HT, with respect to aggression in humans, but also indicate the need for further studies.

1. Introduction

The sex-steroid testosterone (T) and the neurotransmitter serotonin (5-HT) have been implicated in the regulation of aggression in numerous studies in humans and animals. The heterogeneous results have been the source of ongoing debate [1–6].

Animal studies, as well as studies in clinical psychopathological samples, provide strong, though not unopposed, evidence of impaired serotonergic neurotransmission being associated with increased aggression [7,8,1,9]. Results in healthy humans, however, are very heterogeneous [10]. An association between 5-HT activity and measures of aggression has only been found in men, but not in young pre-menopausal women [10,11]. However, one study, using a large community based sample, showed prolactin secretion after fenfluramine to be associated with measures of aggression in a small sub-sample of post-menopausal women as well as in healthy men, while no association was found in pre-menopausal women [10].

Various studies using animal models support the view of the aggression promoting effects of habitual T-levels [5,12–14]. However, as far as humans are concerned, results are mixed [15,14,5]. Many studies in psychopaths and/or criminal samples have shown increased aggression to be associated with higher T-levels. However, in healthy humans, particularly in women, results are inconclusive [16–18,5]. T in humans appears to be only weakly associated with aggression, as concluded by several meta-analyses and reviews [14,16,15,13,5]. Various aspects appear to modulate this association, including gender, the participants' psychological health, age and past experience. In addition, high T-levels have been reported to be associated with social dominance and success rather than aggression [19,1].

In conclusion, neither habitual T-level nor basal central 5-HT activity alone is able to satisfactorily explain individual differences in aggression in healthy subjects. As outlined above, meta-analysis and reviews indicate them to have only weak, inconsistent effects in healthy populations, which are hard to detect in smaller samples.

Numerous animal studies implicate the activity of the serotonergic system to be modulated by T as well as by estrogen (E). The distribution and density of 5-HT receptors, as well as 5-HT levels in the brain, have been shown to be modulated by early life exposure to androgens [20,21]. In adult rats, T and E increased 5-HT-transporter (SERT) and 5-HT_{2a} receptor mRNA and binding sites [22]. T counteracted 5-HT_{1a} receptor down-regulation, induced by glucocorticoid secretion under chronic subordinate stress [23]. The

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anabolic androgenic steroid nandrolone down-regulates 5-HT_{1b} receptor densities, but up-regulates 5-HT_2 receptors in specific brain regions of male rats [24].

Beyond this apparent modulation of the activity of the serotonergic system by sex-steroids, different animal studies showed a behaviorally relevant interaction of the two systems with respect to aggressive behavior. One study used parachlorophanylalanin (PCPA) to deplete 5-HT in young male rats from postnatal day 26 to 40. With the onset of puberty, half of the number of rats were treated with testosterone propionate (TP) and several behavioral measures were assessed. 5-HT was significantly depleted by PCPA and further reduced by treatment with (TP), however to a lesser extent. Irritability was significantly increased in PCPA-treated males. TP alone increased aggression. Aggression under provocation conditions was dramatically increased in male rats treated with PCPA and TP [25]. This result is in line with other studies. One of them showed that T and E modulate the aggression-reducing effects of 5-HT_{1a} and 5-HT_{1b} agonists on inter-male aggression [26]. The authors concluded that in the presence of estrogens, either directly applied or after conversion from T, the inhibition of aggression by the serotonergic agonist was restricted. However, the agonists were effective in decreasing aggression directly mediated by androgens. Further support for an interaction of T and 5-HT in the regulation of aggression comes from a study using non-human primates. As might be expected, CSF T levels were positively associated with overall aggression. On the other hand, CSF 5-HIAA (5-hydroxyindole acetic acid) was negatively associated with impulsive behavior and severe unrestrained aggression. In animals with low CSF 5-HIAA levels, aggressive behavior was increased if they also had increased CSF T levels [27].

From these data, it appears promising to analyze analogous effects of Testosterone and 5-HT in humans, and their role in aggressive behavior; an idea already suggested in earlier reviews [1,6]. From the animal data, showing how habitual T-level might directly modulate 5-HT-system activity on the level of, e.g. receptor densities or 5-HT levels plus the fact that 5-HT is directly associated with aggression in animals, it might be concluded that the 5-HT-system probably acts as a mediator of the association between T and aggression. That is T, might directly down-regulate the expression of certain functional parts of the 5-HT synapse, i.e. 5-HT_{1b} receptor densities, which are thought to mediate the aggression inhibiting effects of 5-HT, and at the same time increase expression rates of those functional parts of the 5-HT-system which are thought to facilitate aggressive behavior such as 5-HT_{2a} receptor densities. However, judging from animal studies on the effects of habitual T and habitual 5-HT activity on aggression, somewhat independent effects of both systems, which add up to substantially increased aggressive behavior in animals showing the combination of increased T-level plus reduced 5-HT activity, appear equally plausible.

2. Aims of the study

No studies on the combined effects of central 5-HT activity and acute T-level on human aggression in healthy participants are available so far. Therefore, we were interested in further elucidating the effects of habitual T-level and central 5-HT activity with respect to the following questions:

(1) Do habitual Testosterone-level modulate central 5-HT activity as measured with an S-citalopram challenge test in healthy men and women, as implicated by some animal studies?
(2) Does 5-HT act as a mediator of the relation between T and aggression, or do both systems independently modulate trait aggression giving rise to additive effects?

3. Materials and methods

3.1. Participants

The sample consisted of 48 healthy volunteers (24 men, 24 women) between 20 and 30 years of age (mean = 23.9 years, SD = 2.53). Prior to participation, participants were informed regarding the substance used and possible adverse side effects. Participants were further informed that the aim of the study was to investigate associations between personality, central serotonergic activity and testosterone, without specifying our intentions to analyze human aggression. After all questions were answered, participants gave their written informed consent. The study was approved by the ethics committee of the German Psychological Association.

In order to ensure psychological and physical health, questionnaires, and – if necessary – additional interviews were administered. Furthermore, participants were required to meet the following criteria: non-smoker, no use of steroidal contraceptives for at least 6 months prior to and during the study, and no intake of any medication containing steroids or interfering with central neurotransmission. Participants received detailed written instructions regarding appropriate behavior the evening before and on the day of the experiments. In particular, subjects were instructed to go to bed before midnight, refrain from the use of alcohol or other drugs and to have lunch before 13:00 h.

3.2. Procedures: S-citalopram challenge tests and habitual testosterone levels

Basal, central 5-HT availability was measured by means of a neuroendocrine challenge test in a placebo controlled, double blind crossover design, using single doses of S-citalopram (10, 20 mg, orally) with cortisol responses as indicators of responsiveness. The usefulness of S-citalopram and citalopram (S-citalopram is the active enantiomere of the racemic mixture citalopram) has been shown by several authors and is discussed in detail elsewhere [28–31].

Figs. 1 and 2 give an overview of experimental procedures and group allocation with respect to drug conditions.

During the three test sessions participants received 10 and 20 mg of S-citalopram in a placebo controlled double blind crossover design. In order to control for effects of the menstrual cycle, women were treated according to the phase of their menstrual cycle, and were randomly assigned to a “follicular” (days 4–6 after onset of menstruation) or “luteal group” (2–4 days prior to onset of menstruation). However, to ensure that data sampling did not exceed 32–36 days (depending on the length of individual menstrual cycle), the second test day took place around ovulation (individual mid-cycle day $\pm$ 1) for all women. As a consequence placebo, 10 and 20 mg were administered in equal proportions during each phase of the menstrual cycle, allowing us to investigate drug effects across all phases of the menstrual cycle in a balanced within-subject design. The time intervals between the three sessions for men were scheduled accordingly, in order to have the same temporal spacing as for women (number of days between experiments = 16.04, SD = 5.47). On each test day, participants entered the laboratory at 15:15 h. Subjects were seated in comfortable armchairs and a first set of data was collected, consisting of a questionnaire measuring basic dimensions of emotional states (visual analog scale, VAS) and bodily symptoms (VAS) to control for possible drug-associated side effects later on. During the completion of the questionnaires, participants were instructed to give a sample of saliva by the use of glass tubes. Immediately afterwards, drug or placebo was administered orally in identical neutral capsules. Further samples were taken at 55, 95, 135, 175 and

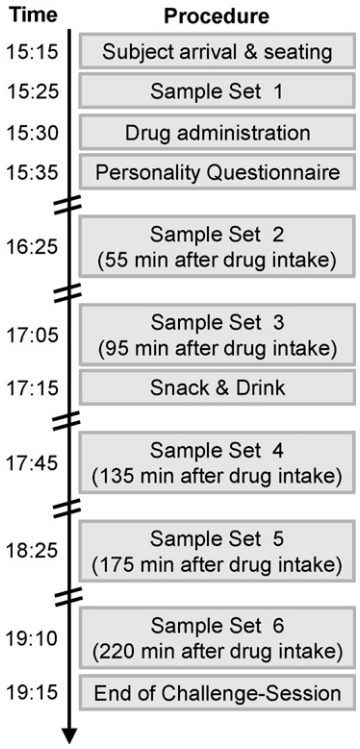


Fig. 1. Flow chart of procedures during each of the three challenge test sessions.

220 min after drug intake. Based on our previous experience using S-citalopram, participants were offered something to eat (soft cake pretzel) 100 min after drug intake, in order to reduce the probability of adverse effects. Moreover, participants were offered 300 ml to drink (carbonated water). Baseline saliva samples (sample set 1) of each test session were used to determine T-levels.

After each test session, saliva samples were centrifuged (10 min, 4000 × *g*) and frozen at −30 °C until assayed. Concentrations of salivary cortisol and testosterone levels were determined by the use of commercial immunoassays (cortisol: IBL, Hamburg, Germany; testosterone: Salimetrics, Pennsylvania, USA). All analyses were performed in duplicates using a fully automated analyzer (Biochem, Labotech, Freiburg, Germany), within the same lot to avoid high inter-assay variation. The intra-assay-variation (CV) was below 5%.

3.3. Psychometric measures

Psychometric assessments were performed on the three test sessions, prior to the onset of drug action. Participants were asked to fill in the following self-report questionnaires: the Buss–Durkee Hostility Inventory (BDHI) [32], the revised Freeburg Personality Inventory (FPI-R) [33], and the Questionnaire for Measuring Factors of Aggression (FAF) [34].

3.4. Statistics and response measures

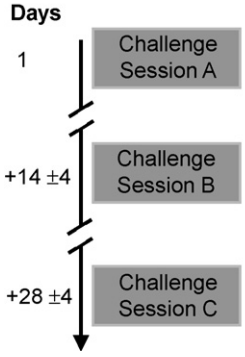
Testosterone data from the three sample days were aggregated for each subject and used for subsequent analysis to gain a valid estimate of habitual levels.

S-citalopram challenge tests were used to index central 5-HT activity. Analyses of variance (ANOVA) for repeated measures were calculated with two within subject factors (factor 1: drug, 3 levels; factor 2: time, 6 levels) to assess the effect of S-citalopram on cortisol concentrations. The single application of this SSRI prolongs the residence time of 5-HT in the synaptic cleft, thereby transiently increasing 5-HT levels in the synaptic cleft, thus leading to a transiently more pronounced activation of post-synaptic receptors, which again is thought to induce the secretion of hormones via activation of the HPA-axis [35]. An increased hormone secretion may be interpreted as an indicator of an up-regulation of post-synaptic receptors, possible due to reduced 5-HT availability [36,37]. Therefore, a high drug-induced CORT response (high CORT-group) is interpreted to indicate decreased 5-HT availability, while a low drug induced CORT response (low CORT-group) indicates increased 5-HT availability. In order to quantify cortisol release after S-citalopram treatment, the area under the curve (AUC) measure was calculated for both drug and placebo conditions ($AUC_{10\,mg}$, $AUC_{20\,mg}$, $AUC_{placebo}$) for each subject. As a measure of central 5-HT availability, placebo corrected response measures for each drug condition ($AUCR_{10\,mg}$, $AUCR_{20\,mg}$) were calculated by subtracting $AUC_{placebo}$ from, $AUC_{10\,mg}$ or $AUC_{20\,mg}$ respectively. Due to gender differences in placebo corrected cortisol release after 20 mg S-citalopram ($AUCR_{20\,mg}$), all subsequent analyses were conducted using the 10 mg data ($AUCR_{10\,mg}$) in order to be able to compare men and women (see also [31]).

Gender differences in T-levels are well known. Furthermore, earlier findings showed gender to modulate associations of T and 5-HT with aggression (also see Section 1). Therefore, all subsequent analyses were conducted separately but analogously for men and women.

Participants were divided into high and low S-citalopram cortisol responders by means of a median split of the $AUCR_{10\,mg}$ (high CORT, low CORT). High and low T-level groups were established by means of a median split of aggregated T-levels (high T, low T). Both procedures for grouping were performed within each gender.

Chi-square analysis was used to analyze possible differences in the proportions of high and low T-level participants with high or low placebo-corrected cortisol response.



	Challenge Session A	Challenge Session B	Challenge Session C	N
permutation I	Placebo	10 mg S-Citalopram	20 mg S-Citalopram	8
permutation II	Placebo	20 mg S-Citalopram	10 mg S-Citalopram	8
permutation III	10 mg S-Citalopram	Placebo	20 mg S-Citalopram	8
permutation IV	10 mg S-Citalopram	20 mg S-Citalopram	Placebo	8
permutation V	20 mg S-Citalopram	Placebo	10 mg S-Citalopram	8
permutation IV	20 mg S-Citalopram	10 mg S-Citalopram	Placebo	8

Fig. 2. Overview of challenge test session schedule and group allocation with respect to drug conditions.

Table 1
Testosterone (T) levels are given by gender and group allocation with respect to high and low T-level.

			Mean	SD	Low CORT-group	High CORT-group
Testosterone (pg/ml)	Low T-group	Men	28.66	1.74	$N=6$	$N=6$
		Women	13.11	1.12	$N=5$	$N=7$
	High T-group	Men	51.10	2.61	$N=6$	$N=6$
		Women	24.46	2.76	$N=7$	$N=5$
AUCR10 mg (arbitrary units)	Low CORT-group	Men	−6.79	4.76		
		Women	−2.02	2.86		
	High CORT-group	Men	13.79	5.91		
		Women	10.25	2.48		

Placebo corrected cortisol (CORT) responses after 10 mg S-citalopram (AUCR 10 mg) are given by gender and group allocation with respect to high and low CORT group. Means and standard deviations (SD) are given. Distribution of high and low T participants in high and low cortisol groups are indicated.

Questionnaire data were subjected to multivariate analysis of variance (MANOVA) with two independent factors (factor 1: high/low T, factor 2: high/low CORT response).

4. Results

4.1. Testosterone concentrations, S-citalopram induced cortisol release and psychometric measures

Aggregated T-levels and placebo corrected S-citalopram induced cortisol responses are given in Table 1, by gender and group allocation.

With respect to S-citalopram induced cortisol responses, analyses of variance (ANOVA) indicated three significant effects (main effect "drug": $F_{(1.31,232.5)} = 11.75$; $p < .01$; $\eta^2 = 0.20$, main effect "time": $F_{(2.63,232.5)} = 13.99$; $p < .001$; $\eta^2 = 0.23$; interaction "drug × time": $F_{(4.93,232.5)} = 5.66$; $p < .001$; $\eta^2 = 0.11$). Results clearly indicate that S-citalopram, dose dependently increases cortisol secretion according to the kinetics of the drug. The occurrence of overall side effects after acute S-citalopram administration was assessed using visual analogue scales at each time point of the experiments requiring subjects to rate the following items: physical well-being, nausea, itching, impaired vision, dysgeusia, headaches, shivering, increased sweating, somnolence, dizziness, constipation, indigestion and diarrhoea. Results reveal no significant drug effects, with respect to aversive effects for the 10 mg condition. However, after treatment with 20 mg S-citalopram, side effects were found (nausea, impaired vision and somnolence). To avoid confounding effects between drug induced and stress induced cortisol responses, all subsequent analyses were obtained for responses after the 10 mg dosage.

As confirmed by ANOVA, men and women did not differ significantly with regard to the psychometric measures assessed (Table 2), or placebo corrected CORT release after 10 mg S-citalopram ($F = 0.562$, $p > .05$, also see Table 1). As expected, they did, however, differ in their T-levels, with men having significantly higher values ($F = 70.27$, $p < .001$ also see Table 1).

4.2. Do habitual T-level modulate central 5-HT activity, as measured with an S-citalopram challenge test, in healthy men and women?

In order to answer the first of our questions raised above, we calculated Pearson correlations between aggregated T-levels and S-citalopram induced cortisol responses. No significant correlation was detected in either gender (men: $r = 0.21$, $p > .05$, woman: $r = -0.26$, $p > .05$, also see Fig. 3).

Furthermore, a chi-square analysis confirmed equal distribution of high and low T participants in high and low CORT groups (men: $\chi^2 = 0.01$, df = 1, $p > .05$, women: $\chi^2 = 0.41$, df = 1, $p > .05$, also see Table 1).

4.3. Does 5-HT act as a mediator of the relation between T and aggression, or do both systems independently modulate trait aggression, giving rise to an additive effect?

Since we did not detect any significant associations between T-level and central 5-HT responsivity as measured with S-citalopram Challenge tests, the requirements for a mediator effect, as postulated by Baron and Kenny, were violated [38]. Therefore, we used MANOVAs to test for main- and interaction-effects of T-levels and 5-HT-responsivity on psychometric measures of aggression for each questionnaire used. In women, no significant effects were detected (BDHI: main effect T-level: $F_{(8,13)} = 0.959$, $p > .05$, Wilks' lambda = 0.629; main effect CORT response: $F_{(8,13)} = 0.807$, $p > .05$, Wilks' lambda = 0.668; interaction T-level × CORT response: $F_{(8,13)} = 0.576$, $p > .05$, Wilks' lambda = 0.738, FAF: main effect T-level: $F_{(6,15)} = 0.713$, $p > .05$, Wilks' lambda = 0.778; main effect CORT response: $F_{(6,15)} = 0.525$, $p > .05$, Wilks' lambda = 0.827; interaction T-level × CORT response: $F_{(6,15)} = 0.173$, $p > .05$, Wilks' lambda = 0.935, FPI-R aggression: main effect T-level: $F_{(1,20)} = 1.093$, $p > .05$; main effect CORT response: $F_{(1,20)} = 2.502$, $p > .05$; interaction T-level × CORT response: $F_{(1,20)} = 1.502$, $p > .05$). In men, the interaction effects for each questionnaire were significant (BDHI: interaction T-level × CORT response: $F_{(8,13)} = 5{,}471$, $p < .05$, Wilks' lambda = 0.229, FAF: interaction T-level × CORT response: $F_{(6,15)} = 3.320$, $p < .05$, Wilks' lambda = 0.430, FPI-R aggression: interaction T-level × CORT response: $F_{(1,20)} = 7.596$, $p < .05$), whereas both main effects were non-significant (BDHI: main effect T-level: $F_{(8,13)} = 0.450$, $p > .05$, Wilks' lambda = 0.783; main effect CORT response: $F_{(8,13)} = 0.665$, $p > .05$, Wilks' lambda = 0.709, FAF:

Table 2
Overview of psychometric measures.

	Men	SD	Women	SD	F	p
BDHI scales						
Indirect hostility	3.17	0.42	2.67	0.43	0.69	0.41
Irritability	5.13	0.45	5.29	0.42	0.07	0.79
Negativism	4.33	0.53	5.13	0.49	1.19	0.28
Resentment	2.04	0.24	2.00	0.29	0.01	0.91
Suspicion	1.29	0.26	1.33	0.33	0.01	0.92
Verbal	2.75	0.49	2.63	0.44	0.04	0.85
Guilt	7.21	0.39	7.83	0.44	1.15	0.29
Assault	3.08	0.39	4.08	0.39	3.33	0.07
Mean scores over scales	3.02	0.18	3.21	0.23	0.41	0.53
FAF scales						
spontaneous aggression	2.92	0.43	3.13	0.42	0.12	0.73
reactive aggression	2.67	0.38	3.00	0.52	0.27	0.61
irritability	4.38	0.56	4.83	0.57	0.33	0.57
self-directed aggression	3.29	0.50	2.96	0.41	0.26	0.61
inhibition of aggression	4.29	0.44	5.17	0.51	1.68	0.20
openness	6.79	0.37	6.88	0.47	0.02	0.89
FPI-R aggression	3.42	0.53	2.83	0.54	0.59	0.45

Means and standard deviations (SD) are given by gender. ANOVA results on gender differences for each questionnaire are given as well.

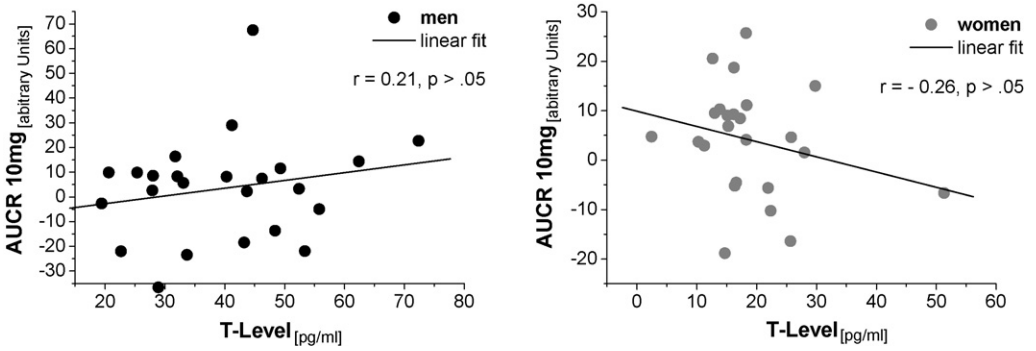


Fig. 3. Scatterblot of T-level versus the measure of central 5-HT availability (AUCR 10 mg, placebo corrected response measures for 10 mg S-citalopram) in male and female subjects.

main effect T-level: $F_{(6,15)} = 1.368$, $p > .05$, Wilks' lambda = 0.646; main effect CORT response: $F_{(6,15)} = 1.548$, $p > .05$, Wilks' lambda = 0.618, FPI-R aggression: main effect T-level: $F_{(1,20)} = 0.03$, $p > .05$; main effect CORT response: $F_{(1,20)} = 0.119$, $p > .05$).

Between-subject-effects for single sub-scales were analyzed for interaction-effects in men (see Table 3).

Post hoc analysis was employed in order to determine the exact nature of significant interactions on the level of questionnaire subscales, and Bonferroni correction for multiple testing was applied (see Fig. 4).

ANOVAs showed men of the "high T + high CORT" group to consistently display increased aggression-scores as compared to those the "low T + high CORT" and "high T + low CORT" groups. Analogous, but logically inverted, effects were seen for the "FAF inhibition of aggression" subscale. These effects, however, were rendered non-significant after Bonferroni-correction for some of the scales entered into post hoc analysis (see Fig. 4).

Interestingly the group of "low T + low CORT" participants also showed increased aggression scores with respect to several subscales from different questionnaires. Indeed this group of men displayed aggression scores mostly comparable to those of the "high T + high CORT" (see Fig. 4). One would expect participants with low T-levels to be rather non-aggressive, as one would expect persons with high 5-HT availability (low CORT group). Exploratory tests of the four groups for differences on personality traits related to aggression showed that the "low T + low CORT" group exhibited significantly increased narcissism scores as compared to the other three groups.

5. Discussion

The first aim of our study was to test whether habitual Testosterone-level would explain variance in central 5-HT activity as measured with a S-citalopram challenge test. This might be expected based on animal studies showing T to modulate the expression of several subcomponents of the 5-HT synaps (e.g. 5-HT_{1b} and 5-HT_{2a} receptor densities). However, our data clearly do not show any association between T-level and 5-HT activity, in either men or women (Fig. 3 and Table 1). This finding, albeit somewhat unexpected from several animal studies cited in the introduction, is in line with other human data. For example, Fink et al. failed to find significant associations between plasma T-level and 5-HT_{1a} receptor binding lateralization in men and women [39]. Furthermore, prolactin responses to a d-fenfluramine challenge test were not associated with T-levels in a sample of male offenders with personality disorders [40]. However human data on the direct association between central 5-HT activity or densities of functional parts of the 5-HT synaps (i.e. different receptors) respectively and habitual T-level is particularly scarce, and therefore rather inconclusive. Nevertheless it would be of particular interest to determine the reason for the failure to replicate results from animal studies in humans. Several lines of reason might be employed. For one thing even though Testosterone is known to modulate expression rates of different parts of the 5-HT synapse (5-HT reuptake transporter, 5-HT_{2a} receptors) it certainly is not the only factor influencing 5-HTy system activity. Therefore, the net effect of T might just be too small to pick up with a challenge test. Furthermore, at least some effects of T on the activity of the serotonergic system appear to be dependent on its conversion to estrogen by aromatse [22]. Future studies should further investigate these issues.

The second focus of our study concerned the combined effects of central 5-HT activity and T-level on human aggression. Since T-levels were not associated with central 5-HT activity the requirements for a mediator effect of 5-HT were not met [38]. We therefore tested whether both systems would independently modulate trait aggression giving rise to additive effects.

Indeed Multivariate analysis revealed two important findings: Neither, 5-HT and T imposed main effects on aggression. Secondly, within males an interaction could be observed in an expected (high aggression in low 5-HT availability + high T) but also in an unexpected direction (high aggression in low T + high 5-HT availability). Interestingly, these effects were shown only for subscales measuring more distinct, direct forms of aggression across various questionnaires (verbal, physical, spontaneous, and reactive aggression as well as general aggression scales comprising several forms of aggression). We consistently did not find effects on scales confounded with other facets of personality, such as self-directed aggression (FAF), guilt (BDHI), which for example is confounded with facets of depressiveness or irritability (BDHI), which is a measure of anger susceptibility. These results are further underlined by the fact that none of the other FPI-R scales were associated with either 5-HT activity or T-level (all $p > .05$, data not shown). Taken together this indicates the high specificity of our findings.

A number of studies reported main effects of T or 5-HT on aggression. However, some only in men, during certain phases of the menstrual cycle in women, or in psychopathological samples (e.g. [41,11,42,10,43,44]). Furthermore, there are contradicting results [45–49]. A broader summary of the data might be found elsewhere, e.g. [14,50,10,13,51]. With respect to T, one should also keep in mind potential effects of embryonic T on adult aggression, which might modulate associations between habitual T-level and aggression. Several recent studies showed embryonic T-levels, as operationalized by the second to fourth digit ratio (2D:4D ratio), to be associated with aggression in healthy adults, albeit mostly in men [52–56]. It would be a stimulating approach to include the 2D:4D ratio, a promising marker of embryonic T-level, as an additional factor in future studies on interactions between 5-HT and T.

There are very few studies on aggression, taking into account effects of T as well as 5-HT in humans. The only study in women analyzed serum T and platelet MAO-activity, which is thought to index central 5-HT turnover capacity, in women with fibromyalgia syndrome [57]. A positive association was found on T with global and verbal aggression as well as with anger. Furthermore, a negative association between platelet MAO activity with verbal aggression was reported. Interaction effects were not reported, possibly due to

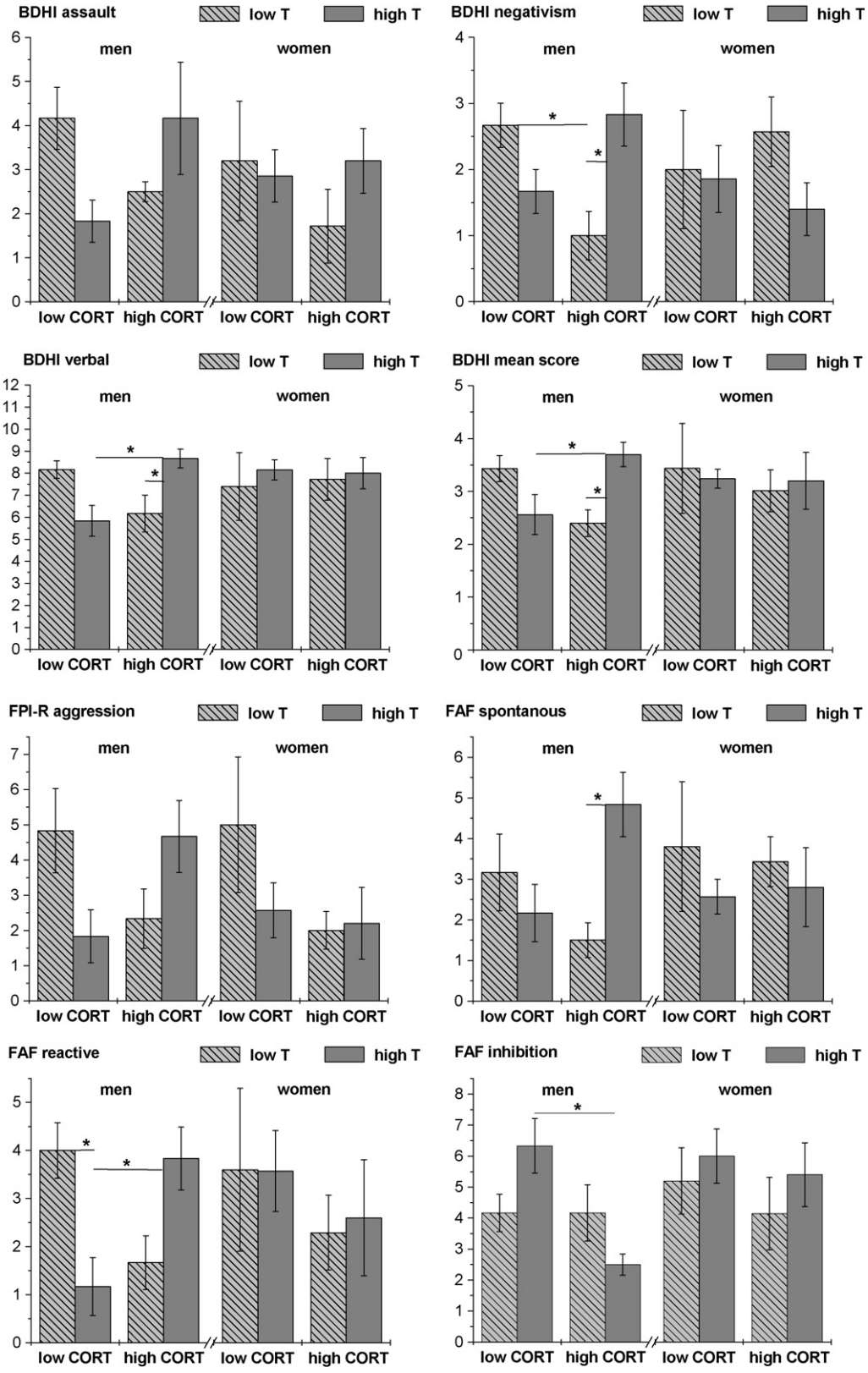


Fig. 4. Interaction effects between 5-HT responsivity and T-level, by gender. Mean scores and standard deviations (SD) of aggression subscales, as a function of central 5-HT responsivity and T-level are given. Asterisks (*) indicate group differences remaining significant after correction for multiple comparisons.

Table 3
Summary of interaction effects (T-level × CORT response) as determined by MANOVA, separated by gender.

	Men		Women	
	$F_{(1,20)}$	p	$F_{(1,20)}$	p
FAF spontaneous aggression	**8.535**	**0.008**	0.113	n.s.
FAF reactive aggression	**17.442**	**0.000**	0.024	n.s.
FAF irritability	3.072	n.s.	0.274	n.s.
FAF self-directed aggression	0.006	n.s.	1.220	n.s.
FAF inhibition of aggression	**7.053**	**0.015**	0.046	n.s.
FAF openness	4.263	n.s.	0.301	n.s.
BDHI				
Assault	**6.667**	**0.018**	1.061	n.s.
Indirect hostility	1.539	n.s.	0.142	n.s.
Irritability	0.851	n.s.	0.085	n.s.
Negativism	**13.762**	**0.001**	0.739	n.s.
Resentment	3.270	n.s.	0.510	n.s.
Suspicion	3.115	n.s.	2.192	n.s.
Verbal	**15.291**	**0.001**	0.059	n.s.
Guilt	0.711	n.s.	0.200	n.s.
Mean scores over scales	**14.651**	**0.001**	0.154	n.s.
FPI-R aggression	**7.596**	**0.012**	1.521	n.s.

Significant interactions are indicated by bold letters.

a sample size of only 14 participants, but concluded that irritability and aggression might be attributable to high T-level in combination with a diminished 5-HT capacity. These data and interpretations are in line with studies using men with personality disorders [58,59] and a study using non-human primates [27], which reached similar conclusions. With respect to females, we did not find support for the proposed interaction in women and our data are in contrast to Prochazka et al. [57]. Possible explanations for this discrepancy might be that in our study healthy young participants were tested, while Prochazka et al. analyzed a patient sample varying in age [57]. As already pointed out, results concerning the effects of 5-HT and T on personality tend to be substantially more robust in patient samples than in healthy participants. Furthermore, influences of fluctuating sexual steroids in women might have masked potential effects. A number of studies implicate the involvement of fluctuating sexual steroids (estrogen, progesterone) [10,44]. Even though we controlled for menstrual cycle, such influences would be a function of *actual* sex-steroid levels, which vary across different participants, even *within* a well-defined phase of the menstrual cycle.

In men, we did find the expected interaction of 5-HT and T for measures of aggression only. Specifically, we expected participants with high T-level *combined* with reduced 5-HT availability to exhibit increased aggression. S-citalopram challenge tests were used to index central 5-HT activity. As already explained above, a single application of S-citalopram transiently increases 5-HT availability in the synaptic cleft. This will induce the secretion of hormones via activation of the HPA-axis [35], via a more pronounced activation of post-synaptic receptors. An increased hormone secretion may be interpreted as an indicator of an up-regulation of post-synaptic receptors, possibly due to reduced 5-HT availability [36,37]. Therefore, a high drug-induced CORT response (high CORT-group) is interpreted to indicate decreased 5-HT availability, while a low drug induced CORT response (low CORT-group) indicates increased 5-HT availability. In terms of our hypothesis, we would expect participants belonging to the "high T + high CORT" group to exhibit increased scores on measures of aggression. We found this prediction to be consistently confirmed in men for various trait measures of aggression. Our data corroborate earlier findings in animal studies showing the combination of high T-level and low 5-HT function to be associated with increased aggressiveness [27,25]. The possible underlying mechanism might be that the aggression promoting effects of T are less respectively, insufficiently counteracted by the aggression suppressing effects of the 5-HT system. The results of men with "high T + low CORT" being non-aggressive is also in line with animal studies, as is the lack of aggression in the "low T + high CORT" group.

Less easy to explain is the finding of relatively consistently increased trait aggression in the "low T + low CORT". Narcissism, characterized by an unstable high self-esteem, has been found to be associated with aggression and hostility [60,61]. Therefore, one might assume the increased aggression in the "low T + low CORT" group to be attributable to particularly high narcissism scores. Explaining these high scores on narcissism in this particular group is, however, beyond our data. There is no literature available on potential involvement of 5-HT and T in the etiology of narcissism. However, our data tentatively point towards such a role 5-HT and T in men.

Certain limitations of our data must be kept in mind. For one thing, our sample was relatively small. A replication should be conducted, in order to confirm our findings. With respect to the female sample, it would be advisable in future studies to test all women in just one menstrual cycle phase. This will enable us to address the possibility of a moderating effect of the menstrual cycle on interaction effects in a more straightforward manner. It might also be promising to measure actual levels of E and P. Furthermore, the use of behavioral measures of aggression rather than self-reports might be an interesting alternative, which would allow testing for the influence of situational effects. It remains a challenging task for future studies to elucidate which 5-HT receptors are responsible for the associations described above.

In conclusion, we were able to show for the first time an interaction effect of 5-HT and T on measures of aggression in healthy men. All in all, our results support the notion of a behaviorally relevant interaction of 5-HT and T with respect to facets of aggression in healthy men.

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