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ORIGINAL ARTICLE

Influence of acute tryptophan depletion on verbal declarative episodic memory in young adult females

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Abstract Diminished synthesis of the neurotransmitter serotonin (5-HT) in the brain has been linked to disturbed memory processes. The present study investigated the effects of diminished central nervous 5-HT synthesis as achieved by an acute dietary tryptophan depletion (ATD) on verbal declarative episodic memory in young women while controlling for the effects of female sex hormones. Eighteen healthy females (aged 20–31 years) participated in a within-subject repeated measures study, with two separate days of assessment spaced at least one individual menstrual cycle apart. On one day, participants were subjected to ATD, thus lowering central nervous 5-HT synthesis. The other day participants received a tryptophan-balanced amino acid load (BAL = control condition). The study was randomized, counterbalanced and double blind in terms of ATD/BAL administration. Measurements took place in the early follicular phase of the participants' menstrual cycle. Estrogen, FSH and LH levels were assessed at baseline. Verbal declarative episodic memory was assessed using a structured word-learning task. Short-term memory, as indexed by immediate recall, was reduced after ATD intake, whereas delayed recall and recognition after a 25-min delay did not show any differences after intake of ATD or BAL. In young women, verbal short-term memory function was more vulnerable to ATD than consolidation processes. In light of the possible interplay between female sex hormones and 5-HT, further studies comparing different menstrual cycle phases are needed.

Keywords Serotonin · Acute tryptophan depletion · Verbal declarative episodic memory · Short-term memory · Long-term memory · Gender · Menstrual cycle · Sex hormones

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Introduction

A dysfunction of the neurotransmitter serotonin (5-HT) is thought to be associated with various psychiatric disorders, including mood and anxiety disorders, attention deficit hyperactivity disorder (ADHD), schizophrenia and borderline personality disorder (Akimova et al. 2009; Kishi et al. 2012; Kötting et al. 2012; Lis et al. 2007; Mette et al. 2013; Zimmermann et al. 2012). Most of these disorders are accompanied by cognitive problems, including memory

impairments, as observed in children as well as adolescents and adults (Porter et al. 2003; Günther et al. 2004). This suggests an influence of altered serotonergic neurotransmission on memory impairment.

In line with these findings, studies support the involvement of serotonergic neurotransmission in memory-related processes (Schmitt et al. 2006). For instance, users of methylenedioxymethamphetamine (MDMA, the drug ecstasy), which causes a reduction in the concentration of serotonin transporters (SERTs) in the brain in the long term, report higher rates of memory problems than ecstasy-naïve controls. The incidence of memory problems is related to the number of occasions on which ecstasy was used (Parrott et al. 2002).

5-HT seems to specifically affect verbal memory, whereas dopamine seems to affect visuo-spatial memory with evidence from both animal and human studies (Castner et al. 2004; Harrison et al. 2004). In a study involving 100 subjects (patients with schizophrenia, patients with bipolar I disorder and healthy individuals), a genetic polymorphism of the 5-HT-transporter gene (SCLC6A4, 5-HTT) was associated with significant effects on verbal working memory performance; whereas the dopamine transporter gene (SLC6A3, DAT) seemed to affect spatial working memory and the catechol-*o*-methyl-transferase (COMT) gene did not exert an influence on either working memory domain (Zilles et al. 2012). Accordingly, a systematic review of the effects on acute tryptophan depletion (ATD, a physiological neurodietary method to safely lower brain 5-HT synthesis in human subjects) on memory, attention and executive functions states that ATD most robustly affects verbal memory, and in particular the consolidation of episodic memory for verbal information (Mendelsohn et al. 2009).

The effects of ATD-induced impairments are larger in females than in males, as evidenced by a pooled analysis of nine ATD studies that focused on the memory-impairing effects of ATD and potential mediating factors such as sex, age and vulnerability for disorders (Sambeth et al. 2007). This particular study indicated not only impairing effects of ATD on delayed recall, but also on immediate recall, an indicator of short-term memory ability. Interestingly, female gender was the only vulnerability factor for serotonergic effects on declarative memory performance.

It could be suggested that female sex steroids are responsible for the previously described sex differences. Estrogen receptors encode a variety of proteins that play a key role in the synthesis and function of nerve growth factors and neurotransmitters, including 5-HT (Lasiuk and Hegadoren 2007). They are widely expressed in the midbrain (Nomura et al. 2005) and the hippocampus (Kalita et al. 2005), which has a high density of 5-HT receptors and receives extensive projections from 5-HT cell bodies (Törk 1990). These observations suggest an interaction between estrogens, 5-HT and memory processes. The hippocampus is primarily engaged in the formation of episodic memories, which store information specific to time and place (Addis et al. 2004). As outlined above, 5-HT affects verbal memory in particular. Estrogen levels fluctuate throughout the menstrual cycle, with the highest levels observed during ovulation. Menstrual phase differences have been observed for 5-HT-transporter and 5-HT2A-receptor binding (Wihlbäck et al. 2004). Some studies on the effects of ATD on verbal episodic memory have tried to control for the influence of sex steroids by limiting measurements to the follicular phase in a subset of their participants that were premenopausal females (Sobczak et al. 2003; Harrison et al. 2004; Kilkens et al. 2004). However, these studies also included postmenopausal women or men. To our knowledge, neither study restricted measurements to young adult women in the early follicular phase nor excluded the direct intake of hormones, such as hormonal contraceptives or other hormonal medication. In a non-ATD study, menstrual cycle influences on verbal working memory were found in women with a natural cycle, but they were not found for females taking birth control pills. In women who take birth control pills, estrogen levels are rather stable throughout the cycle (Rosenberg and Park 2002).

In this study, we investigated verbal declarative episodic memory performance following a reduction in central nervous 5-HT synthesis as achieved by a dietary depletion of the amino acid tryptophan (TRP, the physiological precursor of 5-HT) in young adult females. We controlled for menstrual cycle phase by measuring participants who were free of any hormonal medication during the early follicular phase of their menstrual cycle when on average estrogen levels are low but rising. The effects of ATD on verbal declarative memory in a cohort like the present remain unclear. To our knowledge, this is the first study examining the effects of ATD on verbal declarative episodic memory in premenopausal women all measured in the same phase of their menstrual cycle. However, based on previous findings and pooled analyses (Mendelsohn et al. 2009; Sambeth et al. 2007) we expected immediate and delayed recall performance to be impaired by ATD intake, whereas we assumed recognition performance to remain unaffected by the administered neurochemical challenge procedure. The reduction of central nervous 5-HT synthesis was achieved by applying a well-tolerated body weight-adjusted ATD protocol. To control for the possible mediating effects of altered emotions due to reduced central nervous 5-HT synthesis on memory, an assessment of subjective mood was incorporated into the assessment.

Methods

Study design

The study employed a randomized and counterbalanced, double-blind, within-subject design. On one day, participants were subjected to an ATD beverage, thus lowering central nervous 5-HT synthesis. On the other day participants received a tryptophan-balanced amino acid load beverage (BAL), which served as the control condition. The participants were healthy young adult females who were free of any hormonal medications including oral contraceptives. Both measurements took place during the early follicular phase of the participants' menstrual cycles, when estrogen and progesterone are expected to be at their lowest levels. Days of assessment were spaced at least one individual menstrual cycle apart to control for learning effects and hormonal status. The well established structured word learning task Verbaler Lern- und Merkfähigkeitstest [VLMT, verbal learning memory test (Helmstaedter et al. 2001)] was applied to assess verbal declarative episodic memory. For the assessment of subjective mood, the positive and negative affect schedule [PANAS (Crawford and Henry 2004)] was used.

Ethics statement

The experimental protocol was assessed and approved by the ethics committee of the Medical Faculty of the RWTH Aachen University, Germany, and the study was carried out in accordance with the Declaration of Helsinki. All participants provided written and oral informed consent to participate in the study and were financially compensated at the end of the second test day.

Participants

Eighteen healthy female subjects between 20 and 31 years of age (mean age 24.22 ± 2.9 years) participated in the study. Thirty-two females were interested in participating and were screened via telephone interviews. Five were excluded due to physiological exclusion criteria. Five were unable to participate in the morning. One was excluded due to a DSM-IV diagnosis. Out of the remaining 21, three did not participate due to difficulties in appointment arrangements. Participants were screened for neurological and psychiatric disorders, the latter via the German version of the diagnostic interview for psychological disorders for ICD-10 [Mini-DIPS (Margraf 1994) and the DIPS questionnaire (Margraf et al. 2008)]. All participants were native German speakers and were right-handed. Exclusion criteria included an IQ lower than 85 assessed via the CFT-20R (Weiß 2006) (mean IQ 110.3 ± 10.5), any current or past psychiatric or neurological condition (including head trauma with loss of consciousness), and current medical conditions including migraine, asthma, diabetes, allergies or obesity (mean BMI 23.6 ± 3.7). Current or prior pregnancies were excluded to have a controlled, homogeneous group in terms of hormone levels, and to exclude possible effects of prior pregnancies on current hormone levels and memory performance. Urine drug screens and pregnancy tests before the intake of the ATD/BAL beverages on each study day were conducted to exclude pregnancy and psychoactive drug intake. Only participants free of hormone intake, such as oral contraceptives (no hormonal contraceptive use within at least 6 months), or other hormonal medications for hormonal disorders, such as metabolic or thyroid diseases were included. Both measurements took place during the early follicular phase of participants' menstrual cycles (days 1–7). Participants filled in a menstrual cycle diary starting 3–6 months before the first measurement to match measurement days with menstrual cycle phase. Due to the variability of intra-individual menstrual cycle length and measurement arrangements, two women were premenstrual, menstruating within 48 h after their measurements. Their hormone levels were within the 95 % confidence interval of the distributions of hormone levels of the other participants. The mean cycle length was 28.56 ± 2.69 days. Number of menstrual cycles between measurements was 1.5 ± 0.78. Measurements took place throughout the year with no significant seasonal deviations [$N = 4$ (22.2 %) in spring, $N = 6$ (33.3 %) in summer and $N = 5$ (27.8 %) in autumn, $N = 3$ (16.7 %) in winter]. Participants were asked to arrive after an overnight protein fast and a standard breakfast containing no TRP. Detailed recommendations for such a breakfast were given to all subjects in advance of the study. Participants were further instructed to abstain from alcohol or smoking from the evening before measurement. All participants confirmed that they had followed these recommendations on each day of testing. In a randomized and counterbalanced order, half of the subjects ($N = 9$) received ATD on their first day, and the other half-received ATD on the second day of testing.

Depletion procedure

Due to circadian rhythm-influenced differences in metabolism and synthesis of 5-HT (Halberg et al. 1967; Sánchez et al. 2008), all participants arrived in the morning. ATD/BAL administration took place at 0800 hours, and the structured word-learning task was administered at 1000 hours. With regard to the time point of data acquisition, the used Moja-De ATD procedure has been demonstrated to substantially impair brain 5-HT synthesis and

to lower 5-hydroxyindoleacetic acid (5-HIAA) content (an indirect measure of 5-HT release) in a rodent model (Biskup et al. 2012) in all assessed brain regions at the particular time point of assessment used in the present study. This procedure was also cross-validated by a recent study conducted in people in a comparable age cohort like the one in the present study that showed a robust decrease in total and free TRP influx over the blood brain barrier after 90 min, remaining stable at this level 180 and 240 min later (Dingerkus et al. 2012).

The underlying principle of our reduction in brain 5-HT relied upon lowering the central nervous system availability of the amino acid TRP (Zepf 2013). TRP is the physiological precursor of 5-HT, and it cannot be synthesized by the human body. TRP is an essential amino acid and crosses the blood–brain barrier using the L-1 transport system, where it competes with other large neutral amino acids (LNAAs) that also depend on L-1 to overcome the blood–brain barrier. The administration of a beverage containing LNAAs other than TRP leads to lowered substrate availability for tryptophan hydroxylase 2 (TPH2), the rate-limiting enzyme for central nervous 5-HT synthesis, and results in reduced 5-HT synthesis in the brain. Furthermore, protein synthesis in the liver is stimulated by the administration of amino acids, thus depleting plasma stores of additional TRP concurrent with passive diffusion of amino acids over the blood–brain barrier in both directions (Zepf 2012).

The Moja-De ATD protocol (Biskup et al. 2012; Dingerkus et al. 2012; Kewitz 2002; Moja et al. 1988; Zepf and Poustka 2008; Demisch et al. 2002) was used, in which the relevant amino acids (AAs) are administered in accordance with participants' body weights. The AA quantities were as follows (dosage per 10 kg of body weight): L-phenylalanine (PHE 1.32 g), L-leucine (LEU 1.32 g), L-isoleucine (ILE 0.84 g), L-methionine (MET 0.5 g), L-valine (VAL 0.96 g), L-threonine (THR 0.6 g), and L-lysine (LYS 0.96 g). The BAL beverage contained the same AA quantities with an additional 0.7 g of TRP per 10 kg of body weight.

Calculation of the total TRP influx into the brain

Total TRP influx across the blood–brain barrier depends on TRP concentrations and concentrations of competing LNAAs. It is characterized by unidirectional uptake. To calculate the total TRP influx into the brain, the Michaelis–Menten equation was used. This equation, with a correction for multiple substrate competition (Pardridge 1983; Smith et al. 1987), provides a valid mathematical model to calculate the unidirectional influx rates for TRP from plasma into the brain (Dingerkus et al. 2012; Kewitz 2002).

Blood samples

For the analysis of TRP influx, two blood samples were drawn on each study day. The first took place at baseline prior to administration of ATD/BAL. In order to prevent undesired effects of taking blood on task performance, the second blood sample was drawn after completion of the study day, on average 255 min after beverage intake. In addition, baseline hormonal status [follicle stimulating hormone (FSH), luteinizing hormone (LH) and 17-β estradiol (E2) concentrations] was assessed.

Blood samples were drawn into tubes (EDTA S-Monovette®, Sarstedt, Germany). In order to measure total TRP in relation to other LNAA levels, plasma levels of PHE, TYR, and TRP were measured using enzyme-linked immunosorbent assay (ELISA) kits (Immundiagnostik AG, Bensheim, Germany) in accordance with the manufacturer's instructions. Concentrations of LEU, ILE, and VAL were determined using a commercially available enzyme test kit for BCCAs (Immundiagnostik AG, Bensheim, Germany) according to the manufacturer's instructions. Estradiol, FSH, and LH were analyzed at our local laboratory at the University Hospital of RWTH Aachen University via electrochemiluminescence immunoassay "ECLIA" on a Modular Analytics E170 device (Roche Diagnostics GmbH, Mannheim, Germany), with a day-to-day imprecision of <2.5 % (LH, FSH) and <10 % (estradiol), respectively. Cross-reactivity with other steroid hormones/gonadotropins was <0.1 %. The normal values for estradiol, in pmol/L are 46–607 during the follicular phase, 315–1,828 during the ovulatory phase and 161–774 during the luteal phase, for FSH in U/L 3.5–12.5, 4.2–21.5, 1.7–7.7, and for LH in U/L 2.4–12.6, 14.0–95.6, 1.0–11.4, respectively (data obtained from our local laboratory; Institut für Klinische Chemie und Pathobiochemie, Klinisch-Chemisches Zentrallaboratorium, Uniklinik RWTH Aachen, Germany).

Structured word-learning task: verbal learning memory test

Two hours after the intake of ATD/BAL, when a stable reduction in TRP influx into the brain was achieved (Dingerkus et al. 2012), the VLMT, a structured word learning task, was applied. The VLMT is a modified German version of the originally French well-known Rey's (1964) auditory verbal learning test. The test includes standardization values for different age groups allowing developmental comparisons. Participants listened to a 15-word spoken list. The task was to freely recall the words presented. The test comprised three main aspects of verbal episodic memory, as indexed by verbal short-term memory, verbal long-term memory (including consolidation and

retrieval) and verbal recognition. The test was initiated with a learning phase with immediate recall representing verbal short-term memory (5 consecutive trials of listening to the same word list and giving free recall), followed by an interference phase (listening to a second word list with 15 new words and giving free recall of the second list, then recalling the first list without another presentation) and a delayed recall phase (giving free recall of the first list after a 25-min break). The latter two phases represent the consolidation process followed by a recognition phase. While listening to 50 words, including the first and second word list and new words, participants had to indicate which items they recognized as part of the first word list. Due to the repeated measures design, in counterbalanced order, half of the participants learned list A on the first day of testing and a parallel list B on their second day, and vice versa.

The dependent variables were learning efficiency or immediate recall (cumulative number of correct answers trials 1–5), loss after interference (number of correct recalled words in the last trial of the learning phase minus the number of correct recalled words in the trial after interference), delayed recall (number of freely recalled words after a 25-min break), loss after delayed recall (number of correct recalled words in the last trial of the learning phase minus number of correct recalled words in the trial after delay) and corrected recognition performance [sum of the number of correct recognized words of the first word list minus the sum of the number of false positives (distracters or words of the interference list which is the second word list)]. Moreover, the learning curve, equivalent to the number of correct answers in each of the first five consecutive learning trials, was analyzed post-hoc.

Mood assessment

For mood assessment, a German version of the PANAS was used. Positive affect (PA) and negative affect (NA) reflect dispositional dimensions with PA representing the extent to which a person experiences pleasurable engagement with the environment and high NA representing subjective distress and unpleasurable engagement and low NA the absence of these feelings (Crawford and Henry 2004). Participants rated their momentary mood state on a list of ten positive and ten negative mood-associated adjectives (positive, e.g., proud; negative, e.g., anxious) on a scale from 1 ("not at all") to 5 ("extremely"). Participants gave their reports before the intake of ATD/BAL (baseline; time point 1) and after 2 h, directly before the VLMT (time point 2).

Data analyses

The level of statistical significance was set and maintained at $p < 0.05$. Dependent variables of the VLMT as within-subject variables and challenge procedure (ATD/BAL) order as a between-subject variable were analyzed via two-way mixed ANOVAs. Hyun–Feldt adjusted repeated-measures ANOVAs were conducted for plasma TRP levels, the learning curve in the VLMT and the positive and negative affect scales of the PANAS. In order to compare data between BAL and ATD intake and to compare positive mood scores of day 1 and day 2, regardless of BAL or ATD intake, post-hoc tests were performed using paired t tests for normally distributed data and Wilcoxon's test (z values) for not-normally distributed data. Differences in hormone levels depending upon the days of the menstrual cycle, the effects of hormone levels on differences in TRP influx into the brain and the effects of hormone levels on the VLMT dependent variables were examined using linear regression analyses. Cohen's d was calculated as an estimate for effect sizes for t tests, Wilcoxon tests and F tests and the effect size parameter Cohen's f^2 for regression analyses.

Results

Hormone levels and menstrual cycle

Levels of FSH, LH, and E2 did not differ between ATD and BAL testing days (see Table 1). Within-subject

Table 1 Hormone levels estradiol, FSH and LH in BAL and ATD conditions

	Estradiol (pmol/L)	FSH (U/L)	LH (U/L)	Day of menstrual cycle
BAL	155.22 ± 100.25	4.83 ± 2.11	5.74 ± 2.83	2.7 ± 2.4
ATD	148 ± 61.11	5.65 ± 1.21	7.79 ± 4.17	3.9 ± 1.74
z- or t-value	$z = -0.544$	$t_{(17)} = -1.74$	$z = -1.26$	$t_{(17)} = -1.69$
Significance (p value)	0.586	0.099	0.206	0.110
Cohen's d	0.008	0.87	0.58	0.42

Given are the baseline hormone levels for the BAL and ATD conditions. Data are presented as mean ± standard deviation. Comparison of the two test conditions with t tests (t) or Wilcoxon's test (z) values with p values (p) and Cohen's d as an estimate for effect size

differences in hormone levels between testing days were not modulated by differences in menstrual cycle days (see Table 2). Hence, the days of testing took place during a stable phase with regard to female sex hormones.

Plasma tryptophan

A repeated measures ANOVA indicated a main effect of challenge procedure [$F(1,17) = 320.52$, $p \leq 0.001$, Cohen's $d = 6.20$], a main effect of time [$F(1,17) = 46.56$, $p \leq 0.001$, Cohen's $d = 2.36$] and a time by challenge procedure interaction [$F(1,17) = 240.30$, $p \leq 0.001$, Cohen's $d = 5.37$]. As confirmed by post-hoc analyses, total TRP influx significantly decreased following ATD administration when compared with baseline [67.27 % decrease, ($t_{(17)} = 17.5$, $p \leq 0.001$, Cohen's $d = 8.75$] and was significantly lower after ATD when compared with the BAL condition at 255 min after beverage intake ($t_{(17)} = 17.25$, $p \leq 0.001$, Cohen's $d = 8.63$) (see Fig. 1).

Table 2 Linear regression analysis for differences in hormone levels and differences in days of menstrual cycle between BAL and ATD conditions

	Estradiol (pmol/L)	FSH (U/L)	LH (U/L)
R^2	0.001	0.044	0.011
$F(1,16)$	0.012	0.74	1.993
Significance (p value)	0.913	0.403	0.177
Cohen's f^2	0.001	0.046	0.011

Given are the results of a linear regression analysis for differences in hormone levels (hormone level ATD minus hormone level BAL) and differences in days of cycle (day of cycle ATD minus day of cycle BAL) between ATD and BAL conditions and Cohen's f^2 as an estimate for effect size

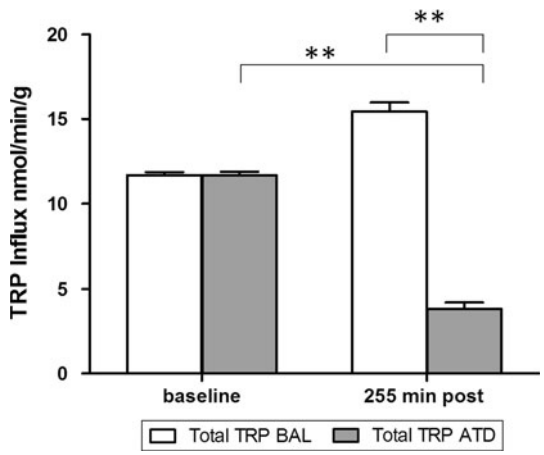

Fig. 1 Influx of total TRP across the blood brain barrier. Given is the influx (nmol/min/g brain tissue) of total TRP across the blood–brain barrier at baseline and 255 min after the intake (255 min post) of acute tryptophan depletion (ATD) and a balanced amino acid load (BAL). Data are represented as mean values ± standard error of mean. ** indicates highly significant differences $p \leq 0.001$

Plasma tryptophan and hormone levels

Differences in total TRP influx between baseline and 255 min after ATD intake were not modulated by baseline estradiol, FSH or LH, regardless of the respective testing day (BAL or ATD), as indexed by regression analyses (see Table 3).

Verbal learning memory test (VLMT)

Verbal short-term memory

Learning efficiency: immediate recall ATD resulted in lowered learning efficiency, indicating reduced immediate recall as observed in a two-way mixed ANOVA revealing a main effect of the neurochemical challenge procedure [$F(1,16) = 6.200$, $p = 0.024$, Cohen's $d = 0.86$]. There was no significant challenge procedure (ATD/BAL) by challenge procedure order (ATD on day 1 vs. ATD on day 2) interaction [$F(1,16) = 1.995$, $p = 0.177$, Cohen's $d = 0.49$] and no significant main effect of challenge procedure order [$F(1,16) = 0.994$, $p = 0.334$, Cohen's $d = 0.35$] (see Fig. 2).

Learning curve For the five consecutive learning trials, a Hyun–Feldt adjusted repeated-measures ANOVA indicated a main effect of time [$F(3,47) = 49.158$, $p \leq 0.001$, Cohen's $d = 0.35$], with a linear increase indicating more words learned with rising trial number [$F(1,17) = 78.443$,

Table 3 Linear regression analyses for differences in TRP-influx levels and baseline hormone levels for BAL and ATD conditions

Δ TRP influx	Estradiol (pmol/L)	FSH (U/L)	LH (U/L)
BAL			
R^2	0.000	0.004	0.061
$F(1,16)$	1.035	0.065	1.035
p value	0.937	0.803	0.324
Cohen's f^2	0.000	0.004	0.065
ATD			
R^2	0.006	0.803	0.002
$F(1,16)$	0.094	1.447	0.028
p value	0.764	0.245	0.870
Cohen's f^2	0.006	4.08	0.002

Given are the results of linear regression analyses for differences in TRP-influx levels (nmol/min/g brain tissue; Δ TRP influx = TRP influx baseline minus TRP influx 255 min after beverage intake) and baseline hormone levels (estradiol, FSH and LH) for BAL and ATD conditions

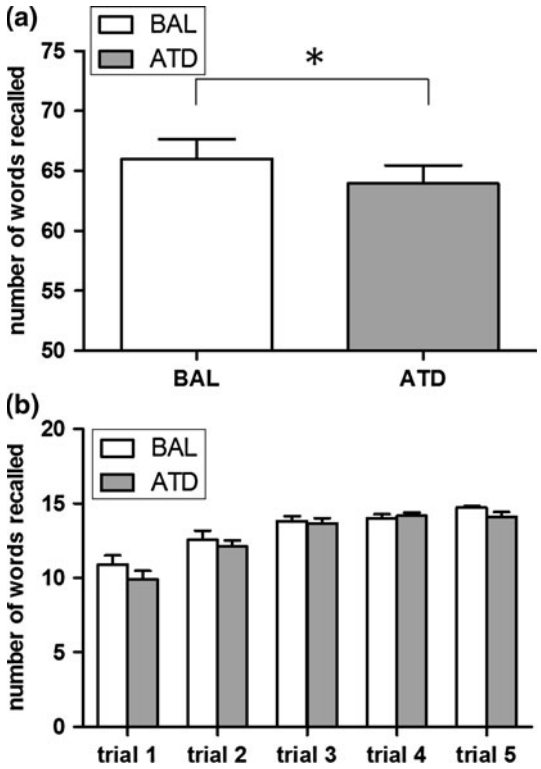

Fig. 2 Learning efficiency and learning curve. Learning efficiency—immediate recall—cumulative number of words recalled during trials 1–5 after the intake of acute tryptophan depletion (ATD) and a balanced amino acid load (BAL); learning curve—number of words recalled in each learning trial. Data are given as mean values ± standard error of mean. * indicates significant differences $p < 0.05$

$p \leq 0.001$, Cohen's $d = 3.07$]. There was also a main effect for the challenge procedure [$F(1,17) = 5.852$, $p = 0.027$, Cohen's $d = 0.84$], indicating fewer words learned after intake of ATD. There was no time, thus trial, by challenge procedure interaction [F (2.479, 42.140) = 1.499, $p = 0.233$, Cohen's $d = 0.42$] (see Fig. 2).

Verbal long-term memory: consolidation and retrieval

Loss after interference No difference was observed in loss after interference between BAL and ATD, as observed in the two-way mixed ANOVA indicating no main effect for the challenge procedure [$F(1,16) = 2.312$, $p = 0.148$, Cohen's $d = 0.53$]. There was a challenge procedure by challenge procedure order interaction [$F(1,16) = 7.491$, $p = 0.015$, Cohen's $d = 0.95$], but no main effect of challenge procedure order [$F(1,16) = 0.302$, $p = 0.590$, Cohen's $d = 0.19$]. A post-hoc univariate ANOVA with the factors test day and challenge procedure indicated a trend for less words forgotten on day 2 compared to day 1 [$F(1,32) = 3.988$, $p = 0.054$, Cohen's $d = 0.69$] (see Figs. 3, 4).

Delayed recall, loss after delayed recall No difference in delayed recall was observed between BAL and ATD. The two-way mixed ANOVA showed no main effect for the challenge procedure [$F(1,16) = 0.126$, $p = 0.727$, Cohen's $d = 0.122$] with no challenge procedure by challenge procedure order interaction [$F(1,16) = 2.016$, $p = 0.175$, Cohen's $d = 0.49$]. In loss after delayed recall, there was a trend for less words forgotten after ATD, when ATD was applied on the second test day. The two-way mixed ANOVA indicated a trend for a main effect for the challenge procedure and a challenge procedure by challenge procedure order interaction [$F(1,16) = 4.000$, $p = 0.063$, Cohen's $d = 0.69$] but no main effect of the challenge procedure order [$F(1,16) = 4.000$, $p = 0.063$, Cohen's $d = 0.69$] (see Figs. 3, 5).

Verbal recognition

Corrected recognition performance Participants' corrected recognition performance did not differ between BAL and ATD. The two-way mixed ANOVA indicated no main effect for the challenge procedure [$F(1,16) = 0.190$, $p = 0.668$, Cohen's $d = 0.15$]. There was no main effect for the challenge procedure order [$F(1,16) = 0.763$, $p = 0.395$, Cohen's $d = 0.30$] and no challenge procedure by challenge procedure order interaction [$F(1,16) = 0.190$, $p = 0.668$, Cohen's $d = 0.15$] (see Fig. 3).

Verbal learning memory test (VLMT) and hormone levels

Variables of the VLMT were not modulated by individual differences in baseline estradiol, FSH or LH levels as indexed by regression analyses (see Table 4). The variable 'corrected recognition performance' showed small inter-individual variance, BAL $s^2 = 0.735$; ATD $s^2 = 1.412$. Hence, the significant p value for corrected recognition performance after ATD and FSH can be ignored.

Positive and negative affect schedule-PANAS

A Hyun–Feldt adjusted repeated-measures ANOVA indicated that mood was not modulated by the challenge procedure (no main effect of ATD/BAL; $F[1,16] = 1.533$, $p = 234$, Cohen's $d = 0.43$). Participants reported higher PA than NA scores [main effect valence $F(1,16) = 115.640$, $p \leq 0.001$, Cohen's $d = 3.73$]. A main effect of time [$F(1,16) = 28.246$, $p \leq 0.001$, Cohen's $d = 1.84$] and a time by valence interaction [$F(1,16) = 14.43$, $p = 0.002$, Cohen's $d = 1.32$] showed lower PA scores 2 h (time point 2) after the intake of BAL and ATD compared to baseline (time point 1), whereas NA did not differ from time point 1 to time point 2 (see Fig. 6).

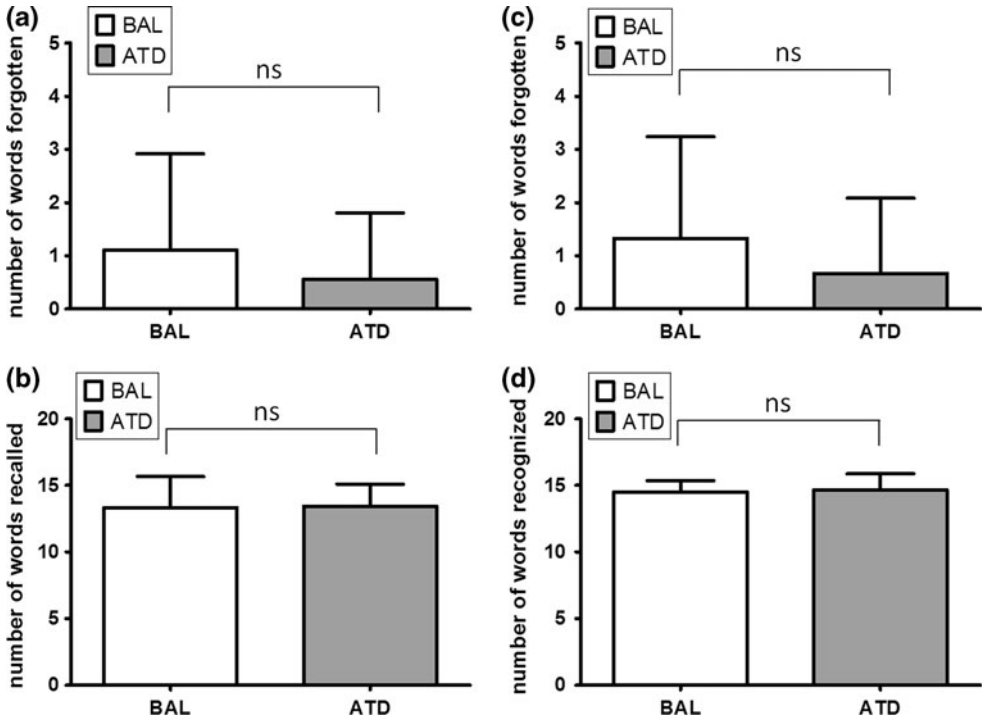

Fig. 3 Loss after interference, delayed recall, loss after delayed recall and corrected recognition performance. Given are the results of **a** loss after interference—number of words forgotten after interference list, **b** delayed recall—number of words recalled after a 25-min break, **c** loss after delayed recall—number of words forgotten after a 25-min break, and **d** corrected recognition performance—number of words correctly recognized minus false positives. Data are represented as mean values ± standard deviation. *ns* indicates non-significant differences

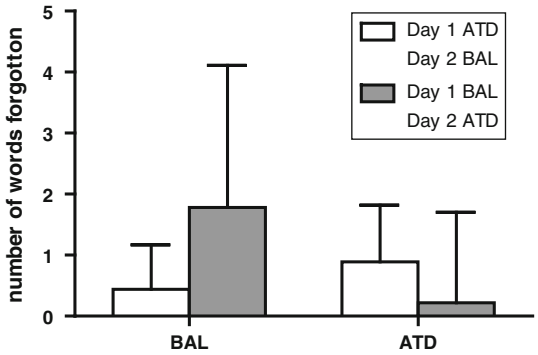

Fig. 4 Loss after interference and challenge procedure order. Given are the results of loss after interference—number of words forgotten after interference list—after BAL and ATD split according to challenge procedure order. The *white column* represents data of participants who received ATD on the first day of testing and BAL on the second and the *grey column* represents data of participants who received BAL on the first day of testing and ATD on the second. Data are represented as mean values ± standard deviation

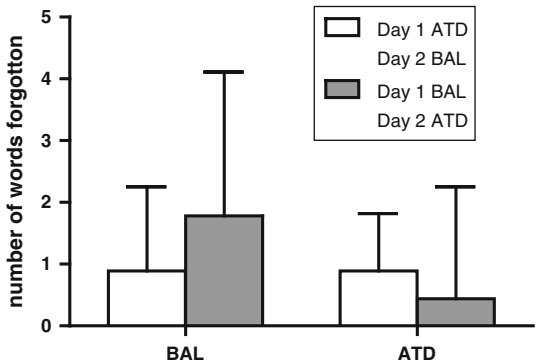

Fig. 5 Loss after delayed recall and challenge procedure order. Given are the results of loss after delayed recall—number of words forgotten after interference list after BAL and ATD split according to challenge procedure order. The *white column* represents data of participants who received ATD on the first day of testing and BAL on the second and the *grey column* represents data of participants who received BAL on the first day of testing and ATD on the second. Data are represented as mean values ± standard deviation

Regarding the lower positive mood scores at time point 2, the reduced learning efficiency after ATD was not modulated by mood at time point 2 (linear regression, no linear trend: $F[1,15] = 0.005$, $R^2 = 0.000$, $p = 0.944$, Cohen's $d = 0.00$). Comparing the positive mood scores from day 1 and 2 at baseline, independent of BAL or ATD intake, positive mood scores were higher at day 1 than day 2 (mean day 1, 30.67 ± 6.46; mean day 2, 27.38 ± 7.45; $t_{(17)} = 2.274$, $p = 0.036$, Cohen's $d = 1.137$). Positive mood scores at time point 2 between day 1 and 2, independent of BAL or ATD intake, did not differ significantly (mean day 1, 24.41 ± 8.30; mean day 2, 25.76 ± 7.11; $t_{(17)} = -0.691$, $p = 0.500$, Cohen's $d = 0.35$).

Discussion

In the current investigation, the body weight-adjusted Moja-De ATD procedure substantially lowered TRP influx

Table 4 Linear regression analyses for the dependent variables of the VLMT and baseline hormone levels for BAL and ATD conditions

	Estradiol (pmol/L)	FSH (U/L)	LH (U/L)
Learning efficiency			
BAL			
R^2	0.033	0.016	0.006
$F(1,16)$	0.541	0.258	0.090
p value	0.473	0.618	0.769
Cohen's f^2	0.034	0.016	0.006
ATD			
R^2	0.122	0.027	0.002
$F(1,16)$	2.228	0.445	0.031
p value	0.155	0.514	0.862
Cohen's f^2	0.139	0.027	0.002
Loss after interference			
BAL			
R^2	0.000	0.002	0.067
$F(1,16)$	0.002	0.026	1.149
p value	0.964	0.873	0.300
Cohen's f^2	0.000	0.002	0.072
ATD			
R^2	0.022	0.061	0.001
$F(1,16)$	0.355	1.032	0.022
p value	0.560	0.325	0.885
Cohen's f^2	0.022	0.065	0.001
Loss after delayed recall			
BAL			
R^2	0.005	0.006	0.049
$F(1,16)$	0.077	0.089	0.821
p value	0.785	0.769	0.378
Cohen's f^2	0.005	0.006	0.051
ATD			
R^2	0.016	0.063	0.006
$F(1,16)$	0.260	1.081	0.104
p value	0.617	0.314	0.751
Cohen's f^2	0.016	0.067	0.006
Corrected recognition performance			
BAL			
R^2	0.006	0.003	0.081
$F(1,16)$	0.094	0.055	1.417
p value	0.763	0.818	0.251
Cohen's f^2	0.006	0.003	0.088
ATD			
R^2	0.018	0.255	0.003
$F(1,16)$	0.299	5.463	0.053
p value	0.592	0.033	0.821
Cohen's f^2	0.018	0.342	0.003

Given are the results of a linear regression analyses for the dependent variables of the VLMT and the baseline hormone levels (estradiol, FSH and LH) for BAL and ATD conditions

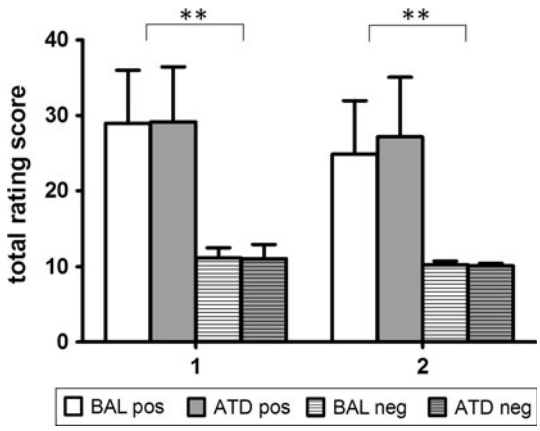

Fig. 6 PANAS total rating scores. Given are the results of the PANAS total rating scores of positive (pos) and negative (neg) mood at baseline (1) and after 2 h (2). Data are represented as mean values ± standard deviation. ** indicates highly significant differences $p \leq 0.001$

into the brain. The impact of female sex hormones was controlled for by measuring during the early follicular phase of participants' menstrual cycles. Neither baseline estradiol, FSH nor LH modulated differences in TRP-influx levels or in VLMT task performance, indicating that the measurements were collected during a stable menstrual cycle phase.

ATD Moja-De ingestion significantly impaired short-term verbal memory in young adult females who were free of hormonal medication during the early follicular phase of their menstrual cycle, as indicated by reduced immediate recall performance during the learning phase in a declarative episodic memory task. Long-term consolidation and recognition memory processes did not appear to be affected by ATD. Challenge procedure order did not influence the results. Furthermore, a possible mediating factor, mood, was not influenced by ATD and reduced immediate recall performance was not modulated by mood.

Most studies as reviewed by Mendelsohn et al. (2009) have shown no effects of ATD on verbal short-term memory. However, the cited studies examined a variety of participants, e.g., elderly participants above the age of 60 (Porter et al. 2005) or remitted depressed patients (Merens et al. 2008). Furthermore, no study mentioned examined females exclusively. Regarding the control for female sex hormones in healthy controls, one study avoided only the premenstrual week in females for measurements (Hayward et al. 2005). The findings of the present study are partially in line with the pooled analysis of nine studies by Sambeth et al. (2007) indicating not only impairing effects of ATD on delayed recall, but also immediate recall, an indicator of short-term memory ability. Included in this particular analysis mentioned was one study examining females exclusively (Harrison et al. 2004) which did not find

immediate recall to be impaired after ATD. However, females were examined in the broader follicular phase with 9 of 13 participants taking oral contraceptives and applying a non-body-weight adapted depletion procedure. The female cohort in our study was tested under strict control for the influence of sex steroids as measurements took place in the early follicular phase of participants' menstrual cycle free of hormonal medication after administration of a well-tolerated depletion procedure. Furthermore, an analysis for postmenopausal aged females showed that ATD also affected immediate recall, which together with the current findings hint towards low estrogen levels being involved in impairing effects of lowered brain 5-HT synthesis on short-term memory.

Information in short-term memory is stored for no longer than one min and requires consolidation for longer storage (Baddeley and Hitch 1974). In the VLMT, long-term memory retrieval and consolidation can be assessed by the variables loss after interference, delayed recall and loss after delayed recall. All three of these variables were unaffected by ATD. Proactive interference was not modulated by ATD, there was a learning effect from day 1 to 2 independent of ATD or BAL. In contrast to most ATD studies that have used the VMLT (Mendelsohn et al. 2009; Sambeth et al. 2007), impaired delayed recall after ATD was not found in the present study indicating consolidation processes remained intact after ATD. In fact, there was a trend indicating less number of words forgotten after delayed recall after ATD but not BAL on the second test day, indicating a facilitated learning effect after ATD, but no trend for improved delayed recall. Fewer words lost might have contributed to the fact that no delayed recall impairments after ATD were observed. Nevertheless, this could be indicative of no consolidation impairments after ATD administration.

A third factor that includes both short-term and long-term components is corrected recognition performance, which requires both working memory operations and stable consolidation because stored information needs to be recognized. In line with the literature as reviewed by Mendelsohn et al. (2009), corrected recognition performance was not affected by ATD. The analysis by Sambeth et al. (2007) revealed impaired delayed recognition after ATD; however, with a main effect of gender indicating that females recognized more words than males which could explain the negative findings in the present study.

The present findings are in agreement with results of an fMRI study reporting reduced activation of the right hippocampus during encoding in an episodic memory task under the influence of ATD, whereas ATD did not affect brain activity in the retrieval phase (van der Veen et al. 2006) in a male cohort, and with males being rather low in estrogen. Our study indicates sensitive ATD-induced encoding impairments. In accordance, applying the same ATD procedure as used in the current study, 5-HT was found to be reduced in the hippocampus in a rodent model (Biskup et al. 2012). Many studies have suggested an important role of 5-HT in hippocampal-dependent learning [e.g., (Buhot et al. 2000)]. Long-term TRP administration in female rats increased 5-HT metabolism in the brain, with the largest increase in the hippocampus, where involvement in learning and memory is well documented. The administration was further associated with an enhancement of spatial working memory in a radial maze test (Haider et al. 2006). Furthermore, the 5-HT type 4 receptor (5-HT4R) modulates cellular memory processes in the hippocampus in humans. A PET study, which used the AVLT comparable to the VLMT used in the present study, found significant negative associations between immediate recall scores and the left and right hippocampal nondisplaceable binding potentials (BPnd) of 5-HT4R and between the right hippocampal BPnds of 5-HT4R and delayed recall (Haahr et al. 2012).

Many psychiatric disorders are accompanied by memory impairments and are associated with brain 5-HT dysfunction. Psychiatric patients often suffer from short-term memory impairments such as in that they struggle to organize their everyday life. Epidemiological findings indicate increased prevalence, incidence and morbidity risk in females for mood and anxiety disorders (Piccinelli and Gomez Homen 1997). Furthermore, schizophrenia in females is associated with hypoestrogenism (Seeman 1996), emphasizing the clinical relevance of the current study. In line with these findings, ATD studies using the VLMT, show gender differences. Studies on potential gender differences relying on comparisons between adult males and females might show different results depending on the composition and recruitment of the female subgroup, in particular if the female group includes premenopausal, menopausal women or both. So far, the existing literature simply states that women are more likely to display larger memory impairments than men (Sambeth et al. 2007). Females have lower central nervous 5-HT synthesis when compared to males, suggesting a disproportionately higher vulnerability for central nervous system 5-HT dysfunction induced by ATD in females (Nishizawa et al. 1997). Sex hormones are possible mediators. Estrogen receptors (ERs) are found both in the hippocampus and in the frontal lobes, which subserve verbal memory, working memory and retrieval. Estrogen treatment in naturally menopausal women caused increased 5-HT activity (Lippert et al. 1996) and improved performance in cognitive domains such as verbal learning and memory (LeBlanc et al. 2001). The present study collected E2, FSH, and LH data. However, there was no association found between hormone parameters and memory performance, possibly

due to the measurement during a stable menstrual cycle phase. Studies confirm that fluctuating sex steroid levels throughout the menstrual cycle affect memory performance. In a non-ATD study, menstrual cycle influences on verbal working memory, as indicated by differences in verbal span score, were found in women with a natural cycle but not in women taking hormonal contraceptives, who maintain rather steady estrogen levels throughout the cycle. Improved verbal working memory was associated with periods of high estrogen (Rosenberg and Park 2002). In rats, estrogen increases the density of 5HT-2a receptors in anterior frontal, cingulate and olfactory cortices (Fink et al. 1996), areas associated with working memory processes. In humans, a neuroimaging study examining premenopausal women free of contraceptives showed better performance in a verbal n-back working memory task in the luteal phase than in the early follicular phase (Joseph et al. 2012). Verbal memory was assessed in the present study during the early follicular phase (low but increasing estrogen levels). Hence, one could speculate that ATD in the early follicular phase might have caused impaired short-term memory performance to a greater extent than in other menstrual cycle phases, such as the ovulation or early luteal phase, when estrogen levels are high. However, this needs to be confirmed in future investigations.

The present study has several and significant methodological advantages in comparison with previous research. Our study controlled for more potential confounding variables than most published studies that used ATD in combination with a memory task. The results of our study might have been less prone to bias due to a more rigorous approach. As outlined previously, data were obtained using a body weight-adjusted ATD protocol, Moja-De, which was previously validated in two strains of mice (Biskup et al. 2012). This ATD protocol is known to be better tolerable than more conventional ATD protocols, in particular regarding vomiting and nausea. None of the participants in this study vomited, nor reported nausea, which is an important methodological advantage of this particular study as these parameters are likely to influence participants' cognitive performance. Fewer side-effects permit applying this ATD protocol to children (Zepf and Poustka 2008) and allow expanding the concept and design to younger people thus enabling a developmental approach; e.g., applying the same ATD procedure, there were no effects of ATD on verbal declarative memory function in boys with ADHD (Zepf et al. 2013). This together with the current findings open the question on what kind of effects of ATD on memory might be found in teenage girls. Furthermore, the time point of assessment could be seen as an advantage as regards preventing potential confounding effects such as fatigue, which is likely to increase with time. However, the current results are drawn from a rather limited sample size, with a small difference in the learning efficiency—immediate recall scores after ATD and BAL administration.

In the present study, the use of a rather short list of 15 words may have caused ceiling effects. Long-term memory performance may not have been sufficiently challenged. Only a few errors were made, indicating that the test may not have been sensitive enough to detect effects of ATD. The repeated-measures design used in the present study allows for the study of within-subject effects of ATD and an estimate for future large-scale studies. Furthermore, the study adds to the existing literature that it is one of a few studies addressing gender-specific aspects by including only premenopausal women in a certain phase of their menstrual cycle. This cohort is not investigated sufficiently as female sex hormones seem to be of great influence regarding ATD and verbal declarative episodic memory.

In summary, the data provide preliminary evidence that verbal short-term memory is more vulnerable to a brain serotonergic dysfunction than verbal long-term memory processes during the early follicular phase of the menstrual cycle. This vulnerability might be mediated by low estrogen levels. Levels of estradiol in the current assessment are still in the low range for estradiol concentrations in the overall menstrual cycle. Future large-scale studies on the effects of ATD on verbal declarative episodic memory in different menstrual cycle phases are needed in naturally ovulating women free of hormone intake to clarify the joint effects of 5-HT and female hormone levels. Research in this domain might be critical for better treatment of memory impairments accompanying psychopathology, such as depression as well as psychopathology intensified by memory impairments in premenopausal female patients.

Conflict of interest F.D.Z. was the recipient of an unrestricted award donated by the American Psychiatric Association (APA), the American Psychiatric Institute for Research and Education (APIRE) and AstraZeneca (Young Minds in Psychiatry Award). He has also received research support from the German Federal Ministry for Economics and Technology, the German Society for Social Pediatrics and Adolescent Medicine, the Paul and Ursula Klein Foundation, the Dr. August Scheidel Foundation, the IZKF fund of the University Hospital of RWTH Aachen University, and a travel stipend donated by the GlaxoSmithKline Foundation. He is the recipient of an unrestricted educational grant, travel support and speaker honoraria by Shire Pharmaceuticals, Germany. In addition, he has received support from the Raine Foundation for Medical Research (Raine Visiting Professorship), and editorial fees from Co-Action Publishing (Sweden). All authors report no conflict of interest.

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