Acta Psychiatrica Scandinavica

Acta Psychiatr Scand 2013: 128: 124–132

DOI: 10.1111/acps.12090

ACTA PSYCHIATRICA SCANDINAVICA

Pages 2 and 5

The impact of acute tryptophan depletion on attentional performance in adult patients with ADHD

Mette C, Zimmermann M, Grabemann M, Abdel-Hamid M, Uekermann J, Biskup CS, Wiltfang J, Zepf FD, Kis B. The impact of acute tryptophan depletion on attentional performance in adult patients with ADHD.

Objective: To date, the impact of the neurotransmitter serotonin (5-HT) on different neuropsychological functions in adults with attention deficit hyperactivity disorder (ADHD) is underinvestigated. We aimed to examine the effects of acute tryptophan depletion (ATD) and the resulting reduction in central nervous 5-HT synthesis on target/non-target discrimination ability and sustained attention in adults with ADHD using an AX-Continuous Performance Test (AX-CPT).
Method: Twenty male patients with ADHD (age: M = 30.25 SD = 9.37) and twenty male healthy controls (age: M = 27.90 SD = 6.01) received ATD on one day and a tryptophan-balanced control condition (BAL) on another day in a double-blind within-subject crossover design. A continuous performance test (AX-CPT) with three conditions (AX, AY, and BX) was administered on both days under depleted and sham-depleted conditions.
Results: In patients omissions increased after ATD when compared with BAL. Patient's reaction time decreased after ATD when compared with BAL, which was contrasted by opposite effects in controls. Patients showed fewer correct responses (AX condition) and showed a higher rate of errors (condition AX_E) independent of ATD or BAL intake.
Conclusion: The present preliminary results are indicative of the contribution of serotonergic neurotransmission to attentional processes in adults with ADHD.

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Key words: ADHD; attention; acute tryptophan depletion; serotonin; AX-CPT

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Accepted for publication January 3, 2013

Significant outcomes

- Reduced central nervous 5-HT synthesis, as achieved by acute tryptophan depletion (ATD), was associated with higher rates of omissions, as measured by a modified AX-Continuous performance test (AX-CPT) in adults with ADHD.
- Adults with ADHD displayed shorter reaction times and committed more omissions in the AX-CPT when compared with healthy controls after administration of ATD, which is indicative of decreased accuracy in attentional performance induced by diminished brain 5-HT synthesis.

Limitations

- Although the present study showed a significant impact of ATD on participant performance in the AX-CPT, the relatively small sample requires replication in future studies with larger samples.
- There is a lack of genetic data, especially regarding polymorphisms related to central nervous system serotonin and dopamine functions.
- Compensational effects of other neurotransmitters (dopamine) on the present behavioral results cannot be ruled out.

Yet it is the standard of care in medicine to give only L-dopa which depletes serotonin

Introduction

Attention deficit hyperactivity disorder (ADHD) is a psychiatric syndrome characterized by persistent inappropriate levels of inattention, as well as impulsivity and hyperactivity. It is associated with functional impairments across multiple academic and social domains and is commonly accompanied by a range of externalizing disorders such as oppositional defiant disorder (ODD) or conduct disorder (CD) as well as internalizing disorders such as social anxiety or major depression (1, 2). ADHD is increasingly acknowledged as a serious psychiatric disorder in adulthood, and ADHD in adult patients is heterogeneous and highly comorbid with other psychiatric disorders, particularly mood disorders, personality disorders, and substance abuse (3, 4). It is known that ADHD in childhood is manifested by marked impairments in both social and academic domains, with different aspects of attention undergoing age-related changes even in healthy children (5). The impairments observed in children and adolescents with ADHD are also apparent in adult patients with the disorder (6). Currently, ADHD is considered a multifactorial psychiatric disorder with a genetic predisposition and changes in catecholaminergic neurotransmission, particularly dopamine (DA) and noradrenaline (NA) (7, 8). These changes are thought to lead to inhibitory and attentional deficits, which in turn contribute to impairments typical of ADHD. Recent studies indicated that the prefrontal cortex (PFC) in particular plays an important role in the underlying pathophysiology of ADHD (9, 10). Many symptoms in patients with ADHD (such as deficits in focused attention, inhibition, and working memory) are comparable with symptoms in patients suffering from frontal lobe damage, a finding that indicates the relevance of frontal subcortical networks (11, 12).

The functions and impairments of these networks have been thoroughly investigated in young people and adults with ADHD (13–16). Because the brain regions of these networks are rich in DA, the DA hypothesis of the underlying pathophysiology of ADHD has received particular attention in the current literature (17). Three dopaminergic pathways have been identified and are suggested to play a major role in symptom manifestation in patients with ADHD. Studies have observed that the mesolimbocortical and nigrostriatal pathways show altered function in ADHD (17). The nucleus accumbens in the mesolimbic pathway was shown to be linked to impulsive behavior (18), whereas dysfunction of the dopaminergic system in nigrostriatal pathways was shown to be related to increased hyperactivity (19). The mesolimbic pathway in particular is essential for selective attention and working memory in patients with ADHD (20). It is generally accepted that the activity of dopaminergic neurons in nigrostriatal pathways and the ventral tegmental area is modulated by central nervous system serotonin (5-HT) function (21). Animal studies using the five-choice serial reaction time task [a task that is thought to be closely related to the continuous performance test (CPT), which is commonly used in human studies] showed the impact of DA and 5-HT on attentional performance. One study reported that manipulations of subcortical DA led to effects on speed and probability of responding (22). Moreover, lesion studies yielded a relationship between 5-HT and impulsivity, particularly with regards to premature responding and decreased omissions (22).

Recent research also suggests that ADHD is associated not only with alterations in catecholaminergic neurotransmission but also with variations in central nervous 5-HT function (23–25). Early studies reported reduced 5-HT uptake in platelets in a sample of children with ADHD (26). Further human and animal studies indicated that lowered central nervous system 5-HT synthesis is associated with higher rates of aggression and impulsivity (27, 28). Oades reported that poor attention and reduced performance on a stop-signal task correlated with reduced serotonin transporter (SERT) binding in children with ADHD (29). Reduced performance on a stop-signal task also correlated with higher distractibility and impulsivity (30). A review of the genetics of ADHD in children and adolescents suggested a genetic foundation for the function of the 5-HT1B receptor in patients with the inattentive ADHD subtype and also for the function of 5-HT2A/C receptors in patients with ADHD (31). It was speculated that in patients with ADHD, there could be ineffective central nervous system 5-HT synthesis, possibly caused by a particular variant of the enzyme tryptophan hydroxylase (31), which is the rate limiting enzyme in central nervous system 5-HT synthesis. However, the influence of changes in central nervous system 5-HT synthesis on ADHD symptoms in adult patients has not been thoroughly investigated as of yet.

Aims of the study

Our first aim was to replicate deficits in sustained attention and behavioral inhibition in adults with ADHD using a modified continuous performance test (AX-CPT). Second, we aimed to investigate whether a diminished central nervous 5-HT

synthesis rate as achieved by acute tryptophan depletion (ATD) has an impact on deficits related to attentional processes as indexed by target/non-target discrimination ability and sustained attention in adults with ADHD.

Material and methods

Sample

The patients were recruited from a pool of male adult patients with ADHD who were receiving treatment at the ADHD out-patient clinic of the Department of Psychiatry and Psychotherapy, University of Duisburg-Essen. The study sample is identical with the one in previous research (32). Patients with an IQ below 85 were not included in the study. IQ was estimated with the 'picture completion' and 'similarities' (33) subtests of the Wechsler Intelligence Scale for adults. A multiple-choice word test, the B version of the German 'Mehrfachwahl-Wortschatz-Intelligenz-Test' (MWT-B), was also administered (34). The MWT-B resembles the English spot-the-word test. Subjects with diabetes, obesity, schizophrenia, substance abuse, mood and anxiety disorders, and other chronic medical conditions were excluded. No patients with a history of drug abuse were included in the study. The control group was recruited by notices at adjacent universities and university hospitals. All patients were diagnosed with ADHD by a trained psychiatrist according to the ICD-10 and DSM-IV-TR criteria prior to the beginning of the study. An assessment was performed as part of the regular diagnostic process of diagnosing patients with ADHD, using questionnaires involving current problems related to attention, hyperactivity, and impulsivity (ADHD Self-rating questionnaire: ADHS-SB; Wender Utah Rating Scale, WURS-K, (35, 36)) as well as questionnaires assessing state and trait anxiety (State-Trait Anxiety Inventory, STAI X1 and X2, (37)), depressive symptoms (Beck's Depression Inventory, BDI, (38)), and baseline levels of impulsivity (Barrett-Impulsiveness Scale (39)). We used self-rating questionnaires because of their generally acknowledged validity and efficient diagnostic properties (36). Controls were excluded from the study if they reached the cut-off score for ADHD (ADHS-SB, WURS-K) or depression (BDI). After applying these exclusion criteria, 20 male adult patients with ADHD (age: M = 30.25 years, SD = 9.37 years) with a confirmed diagnosis of ADHD and 20 healthy male control participants (age: M = 27.9 years, SD = 6.01 years) were included in the study. The groups did not differ significantly in age. The subjects had achieved between 8 and 21 years of formal education (mean duration of formal education was 14.58 years, SD = 3.2 years). Years of formal education did not differ significantly between the groups. All patients with ADHD were instructed to stop their medications during the study after consulting with their psychiatrist. Patients receiving methylphenidate (MPH) were instructed to stop their medication 24 h before each study day, and these patients confirmed that they had followed these instructions. The study was assessed and approved by the Ethics Committee of the Faculty of Medicine of the University Essen-Duisburg and was conducted in accordance with the Helsinki declaration.

Study design

The study design was a double-blind, within-subject, crossover design. The patients received the ATD test and a control condition in a randomized and counterbalanced order on two separate testing days, which were 7 days apart. On one day, the ATD procedure Moja-De was administered within an amino acid beverage lacking tryptophan (TRP), the physiological precursor of 5-HT (40–45). On a further day the participants received the same amino acid mixture with TRP serving as a balanced control condition (BAL). If ATD was administered on day 1, the subjects received the BAL mixture on day 2 and vice versa.

Acute tryptophan depletion

The ATD procedure by Moja et al. (45), a method of diminishing central 5-HT synthesis in human subjects, lowers central 5-HT synthesis by decreasing the influx of TRP over the blood–brain barrier into the brain by orally administering a TRP-free amino acid mixture (46, 47). This procedure was recently validated in an animal model (46) and was also used in studies with young people (48, 49). The reduced influx of free plasma TRP is achieved by changing the plasma concentrations of other long-chained neutral amino acids (LNAAs) and their ratio to free plasma TRP at the L-1 transporter at the blood–brain barrier. Because the influx of LNAAs into the brain works in a competitive manner, changes in plasma concentrations of relevant amino acids result in a diminished influx of TRP into the brain. The decreased uptake of TRP into the CNS leads to a reduced synthesis rate of central nervous system 5-HT (50). A marked reduction in plasma TRP levels after the administration of a 7-amino acid mixture was first described by Moja et al. (45). This study used an

amino acid mixture identical to previous research (51). For the balanced control condition, TRP was added to the mix [both mixtures were optically identical, and the difference in quantity (as the BAL condition also included TRP) was not visible]. The participants were not allowed to eat protein-rich meals starting at 8 p.m. on the day prior to each study day. Immediately after beverage intake (ATD/BAL), the subjects were given a multivitamin candy to counterbalance the aftertaste of the amino acid mixture. One hour after administration of ATD/BAL, all participants received a multivitamin drink containing 30% of the recommended daily dose of nicotine adenine dinucleotide, a TRP-derived vitamin. A soup snack containing no TRP was offered to the patients 2 hours after ATD/BAL intake.

AX-Continuous Performance Test (AX-CPT)

The AX-CPT is a modified computerized continuous performance test (CPT) (52). The experimental paradigm was a variant of an AX-CPT with three stimulus conditions. In the AX-CPT, the subject was instructed to press the left [ctrl] key every time the letter 'A' (cue) was followed by the letter 'X' (target). In any other cue-target combination other than 'AX' (AY, BX), subjects were instructed to press the right [ctrl] key. To increase test difficulty and avoid possible ceiling effects, 90% of the pixels of the target stimulus were removed using a standard picture tool (Adobe Photo Shop CS4®). After the presentation of an exclamation mark (100 ms), the first letter (cue stimulus) appeared on a computer screen for 100 ms. The second letter (target stimulus) was presented for 100 ms after a 700 ms delay. Subjects had to respond by pressing the right or left [ctrl] key within 1000 ms. There was no intertrial interval. Instead, one trial immediately followed the next. The AX condition was presented in 70% of the trials (490 trials). The remaining 30% of the trials (210) were separated into two conditions [condition AY (15% = 105 trials) and condition BX (15% = 105 trials)]. The letter 'Y' represents any non-X letter. The letter 'B' is indicative of a range of other letters that were used randomly in the BX condition. The difficulty for the subjects was that the presentation of the AX condition (70% of the trials) led to predominant response behavior.

The AX-CPT was programmed and presented in MS-DOS 6.0 on a desktop PC with a license for ERTS. Before the main statistical analysis was performed, the median response times, correct responses, and errors for the conditions AX, AY, BX as well as omissions were analyzed using ERTSCODE (BeriSoft Cooperation). Omissions were defined as a lack of response in each trial during the AX-CPT.

Data analysis

All calculations were performed using SPSS version 17.0 (IBM). Between-group comparisons were performed using Student's t-tests. The significance level was set and kept at $P < 0.05$. The main dataset was statistically analyzed using repeated-measures analyses of variance (ANOVAs) for each dependent variable. The following variables were used as within-subject factors: the AX-CPT's correct responses (conditions AX, BX, AY), the error rate (conditions AX_E, BX_E, and AY_E), the number of omissions, and the arithmetic mean of the median reaction time (RT) for correct responses and errors (we used the median because of its insensitivity to statistical spikes). In addition, the group (ADHD vs. controls) and ATD/BAL were included as factors in the ANOVAs. All ANOVAs were corrected using *post hoc* Bonferroni correction and Greenhouse–Geisser adjustments. Effect sizes were calculated with η^2, which estimates the proportion of variance in the dependent variable that is attributable to the group effect. T-tests revealed no significant differences between subjects with or without ADHD in variables of estimated intelligence (IQ) or duration of education (all $P > 0.05$). Thus, the models were not adjusted for intelligence or education. The results of the scores for the WURS-K, ADHS-SB, STAI, BIS (Barratt impulsiveness scale), and BDI questionnaires showed significant differences between patients and controls. BDI was used as a covariate for further analysis (Table 1).

Results

Reaction time

ATD had a significant effect on the subjects' RT in the AX-CPT. An ANOVA for RT showed a significant group-by-challenge interaction ($F(1/35) = 6.65$, $P = 0.005$, $\eta^2 = 0.2$) for the AX condition. The ADHD group's RT decreased when under the influence of ATD (M = 296.10 ms, SE = 21.37) compared with BAL (M = 374.55 ms, SE = 20.66). In the control group, the RT increased for ATD (M = 385.91 ms, SE = 21.08) when compared with BAL (M = 356.7, SE = 20.77).

In addition, the condition AX_E was significantly different between groups ($F(1/35) = 4.81$ $P = 0.03$, $\eta^2 = 0.13$). Participants with ADHD had a shorter

Table 1. Demographic data of the ADHD group and the control group

Variable	ADHD Group M (SD) *(n = 20)*	Control Group M (SD) *(n = 20)*	*p*-value *(t-test)*
Age (years)	M = 30.25 (9.37)	M = 27.90 (6.01)	*n.s.*
Years of education	M = 13.90 (3.401)	M = 15.25 (2.826)	*n.s.*
MWT-IQ	M = 108 (15.875)	M = 109.25 (10.64)	*n.s.*
WIP S	M = 107.4 (12.69)	M = 108.55 (11.15)	*n.s.*
WIP PC	M = 118 (9.78)	M = 116.75 (9.32)	*n.s.*
WURS-K	M = 34.15 (13.73)	M = 11.30 (9.60)	*P < 0.001*
ADHS-SB	M = 30.90 (12.01)	M = 9.05 (6.60)	*P < 0.001*
STAI X1	M = 40.70 (9.13)	M = 33.25 (6.73)	*P < 0.006*
STAI X2	M = 45.77 (12.20)	M = 34.10 (11.31)	*P < 0.003*
BIS	M = 68.92 (10.70)	M = 61.85 (9.91)	*P < 0.038*
BDI*	M = 9.57 (2.24)	M = 4.55 (5.98)	*P < 0.048*

The characteristics of the two analyzed groups are shown. Data are provided for age, years of education, Wechsler Intelligence Scale, similarity subtest (WIP S) and picture completion subtest (WIP PC), multiple-choice word test (MWT-IQ) [resembles the English spot-the-word test], Wender Utah Rating Scale (WURS-K), ADHD self-rating questionnaire (ADHD-SB), State-Trait Anxiety Inventory (STAI X1 and STAI X2), Barratt impulsiveness scale (BIS), and Beck's Depression Inventory. (M) = Mean, (SD) = Standard deviation, (n) = size.
*considered as a covariate in ANOVA.

RT (M = 347.0 ms, SE = 19.45) than controls (M = 409.1 ms, SE = 19.5). Furthermore, there was a significant difference between participants with ADHD (M = 291.9 ms, SE = 15.9) and without ADHD (M = 342.14 ms, SE = 15.9) in condition AY_E ($F(1/35) = 4.74$, $P < 0.04$, $\eta^2 = 0.12$). For all other conditions, the ANOVA showed no significant differences ($P > 0.05$, $\eta^2 < 0.03$).

Correct responses

There was a significant difference between groups in condition AX ($F(1/35) = 13.01$, $P = 0.001$, $\eta^2 = 0.27$). The participants with ADHD provided fewer correct responses (M = 60.27, SE = 1.57) than the participants without ADHD (M = 68.5, SE = 1.57). Furthermore, a significant difference between groups was detected in condition AY ($F(1/35) = 7.73$, $P = 0.009$, $\eta^2 = 0.18$; ADHD group: M = 9.87, SE = 0.58; Control group: M = 7.2, SE = 0.58). For all other conditions, the ANOVA showed no significant differences ($P > 0.05$, $\eta^2 < 0.08$).

Errors

There was a significant difference between groups ($F_{(1/35)} = 9.69$, $p = 0.004$, $\eta^2 = 0.21$) for the condition AX_E. Participants with ADHD had more errors (M = 2.8, SE = 0.6) when compared with controls (M = 0.5, SE = 0.6). For all other conditions, there were no significant differences as analyzed by ANOVA ($P = 0.06$, $\eta^2 < 0.10$).

Omissions

A 1 (omissions) × 2 (challenge: ATD vs. BAL) × 2 (group: ADHD vs. controls) ANOVA showed significant effects for the factors group ($F_{(1/35)} = 15.67$, $P = 0.001$, $\eta^2 = 0.30$) and challenge ($F_{(1/35)} = 6.16$, $P = 0.02$, $\eta^2 = 0.15$). The test of within-subjects contrasts showed a significant linear group-by-challenge interaction ($F_{(1/35)} = 4.26$, $P = 0.05$, $\eta^2 = 0.11$). The number of omissions increased in the group of patients with ADHD after intake of ATD (M = 17.44, SE = 2.55) when compared with BAL (M = 6.20, SE = 2.50). Identical effects were found for the control group (ATD: M = 2.05, SE = 2.55; BAL: M = 1.07, SE = 2.46) (Fig. 1).

Discussion

In this study, diminished central nervous system 5-HT synthesis as indexed by ATD affected target/non-target discrimination ability and sustained attention in adult subjects with ADHD. In patients with ADHD the number omissions increased after ATD administration when compared with BAL, and patient's reaction time decreased after ATD when compared with BAL intake, a finding that was contrasted by opposite effects in controls. Patients showed fewer correct responses (AX condition) and showed a higher rate of errors (condition AX_E) independent of ATD or BAL intake.

Previous studies assessing sustained attention in a Go/No-go paradigm only detected effects between groups, and an effect of ATD on omissions or RT has not been reported previously (51, 53). Few studies to date have investigated the impact of ATD in patients with ADHD. These

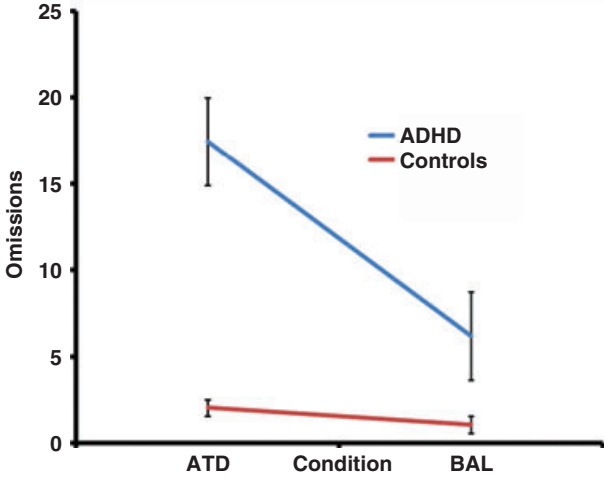

Fig. 1. Given is an interaction between the factors group (adult patients with ADHD vs. adult controls) and challenge (ATD vs. BAL) for omissions as assessed in the AX-CPT.

studies examined the effects of ATD on memory performance (53), reactive aggression in adult patients (32), and reactive aggression in young people with ADHD (54). Sambeth et al. (53) noted that delayed recall in a memory task was impaired after ATD. In contrast to these results, Evers et al. did not show any effects of decreased central nervous 5-HT synthesis on cognitive performance, including verbal learning and recognition in healthy volunteers (51). Our group recently demonstrated an interaction between ATD and reactive aggressive behavior in the present sample. Adult patients with ADHD showed increased reactive aggression in a Point Subtraction Aggression Game after ATD when compared with BAL (32). Further research detected that highly hostile aggressive patients showed increased inhibition errors after acute tryptophan depletion when compared with a control condition (54).

To our knowledge, this study is the first to show an effect of ATD on omissions in adults with ADHD. The slight increase in the number of omissions in the control group after ATD was not as pronounced as in the ADHD group, suggesting that serotonergic pathways in the prefrontal cortex might be more susceptible to manipulation in patients with ADHD than in healthy controls. Consistent with previous work, we identified shorter reaction times and a higher rate of omissions in adult patients with ADHD, confirming the previously established hypothesis of problems with impulsivity and maintaining sustained attention over a long period of time (55–58). With respect to neuropsychological deficits frequently reported in patients with ADHD, the present results are consistent with findings on alterations in executive functioning in these particular patients (59–61).

In accordance with previous reports related to changes in serotonergic neurotransmission in patients with ADHD, diminished central nervous system 5-HT synthesis was associated with changes in omissions and reaction times in these particular subjects in our study. However, with respect to potential 5-HT–DA interactions, recent studies have indicated that serotonergic and dopaminergic pathways project into brain regions (lateral PFC, dorsal anterior cingulate cortex, dorsal striatal regions) that are associated with sustained attention, processing speed, behavioral inhibition, and mediation of inhibition (62). As outlined by Oades et al. (27), there is a lower level of available DA and a 25% reduction in binding characteristics of the SERT in patients with ADHD, particularly in prefrontal regions and the anterior cingulate cortex. Lower SERT binding was associated with poorer attentional performance and higher cognitive impulsivity in patients with ADHD, and 5-HT was shown to modulate impulse control by increasing reaction latency (30). Cheetam and colleagues (63) emphasize that a reduction in 5-HT uptake leads to diminished SERT binding in the PFC.

There is evidence for an altered DA system in ADHD (64–66) and for an influence of 5-HT on dopaminergic pathways (31). There is also evidence supporting the assumption that DA function in the PFC modulates the output to basal ganglia circuits and for the previously mentioned effect of 5-HT on the DA system (67). Furthermore, there is evidence that symptoms such as hyperactivity and poor attentional performance are also linked to changes in DA function in patients with ADHD (68). Studies in patients with ADHD showed that patients displayed more omissions (56–58). Additional findings show that in normal individuals, ATD increased impulsiveness (69). A review of the effects of ATD on memory, executive function, and attention reported that ATD changes memory function in healthy adults (51). With regard to the previously mentioned interaction of 5-HT and DA in ADHD, one could speculate that an ATD-induced imbalance in central nervous system 5-HT synthesis could reduce the modulatory effect of 5-HT on DA. A resulting dysfunction of networks responsible for sustained attention and behavioral inhibition might in turn lead to the observed effects on RT and omissions observed in this study.

This study has advantages over previous research because it included a healthy control group that did not differ from the subjects in terms of education, IQ, or age, which stands in contrast to previous research (54, 70–72). However, there are some methodological limitations when attempting to interpret the data related to ATD and attention. First, the relatively small sample size of this pilot study makes it difficult to draw general conclusions about the effects of ATD on attentional processes. However, as the changes in omissions and reaction time showed the same pattern as expected based on theoretical models, it can be assumed that the investigated sample can be seen as somewhat representative. Nevertheless, future studies are needed to draw general clinical conclusions. Moreover, genetic data, particularly polymorphisms related to the 5-HT system, were not obtained. There is preliminary evidence for an association between altered serotonergic neurotransmission and ADHD symptoms. For example, a variant of the enzyme TPH2, which is essential for central nervous 5-HT synthesis, has been the subject of recent research (31). Further research with significantly larger samples is necessary to establish whether variations in genes linked to

serotonergic neurotransmission affect subjects' AX-CPT performance. Considering the above-mentioned effects of ATD on the AX-CPT performance of adults with ADHD, it should be noted that we did not control for the effects of other neurotransmitters, such as DA or NA. These limitations should be addressed by future research.

The overall complexity of the 5-HT system must be considered when trying to interpret the data obtained in this study. Seven different types of 5-HT receptors have been identified so far (5-HT_1 to 5-HT_7). It remains uncertain whether changes in post- and/or presynaptic serotonergic activity predominate after ATD or whether an inhibitory interneuron permits disinhibition in the control of specific functions of prefrontal serotonergic pathways. Studies using functional magnetic resonance imaging (fMRI) in combination with neurodietary challenge procedures such as ATD (and also, to a further extent, phenylalanine-tyrosine depletion [PTD] to achieve a reduced DA synthesis) are of particular relevance in further disentangling differential and commonly modulated effects of 5-HT and DA on changes in the top-down control of attention-related neurocircuits. Moreover, PET-studies using ATD and PTD, as well as potentially combined monoamine depletion, can be mentioned here as a further avenue to probe the effects of 5-HT-DA interactions on attentional performance with respect to the outlined neurocircuits.

In summary, the present investigation provides preliminary evidence for altered central nervous system 5-HT function in adult patients with ADHD and its association with target/non-target discrimination ability and sustained attention as measured in the AX-CPT. Further research is warranted to examine the specific influence of 5-HT on symptom expression in adults with ADHD.

Declaration of interest

FDZ received an unrestricted award from the American Psychiatric Association (APA), the American Psychiatric Institute for Research and Education (APIRE), and AstraZeneca (the Young Minds in Psychiatry Award). He has previously 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, and the Dr. August Scheidel Foundation as well as a travel stipend from the Glaxo-SmithKline Foundation. He has also received support from the Raine Foundation for Medical Research (Visiting Professorship). Finally, he has received an unrestricted educational grant, speaker honoraria, and travel support from Shire Pharmaceuticals, Germany. BK received honoraria for lectures from Medice and Servier. In addition, he is an advisory board member for Medice and Servier. The other authors have nothing to report or disclose.

References

1. Davidson MA. Adhd in adults: a review of the literature. J Atten Disord 2008;**11**:628–641.
2. Biederman J. Attention-deficit/hyperactivity disorder: a selective overview. Biol Psychiatry 2005;**57**:1215–1220.
3. Klassen LJ, Katzman MA, Chokka P. Adult ADHD and its comorbidities, with a focus on bipolar disorder. J Affect Disord 2010;**124**:1–8.
4. Babcock T, Ornstein CS. Comorbidity and its impact in adult patients with attention-deficit/hyperactivity disorder: a primary care perspective. Postgrad Med 2009;**121**:73–82.
5. Trautmann M, Zepf FD. Attentional performance, age and scholastic achievement in healthy children. PLoS ONE 2012;**7**:e32279.
6. Clarke S, Heussler H, Kohn MR. Attention deficit disorder: not just for children. Intern Med J 2005;**35**:721–725.
7. Barkley RA. Differential diagnosis of adults with ADHD: the role of executive function and self-regulation. J Clin Psychiatry 2010;**71**:e17.
8. Brown TE. ADD/ADHD and Impaired Executive Function in Clinical Practice. Curr Psychiatry Rep 2008;**10**:407–411.
9. Arnsten AF. Fundamentals of attention-deficit/hyperactivity disorder: circuits and pathways. J Clin Psychiatry 2006;**67**(Suppl 8):7–12.
10. Arnsten AF. The emerging neurobiology of ADHD: the key role of the prefrontal association cortex. J Pediatrics 2009;**154**:I–S43.
11. Chudasama Y, Robbins TW. Functions of frontostriatal systems in cognition: comparative neuropsychopharmacological studies in rats, monkeys and humans. Biol Psychol 2006;**73**:19–38.
12. Mega MS, Cummings JL. Frontal-subcortical circuits and neuropsychiatric disorders. J Neuropsychiatry Clin Neurosci 1994;**6**:358–370.
13. Casey BJ, Castellanos FX, Giedd JN et al. Implication of right frontostriatal circuitry in response inhibition and attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 1997;**36**:374–383.
14. Durston S, Tottenham NT, Thomas KM et al. Differential patterns of striatal activation in young children with and without ADHD. Biol Psychiatry 2003;**53**:871–878.
15. Casey BJ, Epstein JN, Buhle J et al. Frontostriatal connectivity and its role in cognitive control in parent-child dyads with ADHD. Am J Psychiatry 2007;**164**:1729–1736.
16. Cherkasova MV, Hechtman L. Neuroimaging in attention-deficit hyperactivity disorder: beyond the frontostriatal circuitry. Can J Psychiatry 2009;**54**:651–664.
17. Genro JP, Kieling C, Rohde LA, Hutz MH. Attention-deficit/hyperactivity disorder and the dopaminergic hypotheses. Expert Rev Neurother 2010;**10**:587–601.
18. Cardinal RN, Winstanley CA, Robbins TW, Everitt BJ. Limbic corticostriatal systems and delayed reinforcement. Ann N Y Acad Sci 2004;**1021**:33–50.
19. Kadesjo B, Gillberg C. Developmental coordination disorder in Swedish 7-year-old children. J Am Acad Child Adolesc Psychiatry 1999;**38**:820–828.
20. Davids E, Zhang K, Tarazi FI, Baldessarini RJ. Animal models of attention-deficit hyperactivity disorder. Brain Res Rev 2003;**42**:1–21.
21. Wood MD, Wren PB. Serotonin-dopamine interactions: implications for the de of novel therapeutic agents for psychiatric disorders. Prog Brain Res 2008;**172**:213–230.

22. Boulougouris V, Tsaltas E. Serotonergic and dopaminergic modulation of attentional processes. Prog Brain Res 2008;**172**:517–542.
23. Aston-Jones G, Gold JI. How we say no: norepinephrine, inferior frontal gyrus, and response inhibition. Biol Psychiatry 2009;**65**:548–549.
24. Retz W, Freitag CM, Retz-Junginger P et al. A functional serotonin transporter promoter gene polymorphism increases ADHD symptoms in delinquents: interaction with adverse childhood environment. Psychiatry Res 2008;**158**:123–131.
25. Oades RD, Sadile AG, Sagvolden T et al. The control of responsiveness in ADHD by catecholamines: evidence for dopaminergic, noradrenergic and interactive roles. Dev sci 2005;**8**:122–131.
26. Stoff DM, Pollock L, Vitiello B, Behar D, Bridger WH. Reduction of (3H)-imipramine binding sites on platelets of conduct-disordered children. Neuropsychopharmacology 1987;**1**:55–62.
27. Oades RD, Lasky-Su J, Christiansen H et al. The influence of serotonin- and other genes on impulsive behavioral aggression and cognitive impulsivity in children with attention-deficit/hyperactivity disorder (ADHD): Findings from a family-based association test (FBAT) analysis. Behav Brain Funct 2008;**4**:48.
28. Westergaard GC, Suomi SJ, Higley JD, Mehlman PT. CSF 5-HIAA and aggression in female macaque monkeys: species and interindividual differences. Psychopharmacology 1999;**146**:440–446.
29. Oades RD. Dopamine-serotonin interactions in attention-deficit hyperactivity disorder (ADHD). Prog Brain Res 2008;**172**:543–565.
30. Oades RD. Dopamine may be 'hyper' with respect to noradrenaline metabolism, but 'hypo' with respect to serotonin metabolism in children with attention-deficit hyperactivity disorder. Behav Brain Res 2002;**130**:97–102.
31. Oades RD. Role of the serotonin system in ADHD: treatment implications. Expert Rev Neurother 2007;**7**:1357–1374.
32. Zimmermann M, Grabemann M, Mette C et al. The effects of acute tryptophan depletion on reactive aggression in adults with attention-deficit/hyperactivity disorder (ADHD) and healthy controls. PLoS ONE 2012;**7**:e32023.
33. Dahl G editor. WIP-Reduzierter Wechsler Intelligenztest: Anwendung, Auswertung, Statistische Analysen, Normwerte. Meisenheim, Germany: Hain, 1981.
34. Lehrl S. Manual zum MWT-B (Mehrfach-Wortschatz-Intelligenztest- Version B, 3. überarb. Aufl.). Balingen: Perimed, 1995.
35. Rösler M, Retz-Junginger P, Retz W et al. Homburger ADHS-Skalen für Erwachsene (HASE). Göttingen: Hogrefe, 2008.
36. Rösler M, Retz W, Thome J, Schneider M, Stieglitz RD, Falkai P. Psychopathological rating scales for diagnostic use in adults with attention-deficit/hyperactivity disorder (ADHD). Eur Arch Psychiatry Clin Neurosci 2006;**256** (Suppl 1):i3–i11.
37. Seifert J. Das deutschsprachige State-Trait Angst Inventar (unveröffentlichtes Manuskript). Trier: Universität Trier, Fachbereich Psychologie, 2002.
38. Beck AT, editor. Beck-Depressions-Inventar (BDI, 2., überarbeitete Aufl.). Göttingen: Hogrefe, 1995.
39. Patton JH, Stanford MS, Barratt ES. Factor structure of the Barratt impulsiveness scale. J Clin Psychol 1995;**51**:768–774.
40. Demisch L, Kewitz A, Schmeck K et al. Methodology of rapid tryptophan depletion (RTD): impact of gender and body weight. Eur Arch Psychiatry Clin Neurosci 2002;**252** (Supplement 2 I/25):11–14.
41. Demisch L, Kaczmarczyk P. Tryptophan metabolism in healthy subjects: influence of pyridoxine after single or repeated administrations. Adv Exp Med Biol 1991;**294**:519–522.
42. Demisch L, Zepf FD, Schmitt M et al. Experimentell induzierte Reduktion der zentralnervösen Serotonin Synthese durch den "Rapid Tryptophan Depletion-Test" (RTD) nach Moja et al. Methodik, Entwicklung sowie Anwendung bei Kindern und Jugendlichen Abstract-Band zur 12. Jahrestagung des Arbeitskreises Biologische Kinder- und Jugendpsychiatrie. Göttingen/Germany, page 31, 2004.
43. Kewitz A. Biochemische Untersuchungen zur Optimierung des "Rapid Tryptophan Depletion Test" (RTD): Eine physiologische Methode zur akuten Verminderung der zentralnervösen Serotonin-Synthese in der psychologischen Forschung. Frankfurt am Main: J. W. Goethe Universität, 2002.
44. Moja EA, Stoff DM, Gessa GL, Castoldi D, Assereto R, Tofanetti O. Decrease in plasma tryptophan after tryptophan-free amino acid mixtures in man. Life Sci 1988;**42**:1551–1556.
45. Moja EA, Rocchi E, Benedetti F et al. Decrease in plasma tryptophan after a tryptophan-free amino acid solution. A comparison between cirrhotic and control subjects. Life Sci 1991;**48**:409–418.
46. Biskup CS, Sánchez CL, Arrant A, Van Swearingen AE, Kuhn C, Zepf FD. Effects of Acute Tryptophan Depletion on Brain Serotonin Function and Concentrations of Dopamine and Norepinephrine in C57BL/6J and BALB/cJ Mice. PLoS ONE 2012;**7**:e35916.
47. Dingerkus VLS, Gaber TJ, Helmbold K et al. Acute tryptophan depletion in accordance with body weight: Influx of amino acids across the blood-brain barrier. J Neural Transmission 2012;**119**:1037–1045.
48. Koetting WF, Bubenzer S, Helmbold K, Eisert A, Gaber TJ, Zepf FD. Effects of tryptophan depletion on reactive aggression and aggressive decision-making in young people with ADHD. Acta Psychiatr Scand 2013;**128**:114–123.
49. Zepf FD, Landgraf M, Biskup CS et al. No effect of acute tryptophan depletion on verbal declarative memory in Young Persons with ADHD. Acta Psychiatr Scand 2013;**128**:133–141.
50. Young SN, Ervin FR, Pihl RO, Finn P. Biochemical aspects of tryptophan depletion in primates. Psychopharmacology 1989;**98**:508–511.
51. Evers EA, Tillie DE, Van Der Veen FM et al. Effects of a novel method of acute tryptophan depletion on plasma tryptophan and cognitive performance in healthy volunteers. Psychopharmacology 2005;**178**:92–99.
52. Carter CS, Van Veen V. Anterior cingulate cortex and conflict detection: an update of theory and data. Cogn Affect Behav Neurosci 2007;**7**:367–379.
53. Sambeth A, Riedel WJ, Tillie DE, Blokland A, Postma A, Schmitt JA. Memory impairments in humans after acute tryptophan depletion using a novel gelatin-based protein drink. J Psychopharmacol 2009;**23**:56–64.
54. Zepf FD, Holtmann M, Stadler C et al. Diminished serotonergic functioning in hostile children with ADHD: tryptophan depletion increases behavioural inhibition. Pharmacopsychiatry 2008;**41**:60–65.

55. Avisar A, Shalev L. Sustained attention and behavioral characteristics associated with ADHD in adults. Appl Neuropsychol 2011;**18**:107–116.
56. Soreni N, Crosbie J, Ickowicz A, Schachar R. Stop signal and Conners' continuous performance tasks: test–retest reliability of two inhibition measures in ADHD children. J Atten Disord 2009;**13**:137–143.
57. Banaschewski T, Yordanova J, Kolev V, Heinrich H, Albrecht B, Rothenberger A. Stimulus context and motor preparation in attention-deficit/hyperactivity disorder. Biol Psychol 2008;**77**:53–62.
58. Advokat C, Martino L, Hill BD, Gouvier W. Continuous Performance Test (CPT) of college students with ADHD, psychiatric disorders, cognitive deficits, or no diagnosis. J Atten Disord 2007;**10**:253–256.
59. Silberstein RB, Farrow M, Levy F, Pipingas A, Hay DA, Jarman FC. Functional brain electrical activity mapping in boys with attention-deficit/hyperactivity disorder. Arch Gen Psychiatry 1998;**55**:1105–1112.
60. Kienle X, Thumser K, Saile H, Karch D. Neuropsychological assessment of ADHD subtypes. Prax Kinderpsychol Kinderpsychiatr 2005;**54**:159–172.
61. Pollak Y, Shomaly HB, Weiss PL, Rizzo AA, Gross-Tsur V. Methylphenidate effect in children with ADHD can be measured by an ecologically valid continuous performance test embedded in virtual reality. CNS Spectr 2010;**15**: 125–130.
62. Vaidya CJ, Stollstorff M. Cognitive neuroscience of Attention Deficit Hyperactivity Disorder: current status and working hypotheses. Dev Disabil Res Rev 2008;**14**:261–267.
63. Cheetham SC, Viggers JA, Slater NA, Heal DJ, Buckett WR. [3H]paroxetine binding in rat frontal cortex strongly correlates with [3H]5-HT uptake: effect of administration of various antidepressant treatments. Neuropharmacology 1993;**32**:737–743.
64. Auerbach JG, Atzaba-Poria N, Berger A et al. Dopamine risk and paternal ADHD symptomatology associated with ADHD symptoms in four and a half-year-old boys. Psychiatr Genet 2010;**20**:160–165.
65. Frank MJ, Santamaria A, O'reilly RC, Willcutt E. Testing computational models of dopamine and noradrenaline dysfunction in attention deficit/hyperactivity disorder. Neuropsychopharmacology 2007;**32**:1583–1599.
66. Mazei-Robinson MS, Blakely RD. ADHD and the dopamine transporter: are there reasons to pay attention? Handb Exp Pharmacol 2006;**175**:373–415.
67. Artigas F. The prefrontal cortex: a target for antipsychotic drugs. Acta Psychiatr Scand 2010;**121**:11–21.
68. Swanson JM, Kinsbourne M, Nigg J et al. Etiologic subtypes of attention-deficit/hyperactivity disorder: brain imaging, molecular genetic and environmental factors and the dopamine hypothesis. Neuropsychol Rev 2007;**17**: 39–59.
69. Walderhaug E, Lunde H, Nordvik JE, Landro NI, Refsum H, Magnusson A. Lowering of serotonin by rapid tryptophan depletion increases impulsiveness in normal individuals. Psychopharmacology 2002;**164**:385–391.
70. Zepf FD, Wockel L, Poustka F, Holtmann M. Diminished 5-HT functioning in CBCL pediatric bipolar disorder-profiled ADHD patients versus normal ADHD: susceptibility to rapid tryptophan depletion influences reaction time performance. Hum Psychopharmacol 2008;**23**:291–299.
71. Zepf FD, Stadler C, Demisch L, Schmitt M, Landgraf M, Poustka F. Serotonergic functioning and trait-impulsivity in attention-deficit/hyperactivity-disordered boys (ADHD): influence of rapid tryptophan depletion. Hum Psychopharmacol Clin Exp 2008;**23**:43–51.
72. Zepf FD, Holtmann M, Stadler C, Magnus S, Wockel L, Poustka F. Diminished central nervous 5-HT neurotransmission and mood self-ratings in children and adolescents with ADHD: no clear effect of rapid tryptophan depletion. Hum Psychopharmacol 2009;**24**:87–94.