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ORIGINAL ARTICLE

Reduced central nervous 5-HT neurotransmission in youth with ADHD influences ratings of a virtual opponents` extraversion – Effects of trait-aggression

Florian Daniel Zepf^{1,2,3}, Tilman Jakob Gaber^{1,2}, Sarah Bubenzer^{1,2},
David Baumann^{1,2}, Katrin Helmbold^{1,2}, Timo Vloet¹, Martin Holtmann^{3,4},
Sophie Magnus^{3,5}, Fritz Poustka³, Lars Wöckel^{1,3,6}

Abstract:

Objective: Evidence from animal and human studies suggests that the neurobiology of aggressive behaviour and extraversion is linked with changes in serotonergic (5-HT) neurotransmission. The present study investigated the effects of diminished central nervous 5-HT neurotransmission following rapid tryptophan depletion (RTD) in youth diagnosed with attention deficit/hyperactivity disorder (ADHD). The study assessed the participants' opponent ratings after the experience of competing against a fictitious opponent in a competitive reaction time game (CRT) whilst under the influence of depletion/placebo. **Method:** 22 boys diagnosed with ADHD were subjected to a double-blind within-subject crossover-design, receiving RTD on one day, and on a further day a tryptophan balanced placebo. 4.5 hours after RTD/placebo intake they were subjected to provocation of aggressive behaviour using the CRT and asked to produce opponent ratings. **Results:** "Low aggressive" boys showed significantly higher extraversion ratings of their fictitious opponent compared to those of "high aggressive" boys under RTD versus placebo. **Conclusion:** The data support evidence that changes in 5-HT neurotransmission are involved in the neurobiological underpinnings of extraversion experienced by children and adolescents with ADHD. Future research with healthy controls and both genders is required to control for developmental and disorder-related effects.

Keywords: Aggression; Serotonin; Rapid Tryptophan Depletion; ADHD; Opponent ratings

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Introduction

Evidence from animal and human studies suggests that changes in serotonergic (5-HT) neurotransmission are involved in the underlying neurobiological processes associated with aggression, extraversion and impulsivity [1-4]. Among those disorders exhibiting increased aggression and extraversion in childhood attention deficit/hyperactivity disorder (ADHD) has been shown to be frequently associated with conduct disorder (CD) and aggression [5, 6, 7]. A variety of neurochemical techniques including the prolactin response to fenfluramine [6-10], the assessment of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in

cerebrospinal fluid [13-18], the 5-HT uptake in platelets [19-20], and the method of rapid tryptophan depletion (RTD) [21-26] have been employed to investigate changes in central nervous 5-HT neurotransmission and their interplay with aggression and extraversion in children.

RTD is a physiological method of reducing central nervous 5-HT synthesis in humans by diminishing the uptake of the 5-HT precursor amino acid tryptophan (TRP) across the blood brain barrier [27-29]. This reduced uptake of TRP is achieved by bringing endogenous TRP in plasma into competition with the amino acids

administered during their uptake into the central nervous system, as both amino acids and TRP use the same active transport system. This competition decreases the influx of TRP over the blood brain barrier into the central nervous system (CNS) [30]. This in turn reduces the central nervous availability of TRP for 5-HT synthesis, whilst the administration of the amino acids increases protein synthesis in the liver, thus taking additional endogenous TRP from plasma stores [27-29]. Overall, the reduced availability of TRP produces a diminished 5-HT synthesis rate in the CNS [27-29, 32].

Several studies have used RTD to investigate the effects of a diminished central nervous 5-HT synthesis rate on aggressive behaviour and extraversion in adults [33-42]. Recent evidence regarding children and adolescents suggests that in ADHD changes in 5-HT neurotransmission influence their susceptibility to react with aggressive behaviour after being provoked in a competitive reaction time game (CRT) [24]. In this particular study trait-impulsivity served as a moderator, because “low impulsive” persons in particular were more susceptible to an increased aggression response under RTD, thus acting much in the way of “high impulsive” patients [24]. Moreover, hostility traits proved to influence behavioural inhibition in boys with ADHD [25]. More indirect forms of aggression and extraversion however, such as the test persons’ experience of rating aggressive behaviour and extraversion of his peers, have not been studied with regard to diminished availability of 5-HT in youth as yet. For this reason the investigation of reactions to opponent extraversion experienced by test persons with ADHD may provide further evidence for the hypothesis that aggressive behaviour and extraversion are influenced by changes in central nervous 5-HT functioning. The present study tested the hypothesis that a diminished central nervous 5-HT turnover influences subject ratings of aggressive behaviour and extraversion of a fictitious opponent. After administering an

Zepf FD^{1,2,3}, Gaber TJ^{1,2}, Bubenzer S.^{1,2}, Baurmann D.^{1,2}, Helmbold K.^{1,2}, Vloet TD¹, Holtmann M^{3,4}, Magnus S^{3,5}, Poustka F³, Wöckel L^{1,3,6}

¹Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, RWTH Aachen University, Aachen /Germany

²JARA Translational Brain Medicine, Aachen & Jülich/Germany

³Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, Goethe University, Frankfurt am Main/Germany

⁴LWL-University Hospital, Department of Child and Adolescent Psychiatry, Psychotherapy and Psychosomatics Ruhr University, Bochum, Hamm/Germany

⁵Department of Child and Adolescent Psychiatry, Johannes Gutenberg University, Mainz/Germany

⁶Center of Adolescent Psychiatry, Clenia Littenheid AG, Littenheid/Switzerland

Correspondence author:
Prof. Dr. med. Florian Daniel Zepf, MD

Juniorprofessor for Translational Neuroscience in Psychiatry and Neurology, Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, RWTH Aachen University/JARA Translational Brain Medicine Neuenhofer Weg 21, D-52074 Aachen, Germany
Tel : +49.241.80.89171 & Fax: +49.241.80.3385504
E-mail: fzepf@ukaachen.de

aggression-provoking task (CRT) to the subject we assessed how the subjects would rate the behaviour of their fictitious opponent. We screened for any potentially moderating influence trait-aggression and –impulsivity might have on extraversion. The data referring to the CRT are the subject of other publications [23-24]. The authors note that a healthy control group would have been necessary to detect influences on the present findings specifically related to ADHD.

Material and methods

Participants and study design

The study employed a double blind within-subject crossover design, with the administration of RTD and the placebo condition as a within-subject repeated measures factor. The criterion for inclusion was a diagnosis of ADHD in accordance with ICD-10 criteria, co-morbid CD was also accepted. Exclusion criteria were

chronic medical conditions (i.e. asthma, diabetes, obesity, schizophrenia, substance abuse, mental retardation, pervasive developmental disorder), and an IQ below 85 so as to ensure the persons' understanding of psychological testing and task related instructions. The study was carried out in accordance with the Helsinki Declaration and was assessed and approved by the Ethics Commission of the Faculty of Medicine of the J.W. Goethe University Frankfurt/Main. The patients and their parents were given a complete description of the investigation, and gave both verbal and written consent. Initially 27 patients were admitted to the study, but 4 patients refused to participate, and 1 patient withdrew from the study because of oppositional behaviour, so that 22 patients comprised the study sample (mean age 10.9 yrs, range 9-15 yrs) analyzed. In this sample 11 patients were referred to us from a local Child and Adolescent Psychiatrist in private practice, 13 patients were recruited from a counseling group for aggressive behaviour. In our sample 4 boys had not been receiving any medication, the remaining 18 boys had been receiving methylphenidate (MPH) for treatment. The MPH treatment was discontinued on each study day, and was taken as prescribed between the two study days (half-life of MPH approx. 3 hrs. in children and adolescents [43]). The characteristics of the analyzed study sample are given in Table 1.

Table I. Characteristics of the analyzed study sample (n = 22).

Parameters	Mean \pm SD
Weight (kg)	40.9 \pm 9.2
BMI	17.6 \pm 2.1
Age (yrs)	10.9 \pm 1.8

RTD procedure

The used RTD procedure Moja-De [24-26, 29, 44, 58] was administered to the patients in an

aqueous suspension as an amino acid drink. The amino acids were dosed according to the body weight of the participants. The RTD drink consisted of L-Phenylalanine (1.32g/10kg), L-Leucine (1.32g/10kg), L-Isoleucine (0.84g/10kg), L-Methionine (0.5g/10kg), L-Valine (0.96g/10kg), L-Threonine (0.6/10kg), and L-Lysine (0.96g/10kg). The placebo drink contained the same amino acids plus 0.7g/10kg L-Tryptophan. The RTD and the placebo amino acid drink were administered on 2 separate study days with a period of at least 7 days between. A detailed description of the RTD procedure Moja-De is given elsewhere [24-26].

Rating procedure

The patients played against a fictitious opponent using a PC network-based computerized CRT [24, 45]. The boys were told that they were playing against a real opponent of the same age and gender who was sitting in a different room of the building. They were also told that the two computers were connected via LAN. In the CRT, the task was to press a button as soon as a visual stimulus appeared on the computer screen (a football). The winner of each trial won 50 points and was allowed to subtract points from 0 to 100 from the opponent's account. However, there was no real opponent, and the order of winning and losing trials and the level of provocation were both prearranged. The level of provocation was indexed by the amount of points the participant lost in losing a trial (high and low provocation, see below). After the game, they were asked to rate how they experienced their virtual/fictitious opponent while still considering him to be a real person. Ratings were carried out using an in-house rating scale ([46], see also Fig. 1).

The items of this rating scale are depicted in Table 2. The subjects had 5 minutes to complete the rating scale on both days of the investigation. The sub-scales employed for the rating scale were those for neuroticism (*NE*), extraversion (*EX*), charm (*CH*) and aggression (*AG*).

Table II. Sub-scales of the in-house rating scale which the subjects completed after the competitive reaction time game (CRT) in order to characterize the behaviour of their fictitious opponent.

Sub-scale	Items (How ... is your opponent?)
Neuroticism (<i>NE</i>)	Anxious - courageous
Extraversion (<i>EX</i>)	Lively – calm (3/12)
Charm (<i>CH</i>)	Friendly – mean (5/10)
Overall aggression (<i>AG</i>)	Aggressive – quiet/peaceful (6/9) Waspish – helpful (7/8)

Assessment of trait-aggression and -impulsivity

Trait-aggressive behaviour was assessed in advance of RTD/placebo intake using the adapted German study version of the Buss-Perry Aggression Questionnaire AQ-G [47, 48]. The AQ-G comprised the sub-scales “verbal aggression” (VA_{AQ-G}), “physical aggression” (PA_{AQ-G}), “anger” (AN_{AQ-G}), “hostility” (HO_{AQ-G}) and an overall “aggression-score”. The trait-impulsive sub-dimensions “venturesomeness” (VE_{IVE}) and “impulsivity” (IM_{IVE}) were also assessed in advance under baseline conditions using the German adaptation of the “I6 Impulsivity Questionnaire” (IVE; [49-51]).

Data analysis

The assessment of the mean RTD-effect for each scale of the in-house rating scale involved calculating the difference in opponent ratings from RTD to placebo (Δ) for each subject. The influence of trait-aggressive and -impulsive characteristics on the RTD-effect for each particular subscale of the in-house rating scale was analyzed using bivariate Pearson

correlations between the AQ-G subscales (VA_{AQ-G} , PA_{AQ-G} , AN_{AQ-G} , HO_{AQ-G}) and the abovementioned RTD-effects (Δ). The level of statistic significance was set and kept at $p < 0.05$, normality of data was assessed using Kolmogorov-Smirnov’s “Goodness of fit” test. Because of the explorative nature of this investigation the results did not require any corrections in terms of α -adjustment. Potential influences different characteristics of the study sample might have made on significant findings were controlled within bivariate Pearson correlations between the AQ-G subscales and detected RTD-effects. The findings were also controlled for potential effects of order within bivariate Pearson correlations between the abovementioned parameters and age, BMI and the order of RTD/placebo administration.

Results

Influence of trait-aggression

There was a significant negative correlation between PA_{AQ-G} and the RTD-effect on extraversion ratings (Δ_{EX} ; $r_{xy} = -0.511$, $p = 0.015$). This relationship is depicted in Fig. 1. A further negative correlation was detected between VA_{AQ-G} and the RTD-effect on extraversion ratings (Δ_{EX} ; $r_{xy} = -0.466$, $p = 0.029$; see Fig. 2). Following this, both baseline verbal and physical aggression scores in the AQ-G were associated with the RTD-effect on extraversion ratings. Low scorers on baseline VA_{AQ-G} and PA_{AQ-G} experience showed higher extraversion ratings under RTD as compared to placebo. A stepwise linear regression between VA_{AQ-G} , PA_{AQ-G} and Δ_{EX} as a dependent variable indicated that PA_{AQ-G} was the main factor, explaining approximately 26% of the shared intraindividual variance as regards the RTD-effect on extraversion ratings ($r_{xy} = -0.511$; $p = 0.015$, scores for VA_{AQ-G} had been excluded by the stepwise linear regression). The overall aggression score of the AQ-G was significantly

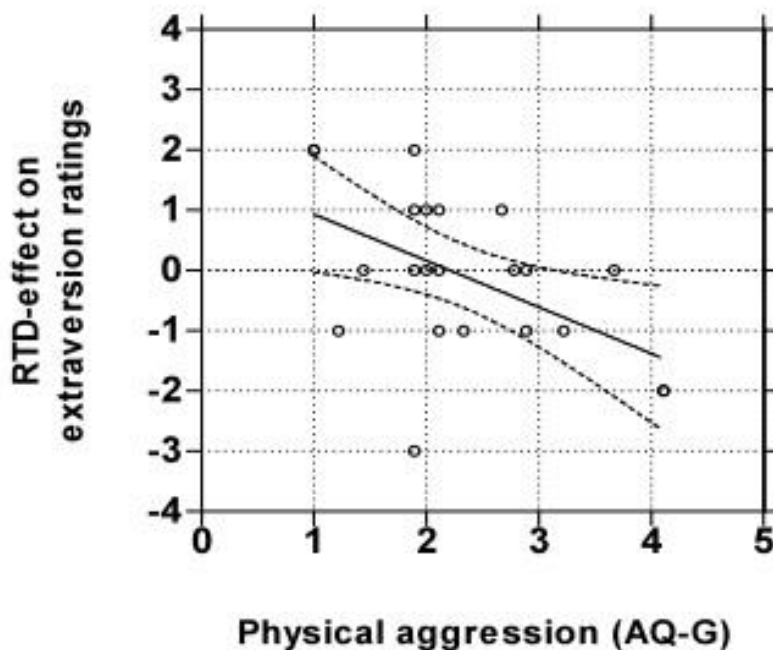


Figure I. Linear correlation/regression with 95% error interval between the baseline physical aggression score (PA_{AQ-G}) and the RTD-effect on extraversion opponent ratings after a competitive reaction time game (CRT).

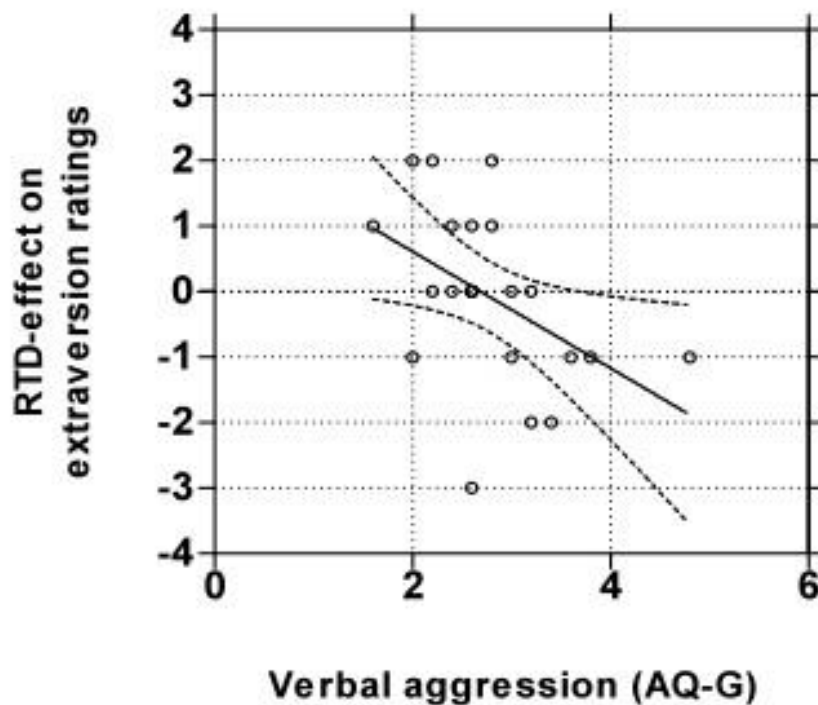


Figure II. Linear correlation/regression with 95% error interval between the baseline verbal aggression score (VA_{AQ-G}) and the RTD-effect on extraversion opponent ratings after a competitive reaction time game (CRT).

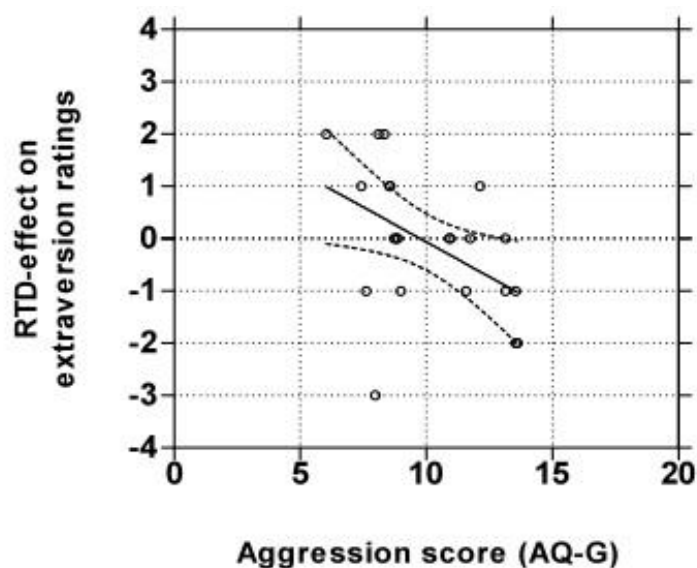


Figure III. Linear correlation/regression with 95% error interval between the baseline overall aggression score (AG_{AQ-G}) and the RTD-effect on extraversion opponent ratings after a competitive reaction time game (CRT)

negatively correlated with the RTD-effect on extraversion ratings (Δ_{EX} ; $r_{xy} = -0.469$, $p = 0.028$). This significant relationship is depicted in Fig. 3.

As the aggression score of the AQ-G is a composite score of different aggressive subscales it was not included in the stepwise linear regression analysis. There were no further significant correlations between the different subscales of the AG-Q and the RTD-effect on any opponent ratings.

Influence of trait-impulsivity

There were no significant relationships detected between the two impulsivity-related IVE subscales (VE_{IVE} and IM_{IVE}) and the RTD-effect on opponent ratings.

The effects of sample characteristics (age, BMI) and study-related influences (order of RTD/placebo administration) were controlled for the significant effects found. We performed bivariate Pearson correlations between AQ-G scales which were found to influence the behavioural results described above, the RTD-effect on extraversion ratings (Δ_{EX}) and age,

BMI and order of RTD/placebo administration. All these correlations remained insignificant.

Discussion

The present results indicate that changes in 5-HT neurotransmission in boys with ADHD influenced their extraversion ratings of a fictitious opponent. These results provide a biological approach to somewhat indirect aspects of aggressive behaviour in terms of extraversion ratings, in particular regarding 5-HT functioning in children and adolescents. Both verbal and physical aggression contributed to these findings, with physical aggression explaining most of the shared intraindividual variance (approx. 26%), which corresponds to findings studying trait-impulsivity (approx. 29% see also [24]).

The fact that impulsive sub-dimensions assessed in the IVE did not influence any RTD-effects on opponent ratings tends to support the hypothesis that the ratings obtained mostly assessed indirect aspects of aggression. In contrast, the CRT was shown to provoke a more impulsive and aggressive response. Moreover, the patients played against their fictitious opponent in a competitive and stressful situation, which may

also have triggered an impulsive response style in the CRT [23, 24]. The fact that in the comparison of the two treatment conditions (RTD vs. placebo) no other dimension of the rating scale showed a significant difference also deserves careful consideration. Particularly the finding that the sub-scale aggression (AG) in the rating scale was not associated with the RTD-effect could be due to expected social desirability of the participants. One could also speculate that the extraversion dimension of the rating questionnaire represents more indirect aspects of experienced opponent aggression.

When considering the role played by TRP (the physiological precursor of 5-HT) in influencing aggressive behavioural properties, recent research has suggested that TRP metabolism may also play an evolutionary part, and that TRP may not only serve as a nutrient but also as a bona fide signalling amino acid to the central nervous 5-HT synthesis rate [52]. The fact that plasma TRP was positively correlated with bodyweight in boys but not in girls [44] may add a gender-related perspective to this hypothesis. This could be of particular relevance in view of the higher prevalence of disruptive behaviour disorders in boys compared to that in girls. This perspective should be considered particularly when interpreting the present findings for children with ADHD from a developmental viewpoint, because lower 5-HT responsivity in childhood was shown to predict the later development of antisocial personality disorder [53]. As “low aggressive” patients are more susceptible to higher negative opponent ratings under RTD and “low impulsive” patients are more vulnerable to reactive aggression [24], one could speculate that a 5-HT modulated vulnerability to behave aggressively may represent a developmental change in central nervous 5-HT regulation in patients with ADHD. However, at this stage this hypothesis is only speculative, but further investigation of this relationship demands a healthy control group to

substantiate this hypothesis further, in particular as regards disorder-related effects.

The present investigation has several limitations. In particular the limited size of the sample must be considered when interpreting the data obtained under depleted and sham depleted conditions. However, the fact that the intake of the RTD and the placebo amino acids was implemented in the study design as a within-subject repeated measures factor is a major advantage over single-blind designs (which would have allowed larger samples more easily), because the individual RTD-effect on reduced TRP availability and subsequently altered central nervous 5-HT neurotransmission was assessed and compared with that of other patients and set in relationship to their baseline aggression and impulsivity ratings.

A major drawback is the fact that the study only comprised a sample of boys. This should be avoided in future studies, above all for the developmental approach reasons previously outlined which suggest that TRP and 5-HT metabolism may have influenced disturbed social behaviour, increased irritability and a lack of impulse control. However, as humans with somatic and inflammatory diseases associated with lowered TRP levels can also show similar behavioural abnormalities, changes in central nervous 5-HT neurotransmission could be regarded as a marker for a neurochemical vulnerability, especially where aggression is concerned. Our study had no healthy control group, raising the question whether the present findings are due to the factor ADHD as a disorder, or whether healthy volunteers might produce similar findings. Future studies should therefore plan the use of a healthy control group, particularly because the used RTD procedure Moja-De was well tolerated. The fact that no plasma concentrations of TRP were obtained under depleted and sham depleted conditions prompts one to note that the RTD procedure

Moja-De used has been proved to produce an at least equal or even stronger reduction in brain 5-HT synthesis when compared to other RTD protocols previously used [44, 54-56]. Moreover, as discussed previously [57], genetic influences such as polymorphisms of the monoamine oxidase A (MAO-A) or the tryptophan-hydroxylase 2 (TPH-2) could provide further evidence on the underlying neurochemical and genetic mechanisms of aggression and impulsivity in children and adolescents. Nevertheless, the limited sample size does not allow any significant conclusions regarding MAO-A and TPH-2 polymorphisms. Thus future studies should aim at acquiring significantly larger samples to investigate the effects of RTD in combination with potential 5-HT-related genetic polymorphisms.

A further limitation of the present investigation is the omission of psychosocial influences on aggression in our assessment, prompting the question whether potential interactions between biological and psychosocial vulnerability influence the development of psychiatric disorders involving aggression. However, a recent study [57] has taken up the role of psychosocial influences on the development of aggressive behaviour and shown that the interaction between both biological (including serotonergic responsivity) and psychosocial risk factors is associated with parent-rated aggression. It could be important to do follow-up studies on the predictive value of an acute 5-HT dysfunction by means of RTD for aggression and later development of psychiatric disorders or specific symptom clusters in adulthood to investigate the role of 5-HT in ADHD further, not only from a disease-specific viewpoint, but also because developmental characteristics are concerned.

Conclusion

In summary, along with existing data the present investigation provides supporting evidence for the hypothesis that alterations in 5-HT

neurotransmission influence aspects of aggression and extraversion in children and adolescents with ADHD both directly and indirectly. Further studies are warranted in order to replicate the present results. Future investigations should include female subjects and healthy controls in their participant groups.

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Declaration of interest

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Conflict of interest/Disclosure: FDZ was the recipient of an unrestricted award donated by the American Psychiatric Association (APA), the American Psychiatric Institute for Research and Education (APIRE), and Astra Zeneca ("Young Minds in Psychiatry Award"). He has also received research support from the Federal Ministry of Economics and Technology (Bundesministerium für Wirtschaft und Technologie, BMWi), the German Society for Social Pediatrics and Adolescent Medicine (Deutsche Gesellschaft für Sozialpädiatrie und Jugendmedizin, DGSPJ), and from the Paul and Ursula Klein Foundation which was unrelated to the present investigation. He was the recipient of a travel-stipend donated by the GlaxoSmithKline Foundation and an unrestricted educational grant donated by Shire pharmaceuticals. MH is a member of advisory boards for Eli Lilly & Co., Novartis, and Bristol-Myers Squibb, and received speaker honoraria from Eli Lilly & Co., AstraZeneca, and Shire. FP is a member of Advisory Boards of Lilly, Janssen Cilag, Astra Zeneca, and Novartis. LW received has research support from the Dannon Institute Nutrition for Health (Institut Danone Ernährung für Gesundheit, e.V.) which is unrelated to the present study. The other authors have nothing to disclose.

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