The effects of dietary omega-3 polyunsaturated fatty acid supplementation on attention and impulsivity in an animal model of attention deficit/hyperactivity disorder (ADHD)

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ABSTRACT

Background: Attention deficit/hyperactivity disorder (ADHD) is one of the commonest psychiatric disorders in children and adolescents. The main symptoms of ADHD are hyperactivity, inattention and impulsivity. Both etiology and neurobiological basis of ADHD are unknown. In this context, long-chain polyunsaturated fatty acids (LC-PUFAs), especially omega-3 (n-3) PUFAs, have become a focus of interest. The symptoms of ADHD have been suggested to be associated with a deficiency of n-3 PUFAs. In addition, the impact of a supply of dietary n-3 PUFAs in the treatment of ADHD has frequently been discussed.

Objective: The aim of the present study was to examine the influence of n-3 PUFA supplementation on attention and impulsivity in the spontaneously hypertensive rat (SHR) which has been proposed to be a valid genetic animal model of ADHD.

Methods: Seven-week-old male SHRs were randomly divided into two groups of 15 rats and fed one of two experimental diets (n-3 PUFA-enriched or n-3 PUFA-deficient) prior to and during behavioral testing. Attention and impulsivity were assessed using a three-choice-serial-reaction-time-task (3CSRTT) which is based on the five-choice-serial-reaction-time-task. The experiment was performed with three-month-old rats.

Results: Our findings demonstrate a marked difference between groups regarding impulsivity but not attention. The n-3 PUFA-enriched diet significantly reduced impulsivity in SHRs compared with rats fed with the n-3 PUFA-deficient diet.

Conclusion: The present data show a decrease in impulsivity following a dietary n-3 PUFA supplementation, but no changes in attention. A possible explanation for these results is that the attention displayed by SHR may not be linked to n-3 PUFA supply. It is important to note that inattention and impulsiveness are two of the main symptoms of ADHD. Our results

regarding dietary n-3 PUFA supply may support the positive findings in human studies demonstrating that n-3 PUFA administration can improve the cognitive or behavioral symptoms in children with ADHD.

Key words: omega-3 polyunsaturated fatty acid, animal model, spontaneously hypertensive rat, attention, impulsivity, attention deficit/hyperactivity disorder

INTRODUCTION:

Attention-deficit/hyperactivity disorder (ADHD) is one of the most frequently diagnosed psychiatric disorders of childhood and adolescence [1, 2]. This mental disorder affects about 3-5 % of all children and adolescents [3]. According to the recently published 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) [4], ADHD has a relatively heterogeneous clinical profile which manifests itself before the twelfth year of life. This disorder is characterized by excessive motor activity, inattention with an increase in distractibility, impulsiveness and disturbances of psychosocial adaptation [5-8]. Dysfunctioning of noradrenergic and dopaminergic neurotransmission appears to play an important role in the pathophysiology for ADHD [9-12]. The etiology and underlying neurobiology are still unknown [13].

Nutrition including the role of long-chain polyunsaturated fatty acids (LC-PUFAs) has become a focus of debate regarding the possible causes of ADHD. LC-PUFAs such as omega-6 (n-6) and omega-3 (n-3) PUFAs play a significant role in the development and functioning of the brain [14]. Deficits and imbalances of these fatty acids can therefore have a significant impact on mental illness [15]. In this context, LC-PUFAs have become a focus of research interest in ADHD [15].

Some studies have shown that blood plasma n-3 PUFA levels are reduced in children and adults with symptoms of ADHD [16, 17]. These deficits are also linked to behavioral problems and learning difficulties [18]. In comparison with a control group, decreased levels of arachidonic acid (AA, C20:4 n-6) and docosahexaenoic acid (DHA, C22:6 n-3) were found in erythrocytes of children and adolescents with ADHD [16, 17, 19, 20]. Further studies could demonstrate an increase in the ratio of n-6/n-3 PUFA (AA/eicosapentaenoic acid (EPA, C20:5 n-3)) in the plasma of children and adults with ADHD [16, 17, 21]. In addition, supplementation studies with LC-PUFA found improvements in behavioral ratings [22-24] which were associated with a decrease in the plasma AA/EPA ratio [24]. Randomized controlled experiments have shown that n-3 PUFA administration can reduce impulsivity and attention problems in children with ADHD symptoms compared with a placebo group [25-28].

Human studies have suggested a link between n-3 PUFAs and ADHD [e.g. 18]. Humans are incapable of synthesizing PUFAs and dietary intake is therefore necessary [29, 30]. However, it is difficult to regulate PUFA ingestion in humans and a possible connection between n-3 PUFAs and ADHD can be more easily assessed in animal studies. It has been shown that long-term deficiency of n-3 PUFAs in rats causes changes in central dopaminergic neurotransmission [31-33]. Dopamine appears to play an important role in the neurobiology of ADHD [34]. In addition, major alterations caused by an n-3 PUFA shortage were observed in the frontal cortex of animals [32]. The underlying mechanisms are not

completely understood. Altered neurotransmission seems to be caused by changes in the concentration of LC-PUFAs in the neuronal membrane [35, 36].

Several studies have shown that n-3 PUFA deficiency in rodents is associated with an increase in activity [37, 38], impairments in spatial learning [39, 40], working memory [41] and olfactory discrimination learning [42] as well as with aggression and depression [43]. These findings suggest that a depletion of n-3 PUFAs has an influence on cognitive processes and behavior. Chalon et al. [44] demonstrated that n-3 PUFA supplementation reduced motor activity in rats. Carrie et al. [45] showed that the intake of n-3 PUFAs leads to a decrease in locomotor activity among older mice. In addition, a marked difference in open field locomotor activity was observed between spontaneously hypertensive rat (SHR) fed with n-3 PUFA-deficient food and SHR on an n-3 PUFA-enriched diet [46]. Furthermore, several studies observed cognitive improvements in n-3 PUFA-depleted rats receiving a supplementation diet at birth and after weaning [47-50]. Dervola et al. [51] have demonstrated that an n-3 PUFA supply in SHR dams decreases the non-reinforcement spontaneous locomotion and enhances the reinforcement-induced attention in the progeny of male SHRs. These investigations suggest that the supply of n-3 PUFAs can lead to a decrease in locomotor activity and may have some impact on cognitive performance in animals.

However, only a few animal studies have investigated LC-PUFAs and behavior with regard to the ADHD main symptoms. A link has been demonstrated between n-3 PUFAs and changes in motor activity in animals [37, 38, 46]. To our knowledge, there is only one study that has assessed attention deficits, impulsivity and hyperactivity aspects [51]. Similarly, the beneficial effect of n-3 PUFA supplementation has not yet been demonstrated. Since ADHD has a genetic basis, more attention should be paid to genetic animal models of ADHD such as the SHR [52, 53], particularly as these rodents show a natural lack of DHA in their neuronal cell membranes [54].

The aim of the present study was to investigate the effect of an n-3 PUFA-enriched diet versus an n-3 PUFA-deficient diet on attention and impulsivity in SHRs using the three-choice-serial-reaction-time-task (3CSRTT).

METHODS:

Animals: Seven-week-old male SHR were obtained from Charles River Laboratories (Sulzbach, Germany). They were kept in standard cages on a 12 h light-dark cycle (light from 7:00 h – 19:00 h). Room temperature was maintained at 22 °C +/- 2 °C with an average humidity of 50 %. Water and food were provided ad libitum. Access to food was restricted during behavioral testing in order to increase the animals' motivation. Body weight was measured daily. A weight reduction of more than 10-15 % was avoided in order to prevent stress [55, 56] and subsequent changes in the dopaminergic system [57].

The rats were randomly divided into two groups of 15 rats and fed one of two experimental diets from the day of their arrival and throughout the testing period (3CSRTT), i.e the diets were provided for a total of 16 weeks (six weeks after arrival and 10 weeks of testing). The two experimental diets, the n-3 PUFA-enriched (n-3 Rich) diet and the n-3 PUFA-deficient (n-3 Def) diet, were prepared by Ssniff, Soest, Germany. Both diets were based on the American Institute of Nutrition–93 G (AIN93G) and meet all the current nutrition standards for rat growth [58]. The fatty acid composition of the diets is shown in Table 1. The diets were stored at −20 °C and provided fresh daily. The experiment was performed with three-month-old rats.

Table 1. Fatty acid composition of the experimental diets (Ssniff Spezialdiäten, Soest, Germany)

	n-3 PUFA-enriched diet	n-3 PUFA-deficient diet
Energy (Atwater), MJ/kg*	17.1	17.1
kJ% protein	18	18
kJ% carbohydrates	60	60
kJ% Fat	22	22
Fatty acids, % of the diet		
C 6:0	0.05	0.05
C 8:0	0.59	0.62
C 10:0	0.47	0.49
C 12:0	3.48	3.64
C 14:0	1.35	1.41
C 16:0	0.84	0.85
C 18:0	0.29	0.28
C 20:0	0.01	0.01
C 18:1	0.82	0.77
C 18:2 n6	**1.54**	**1.58**
C 18:3 n3	**0.27**	**0.01**

* physiological fuel value

Apparatus: The task is based on the five-choice-serial-reaction-time-task [59, 60]. In the present study, a three-choice variant of the paradigm was used (holes no. 3, 5, 7). The remaining unused holes were covered. The experiment was performed using four ventilated wooden chambers (Campden Instruments, Loughborough, Leicestershire, England) which included four stainless steel boxes (26 x 26 cm horizontally and approximately 30 cm high). The task was performed with a house light (3-Watt light bulb), three cue holes (each about 2 cm in diameter) with internal light-emitting diodes (3 Watt) and an illuminated food tray on the wall opposite to the cue holes. The three cue holes were arranged horizontally on one curved rear wall and placed about 2 cm from the floor (stainless steel grid). The distance between adjacent holes was 6 cm. Nose pokes into the cue holes and the food tray were registered by photocells. A correct response, consisting of a nose poke into a lit cue hole was rewarded with a food pellet (45 mg dustless sucrose pellets, Bio-Serv, Frenchtown, New Jersey, USA), which was delivered into the food tray. Premature responses during the intertrial interval (ITI), incorrect responses into an unlit cue hole or not responding after

stimulus presentation (an omission) resulted in a 5-second time-out period in which the house light was turned off. The start of the next trial was signaled automatically by illumination of the house light and food tray, and began with a nose poke of the rats into the food tray.

Procedure: The 3CSRTT comprised three phases – habituation (2 days), training (8 weeks) and test (5 days) (see Instruction Manual for MKII Rat 5CSRT [61]). After completion of the training, the stimulus duration was fixed at 2 s regardless of the performance. A session ended after either 30 minutes or 60 rewards, whichever occurred first. The ITIs were varied randomly between 1.5 s, 2.5 s, 3.5 s, 4.5 s (short ITI) in order to assess attention and between 5.5 s, 6.5 s, 7.5s, 8.5 s (long ITI) to quantify impulsivity.

Statistics: The following parameters concerning impulsive behavior were analyzed: rate of premature responses (ratio of number of premature responses/total number of trials completed), number of premature responses (nose poke responses during ITI), number of time-out responses (nose poke responses during time-out period). The following parameters concerning activity of responses were examined: total number of trials completed and total correct and incorrect responses. In addition, the following parameters regarding attention were investigated: number of correct responses, number of commission errors (false reaction) and number of omission errors (missed reactions). Statistical analyses were performed using the nonparametric Mann-Whitney U-Test (between subjects design), an alpha level of 0.05 was applied. A mean value for each animal was calculated in order to compare the differences between the n-3 fatty acid groups. The analyses were carried out using the statistical package PASW 18.0 for Windows.

Ethics: The present experiment was carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23, revised 1985).

RESULTS:

Body weight: The two experimental diets, i.e. n-3 Rich and n-3 Def, had no significant effect on the body weight of the rats (data not shown).

3CSRTT: In regard to the comparison between the n-3 Rich and the n-3 Def groups, statistically significant differences were found in the "rate of premature responses". The ratio of the number of premature responses to the total number of trials completed is significantly higher in the n-3 Def group than in the n-3 Rich rats. There are no significant differences in the nose poke responses during ITI and the number of time-out responses. However, the n-3 Def rats showed more premature responses than those in the n-3 Rich group. Furthermore, the n-3 Rich group showed fewer nose poke responses during the time-out period than the n-3 Def animals. All values regarding the rate of premature responses, the number of premature responses and the number of time-out responses of the n-3 Rich and the n-3 Def groups are given in Table 2.

Table 2. Effects of n-3 PUFA deficiency and supplementation on impulsivity in the 3CSRTT test (long ITI) in SHR

	Rate of premature responses	**Number of premature responses**	**Number of time-out responses**
n-3 Rich group	0.88 ± 0.34 [*]	13.65 ± 1.39	10.07 ± 1.31
n-3 Def group	1.23 ± 0.20	16.73 ± 1.87	14.57 ± 1.79
p-value	*0.007*	*0.245*	*0.085*
z-value	-2.717	-1.162	-1.723

n-3 Def group = n-3 PUFA-deficient group; n-3 Rich group = n-3 PUFA-enriched group;
[*]$p \leq .05$ compared to n-3 Def group; data are means ± standard errors for 15 rats per group

The total number of trials completed is significantly higher in the n-3 Rich group than in the n-3 Def rats. Similarly, the total correct and incorrect responses were significantly higher in the n-3 Rich group than in the n-3 Def group. The total number of trials completed and the total correct and incorrect responses are presented in Table 3.

Table 3. Effects of n-3 PUFA deficiency and supplementation on the activity of responses in the 3CSRTT test (long ITI) in SHR

	Total number of trials completed	**Total correct and incorrect responses**
n-3 Rich group	25.72 ± 2.05 [*]	22.33 ± 1.89 [*]
n-3 Def group	19.36 ± 2.16	16.67 ± 2
p-value	*0.021*	*0.034*
z-value	*2.302*	*2.116*

n-3 Def group = n-3 PUFA-deficient group; n-3 Rich group = n-3 PUFA-enriched group;
[*]$p \leq .05$ compared to n-3 Def group; data are means ± standard errors for 15 rats per group

There were no statistically significant differences concerning the number of correct responses or the number of commission and omission errors between groups. However, the n-3 Rich rats made more correct responses than the n-3 Def group in the test. Furthermore, the n-3

Rich group made fewer commission errors and more omission errors than the n-3 Def animals. The number of correct responses, commission and omission errors of the n-3 Rich and the n-3 Def groups are given in Table 4.

Table 4. Effects of n-3 PUFA enrichment and deficiency on attention in the 3CSRTT test (short ITI) in SHR

	Number of correct responses	**Number of commission errors**	**Number of omission errors**
n-3 Rich group	23.32 ± 1.28	2.79 ± 0.29	7.68 ± 0.80
n-3 Def group	22.61 ± 1.44	3.17 ± 0.34	6.88 ± 0.77
p-value	0.852	0.440	0.262
z-value	0.187	-0.771	1.121

n-3 Def group = n-3 PUFA-deficient group; n-3 Rich group = n-3 PUFA-enriched group; data are means ± standard errors for 15 rats per group

DISCUSSION:

Up until now the effects of n-3 PUFA supplementation on impulsive behavior and attention in rats have not sufficiently been investigated. The aim of the present study was therefore to elucidate the role of n-3 PUFAs by assessing impulsivity and attention in the SHR model with the 3CSRTT.

In the testing phase, there were no significant differences in body weight between the two n-3 groups. The differences in behavior between the n-3 groups are therefore not confounded by body weight. The present study attempted to investigate the effects of n-3 PUFA supplementation on impulsive behavior in rats as measured with the 3CSRTT. In comparison with the n-3 Def rats, the n-3 Rich diet significantly decreased the ratio of number of premature responses to total number of trials completed. This data suggests that an n-3 PUFA diet can improve impulsive behavior in SHR. Similar findings were reported by a study that investigated the effect of n-3 PUFA supplementation on impulsivity in SHR [51].

However, the choice-serial-reaction-time-task (CSRTT) assesses more parameters regarding impulsive behavior, i.e. the number of premature responses and the number of time-out responses. In regard to these factors no significant differences between n-3 groups were observed, although both parameters may show some improvements concerning impulsive behavior. The n-3 Def rats made more premature responses and more nose poke responses during the time-out period than the n-3 Rich group. This may indicate that impulsive behavior was reduced by dietary n-3 PUFA supplementation. This profile of results is consistent with previous animal studies demonstrating a recovery from PUFA deficiency by a supplementation diet [47-50]. However, as neither the number of premature responses

nor the rate of time-out responses showed significant effects in the present study, our results call for further investigations.

Possible effects of dietary n-3 PUFAs on impulsive behavior in rats were insufficiently examined in the past. Previous studies investigating the effects of impulsivity on behavior used premature responses as sole parameter [62, 63]. In our study, we suppose that it may be important to distinguish between impulsive premature responses and the generally increased response activity [64]. The CSRTT provides no valid parameters of the response activity. However, two parameters might indicate a general response activity, i.e. the total number of trials completed and the total correct and incorrect responses. The total number of trials completed is significantly higher in the n-3 Rich group than in the n-3 Def rats. In addition, the total correct and incorrect responses were significantly higher in the n-3 Rich group than in the n-3 Def group. Both parameters might show a significantly enhanced response activity of the n-3 Rich rats. One could say that rats fed with an n-3 PUFA-enriched diet show more rather than fewer answers, which may be associated with an enhanced motivation (being less "off-task").

Although the n-3 Rich rats were more active, as demonstrated by the total number of trials completed and the total correct and incorrect responses, they showed a significantly reduced premature behavior, as indicated by the "rate of premature responses". Moreover, the most commonly discussed parameters, i.e. the number of premature responses and the number of time-out responses, showed an effect in the same direction, although these comparisons failed to be statistically significant. These findings indicate that the rats' impulsive behavior cannot be seen as a simple result of an increase in activity [65]. Taken together, one may speculate that an n-3 PUFA supplementation cannot only reduce premature responses but can probably also increase the motivational state in the 3CSRTT. It would therefore be of interest to assess motivation and discuss it separately from impulsivity in future studies.

In regard to the effect of n-3 PUFA supplementation on the cognitive performance in the 3CSRTT, the present results indicate no significant improvements in attention in the SHR model. The attention parameters such as the number of correct responses and commission errors did not significantly differ between the n-3 groups.

The exact timing of the n-3 PUFA supply may be seen as a factor responsible for the low level of responses concerning attention in the SHR following the n-3 PUFA-enriched diet. In our study, the n-3 PUFA supplementation began post-weaning. A certain amount of n-3 PUFAs is accumulated in the nervous system of adult mammals and an increase occurs in the prenatal and early postnatal period, namely during brain growth [66-68]. This leads us to assume that an enrichment of n-3 PUFAs in the SHR has more important effects prenatally than in the post-weaning period. However, the aim of the present study was rather to investigate the effect of supplementation in terms of treatment. Further studies using the SHR model, especially in the prenatal phase, appear to be worthwhile.

In summary, one could conclude that the n-3 PUFA-enriched diet reduced the rats' impulsivity. However, dietary n-3 PUFA enrichment was compared with n-3 PUFA deficiency in this study. The question of whether or not an n-3 PUFA-deficient diet has some side-effects which can also influence the behavior of n-3 Def animals remains unanswered. It needs therefore to be investigated whether this effect can also be found when a balanced diet is used as control.

CONCLUSION:

Taken together, the present findings show that the n-3 PUFA-enriched diet administered post -weaning had no significant effects on attention in SHRs as assessed in the 3CSRTT. In contrast, the data suggest a positive influence of the n-3 PUFA-enriched diet on impulsive behavior. Impulsiveness is one of main symptoms of ADHD. The present results may support the positive findings in previous human supplementation studies which demonstrated that the administration of n-3 PUFAs might improve the cognitive or behavioral symptoms in children with ADHD and may have some therapeutical effects [23, 24, 69-71].

Abbreviations: arachidonic acid (AA), attention deficit/hyperactivity disorder (ADHD), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), long-chain polyunsaturated fatty acid (LC-PUFA), omega-3 polyunsaturated fatty acid (n-3 PUFA), omega-6 polyunsaturated fatty acid (n-6 PUFA), spontaneously hypertensive rat (SHR), three-choice-serial-reaction-time-task (3CSRTT)

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Authors' Contributions: All authors contributed to this study.

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