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SHORT COMMUNICATION

The effects of nutritional polyunsaturated fatty acids on locomotor activity in spontaneously hypertensive rats

Joachim Hauser · Ewelina Makulska-Gertruda · Andreas Reissmann · Thomas-A. Sontag · Oliver Tucha · Klaus W. Lange

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Abstract The present study investigated the effects of nutritional omega-3 polyunsaturated fatty acids on locomotor activity in spontaneously hypertensive rats (SHRs), which are used as an animal model of attention-deficit/hyperactivity disorder (ADHD). For 6 weeks, two groups of randomly assigned SHRs received food either enriched with or deficient in omega-3 fatty acids (based on the American Institute of Nutrition—93 G/AIN93G). Using an open field, locomotor activity was subsequently assessed for 6 days. A marked difference in locomotor activity as assessed by the distance travelled in the open field was found between the two groups of rats. In comparison with rats fed with omega-3 fatty acid-enriched food, the animals on the omega-3 fatty acid-deficient diet showed a significantly higher locomotor activity. The present findings demonstrated that nutritional enrichment with omega-3 fatty acids was associated with reduced motor activity in an established animal model of ADHD and support the notion that omega-3 polyunsaturated fatty acids may play a role in the pathophysiology of ADHD.

Keywords Spontaneously hypertensive rat · SHR · Animal model · Nutrition · Polyunsaturated fatty acid · PUFA · Omega-3 fatty acid · Attention-deficit/hyperactivity disorder · ADHD

J. Hauser · E. Makulska-Gertruda · A. Reissmann · T.-A. Sontag · K. W. Lange (✉)
Department of Experimental Psychology, University of Regensburg, 93040 Regensburg, Germany
e-mail: Klaus.Lange@ur.de

O. Tucha
Department Clinical and Developmental Neuropsychology, University of Groningen, Groningen, The Netherlands

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common psychiatric disorders in childhood and adolescence and is characterized by attention problems, impulsivity and hyperactivity. Approximately 3–5 % of school children and adolescents are affected by ADHD, the prevalence is higher in boys than in girls (Barkley 2006; Clements et al. 2003; Lange et al. 2007, 2010; Paule et al. 2000). Environmental, social, genetic and neurobiological factors appear to be relevant in the aetiology of ADHD (Barkley 2006; Biederman and Faraone 2005; Vancassel et al. 2007; Wankerl et al. 2014).

Polyunsaturated fatty acids (PUFAs) are known to play an important role in neuronal development and functioning of the central nervous system (Schuchardt et al. 2010; Wainwright 2002). Long-chain PUFAs such as eicosapentaenoic acid (EPA, C20:5ω-3), docosahexaenoic acid (DHA, C22:6ω-3) and arachidonic acid (AA, C20:4ω-6) have an influence on numerous neuronal processes, such as membrane fluidity and regulatory processes of gene expression (Schuchardt et al. 2010; Wainwright and Huang 2002; Wainwright 2002). Studies in humans indicate that a deficiency of n-3 fatty acids leads to an imbalance of the n-3/n-6 PUFA ratio, affects neurocognitive abilities and is associated with developmental disorders such as ADHD (Schuchardt et al. 2010; Wainwright and Huang 2002; Wainwright 2002; Stevens et al. 2003). In this context, a possibly impaired metabolism of PUFAs has been discussed as a potential risk factor for the development of ADHD (Schuchardt et al. 2010; Wainwright and Huang 2002). The findings of studies addressing the possible therapeutic effects of dietary PUFAs in children and adolescents with ADHD are not conclusive (Hirayama et al. 2004; Voigt et al. 2001; Sorgi et al. 2007; Schuchardt et al. 2010).

Besides neurotoxic animal models of ADHD (e.g. Hauser et al. 2012; Sontag et al. 2008, 2011), the spontaneously hypertensive rat (SHR) is a widely accepted model (Sagvolden 2000; Sontag et al. 2010) with characteristic behavioural symptoms (Wainwright and Huang 2002) and a decrease in dopaminergic function in the prefrontal cortex (Russell et al. 1995). In addition, SHRs have been found to have lower brain levels of omega-3 (n-3) polyunsaturated fatty acids (PUFAs) than their commonly used control, the Wistar-Kyoto (WKY) rat (Mills and Huang 1992; Wainwright and Huang 2002). This finding might suggest a role of n-3 PUFAs in ADHD.

One of the core symptoms of ADHD is hyperactivity. Locomotor activity in adult rats has been shown to be affected by dietary n-3 PUFA content, with rats on an n-3-deficient diet showing significantly increased activity in an open field compared with controls (e.g. Umezawa et al. 1995; Levant et al. 2004; Lange et al. 2013). While most authors have reported the activity in the entire open-field arena, Kelley (1993) suggested to divide the test field into a central and peripheral area because this approach may provide more detailed information regarding locomotor, exploratory and anxiety-like behaviour. For example, without a modification of total locomotion, an increase in locomotor activity in the centre can be interpreted as a reduction in anxiety, while a decrease indicates higher anxiety (Prut and Belzung 2003).

The aim of the present study was to investigate in SHRs the effects of an n-3 PUFA-enriched and an n-3-deficient diet on locomotor activity as assessed by analysing the distance travelled in both centre and periphery of an open field.

Materials and methods

Animals

In the present study, 30 seven-week-old male SHRs were used (Charles River, Sulzbach, Germany). The rats were housed in groups of five animals per cage and kept on a 12:12-h light–dark cycle (room temperature 22 °C; humidity 50 %). They were given free access to water and the experimental diet, except during the behavioural testing period. At the beginning of the behavioural experiments, water was provided ad libitum, but food was restricted in order to prepare the animals for the experiments and to increase their motivation.

The rats were randomly assigned to two diet groups, i.e. diet 1 ($n = 15$) enriched with n-3 PUFAs (Ssniff, Soest, Germany; based on the American Institute of Nutrition—93 G (AIN93G), for details see Table 1) or diet 2 ($n = 15$) deficient in n-3 PUFAs (see Table 1). The experimental diets were provided for 6 weeks and during the subsequent behavioural test phase.

Table 1 Fatty acid composition of the experimental diets based on the AIN93G composition (ssniff Spezialdiäten GmbH)

Product No.	n-3-enriched diet *customized*	n-3-deficient diet *customized*
Energy (Atwater) (MJ/kg)[a]	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

[a] Physiological fuel value

Apparatus

The locomotor activity of rats was measured in an open field. The open field used consisted of a rectangular arena (size 82.5 × 82.5 cm, with walls 40 cm high), made of dark grey synthetic material. The locomotor activity was recorded by a fixed digital video system. These data were digitized and analysed using the video tracking system ETHOVISION 3.0 (Noldus, Wageningen, the Netherlands). The distance travelled (in centimetres) in the square centre (side length 42.5 cm, distance to side walls 20 cm) and the periphery (remaining area surrounding the centre) of the open field was recorded over 15 min.

Procedure

Rats were tested once daily for six consecutive days. Behavioural testing was performed during the light period between 9 a.m. and 4 p.m. Each rat was individually placed in the centre of the open field and allowed to explore freely for 15 min. Prior to each trial, all open-field surfaces were cleaned with 70 % alcohol in order to remove odours and/or residues of rats tested previously. The rats were tested each day in a random order. The body weight of animals was measured daily in order to ensure normal weight gain and to prevent a weight loss of more than 10 %, which was never

the case. At the time of behavioural testing, there were no significant differences in body weight between the two experimental groups (n-3-deficient group: mean ± SE 319.3 ± 3.9; n-3-enriched group: mean ± SE 323.5 ± 4.0; $p = 0.494$; $Z = -0.68$).

Statistics

Statistical analysis of differences between the two groups (n-3 PUFA-enriched and n-3 PUFA-deficient) was performed with Mann–Whitney *U* test using the Statistical Package for Social Sciences 17.0 (SPSS) for Windows. An alpha level of .05 was applied.

Ethics

This study was performed in accordance with national laws (German Law on Protection of Animals) and European laws, and the principles of laboratory animal care (NIH Publication No. 86-23, revised 1985).

Results

The distance travelled of the n-3-deficient and n-3-enriched groups in the entire arena, centre and periphery of the open field on the 6 days of testing are presented in Table 2.

The distance travelled of the n-3-enriched SHRs in the entire arena within 15 min was significantly lower on days 2 and 6 and over all days than in the n-3-deficient controls. In the centre of the open field, statistically significant differences between the groups were found on days 1, 2, 4, 6 and 1–6. Here, the n-3-enriched group showed lower activity. With regard to the periphery, the rats fed with an n-3-enriched diet showed a significantly reduced activity on day 2 only.

Discussion

In the present study, the effects of a diet rich or impoverished in n-3 fatty acids on locomotor activity in an open field were investigated. In common with previous studies (Levant et al. 2004; Lange et al. 2013), we used enriched

Table 2 Distance travelled (in centimetres) in the open field (arena, centre and periphery) of the n-3 PUFA-deficient and n-3 PUFA-enriched SHR groups (means ± SE)

	n-3-deficient group	n-3-enriched group	*p* value	*Z* value
Arena				
Day 1	5,784.00 ± 141.05	5,482.68 ± 136.19	0.110	−1.60
Day 2	6,179.43 ± 182.96	5,215.85 ± 199.07*	0.002	−3.09
Day 3	5,200.39 ± 171.29	5,285.70 ± 224.87	0.983	−0.20
Day 4	5,726.02 ± 121.63	5,343.18 ± 229.80	0.093	−1.68
Day 5	5,316.70 ± 239.75	4,779.20 ± 184.09	0.085	−1.72
Day 6	5,531.15 ± 253.68	4,640.99 ± 161.75*	0.017	−2.39
Days 1–6 (mean)	5,622.95 ± 123.88	5,124.60 ± 121.04*	0.017	−2.39
Centre				
Day 1	1,416.33 ± 61.91	1,185.24 ± 63.49*	0.021	−2.30
Day 2	1,149.03 ± 89.73	859.75 ± 76.83*	0.036	−2.09
Day 3	961.10 ± 102.93	736.69 ± 78.64	0.130	−1.51
Day 4	1,105.98 ± 96.56	668.63 ± 87.15*	0.005	−2.80
Day 5	930.45 ± 107.08	625.01 ± 66.40	0.054	−1.93
Day 6	922.78 ± 119.56	482.23 ± 53.17*	0.011	−2.55
Days 1–6 (mean)	1,080.95 ± 72.56	759.59 ± 40.45*	0.002	−3.13
Periphery				
Day 1	4,367.68 ± 143.67	4,297.43 ± 123.16	0.660	−0.44
Day 2	5,030.33 ± 156.61	4,356.10 ± 146.36*	0.007	−2.72
Day 3	4,239.29 ± 165.27	4,549.01 ± 168.90	0.206	−1.27
Day 4	4,620.03 ± 142.72	4,674.55 ± 157.72	0.101	−1.64
Day 5	4,386.25 ± 176.06	4,154.19 ± 177.46	0.272	−1.10
Day 6	4,608.36 ± 208.62	4,158.77 ± 151.02	0.178	−1.35
Days 1–6 (mean)	4,541.99 ± 106.13	4,365.01 ± 98.04	0.272	−1.10

* $p \leq 0.05$ compared to n-3-deficient group, Mann–Whitney *U* test

and deficient food compositions on the basis of AIN93G (for details, see Table 1). We used SHRs in order to elucidate the possible role of nutritional n-3 PUFAs in locomotor activity because this rat strain is an established animal model of ADHD (Sagvolden 2000; Sontag et al. 2010). Over the 6 days of behavioural testing, the present results show a significant difference in locomotor activity between the two groups of SHRs when the entire arena or the centre of the open field is included in the statistical analysis. Compared with SHRs on an n-3 PUFA-enriched diet, the rats on the deficient diet showed higher motor activity. No significant difference for the total of 6 days was found in the periphery of the open field.

In general, the significant reduction in locomotor activity following an n-3 PUFA-enriched diet in our study could be interpreted as an improvement of hyperactivity. However, dietary n-3 enrichment was compared with n-3 deficiency, and it needs to be investigated whether this effect can also be found when a balanced diet is used as control.

It is of particular interest that the difference in locomotor activity between the two groups was seen in the centre but not in the periphery of the open field. Rats spontaneously prefer the periphery of an open field to activity in the centre (Prut and Belzung 2003). One would expect alterations in pure motor activity to be similar in all parts of an open field or preferably to be seen in the periphery. In a broader sense, psychomotor activity relates to movement or muscular activity associated with mental processes, especially affects. Psychomotor activity may be influenced by—among others—motivation, novelty seeking and anxiety. For example, an increase in time spent in the centre part of an open field has been shown to indicate anxiolysis (Prut and Belzung 2003). Novelty seeking in rats defined as enhanced specific exploration of novel situations can be assessed by their response to novel environments such as an open field. When given the choice between novel and familiar environments, animals frequently choose to spend more time in novel environment (Hughes 1968). An increase in the amount of time spent in the centre of the open field might, for example, indicate pronounced novelty seeking.

In the present study, behavioural differences between the two diet groups may therefore be caused by alterations in motor activity per se, novelty seeking, anxiety or other affective states, i.e. the rats travelling longer distances in the centre of the open field might be novelty seekers or less anxious. The underlying cause of hyperactivity in animal models such as the SHR might be different from that in patients with ADHD. When considering these aspects, it may be difficult to assess the face validity (i.e. phenomenological similarity between animal model and human disorder, see Sontag et al. 2010) of animal models with regard to hyperactivity.

In conclusion, the present results showed a markedly lower activity following an n-3 PUFA-enriched diet in SHRs than with an n-3-deficient diet. Nutritional n-3 PUFAs appear to affect locomotor activity in SHR. These findings call for further investigation into the role of n-3 fatty acids in other behavioural aspects.

References

Barkley RA (2006) Attention-deficit hyperactive disorder: a handbook for diagnosis and treatment. Guilford, London

Biederman J, Faraone SV (2005) Attention-deficit/hyperactivity disorder. Lancet 366(9481):237–248

Clements KM, Girard TA, Xing HC, Wainwright PE (2003) Spontaneously hypertensive and Wistar Kyoto rats differ in delayed matching-to-place performance and response to dietary long-chain polyunsaturated fatty acids. Dev Psychobiol 43:57–69

Hauser J, Sontag TA, Tucha O, Lange KW (2012) The effects of the neurotoxin DSP4 on spatial learning and memory in Wistar rats. Atten Defic Hyperact Disord 4:93–99

Hirayama S, Hamazaki T, Terasawa K (2004) Effect of docosahexaenoic acid-containing food administration on symptoms of attention-deficit/hyperactivity disorder—a placebo-controlled double-blind study. Eur J Clin Nutr 58(3):467–473

Hughes RN (1968) Behaviour of male and female rats with free choice of two environments differing in novelty. Anim Behav 16:92–96

Kelley AE (1993) Locomotor activity and exploration. In: Sahgal A (ed) Behavioural neuroscience: a practical approach, vol II. Oxford University Press, New York, pp 1–19

Lange KW, Tucha L, Walitza S, Gerlach M, Linder M, Tucha O (2007) Interaction of attention and graphomotor functions in children with attention deficit hyperactivity disorder. J Neural Transm Suppl 72:249–259

Lange KW, Reichl S, Lange KM, Tucha L, Tucha O (2010) The history of attention deficit hyperactivity disorder. Atten Defic Hyperact Disord 2:241–255

Lange KW, Makulska-Gertruda E, Reisinger J, Sontag TA, Hauser J (2013) Dietary omega-3 fatty acids and locomotor activity in an animal model of attention deficit hyperactivity disorder (ADHD). Func Foods Health Dis 3:223–229

Levant B, Radel JD, Carlson SE (2004) Decreased brain docosahexaenoic acid during development alters dopamine-related behaviors in adult rats that are differentially affected by dietary remediation. Behav Brain Res 152:49–57

Mills DE, Huang YS (1992) Metabolism of n-6 and n-3 polyunsaturated fatty acids in normotensive and hypertensive rats. In: Sinclair A, Gibson R (eds) Essential fatty acids and eicosanoids. AOCS, Champaign, IL, pp 345–348

Paule M, Rowland A, Ferguson S, Chelonis J, Tannock R, Swanson J, Castellanos F (2000) Attention deficit/hyperactivity disorder: characteristics, interventions, and models. Neurotoxicol Teratol 22:631–651

Prut L, Belzung C (2003) The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol 463:3–33

Russell V, de Villiers A, Sagvolden T, Lamm M, Taljaard J (1995) Altered dopaminergic function in the prefrontal cortex, nucleus accumbens and caudate-putamen of an animal model of attention-deficit hyperactivity disorder—the spontaneously hypertensive rat. Brain Res 676:343–351

Sagvolden T (2000) Behavioral validation of the spontaneously hypertensive rat (SHR) as an animal model of attention-deficit/hyperactivity disorder (AD/HD). Neurosci Biobehav Rev 24:31–39

Schuchardt JP, Huss M, Stauss-Grabo M, Hahn A (2010) Significance of long-chain polyunsaturated fatty acids (PUFAs) for the development and behaviour of children. Eur J Pediatr 169:149–164

Sontag TA, Hauser J, Kaunzinger I, Gerlach M, Tucha O, Lange KW (2008) Effects of the noradrenergic neurotoxin DSP4 on spatial memory in the rat. J Neural Transm 115:299–303

Sontag TA, Tucha O, Walitza S, Lange KW (2010) Animal models of attention deficit/hyperactivity disorder (ADHD): a critical review. Atten Defic Hyperact Disord 2(1):1–20

Sontag TA, Hauser J, Tucha O, Lange KW (2011) Effects of DSP4 and methylphenidate on spatial memory performance in rats. Atten Defic Hyperact Disord 3:351–358

Sorgi PJ, Hallowell EM, Hutchins HL, Sears B (2007) Effects of an open-label pilot study with high-dose EPA/DHA concentrates on plasma phospholipids and behavior in children with attention deficit hyperactivity disorder. Nutr J 6:16

Stevens LJ, Zhang W, Peck L, Kuczek T, Grevstad N, Mahon A, Zentall SS, Arnold LE, Burgess JR (2003) EFA supplementation in children with inattention, hyperactivity, and other disruptive behaviors. Lipids 38:1007–1021

Umezawa M, Ohta A, Tojo H, Yagi H, Hosokawa M, Takeda T (1995) Dietary alpha-linolenate/linoleate balance influences learning and memory in the senescence-accelerated mouse (SAM). Brain Res 669:225–233

Vancassel S, Blondeau C, Lallemand S, Chador M, Linard A, Lavialle M, Dellu-Hagedorn F (2007) Hyperactivity in the rat is associated with spontaneous low level of n-3 polyunsaturated fatty acids in the frontal cortex. Behav Brain Res 180:119–126

Voigt RG, Lorente AM, Jensen CL, Fraley JK, Berretta MC, Heird WC (2001) A randomized, double-blind, placebo-controlled trial of docosahexaenoic acid supplementation in children with attention-deficit/hyperactivity disorder. J Pediatr 139(2):189–196

Wainwright PE (2002) Dietary essential fatty acids and brain function: a developmental perspective on mechanisms. Proc Nutr Soc 61:61–69

Wainwright PE, Huang YS (2002) Polyunsaturated fatty acids and brain function: what are the questions and do we have answers? In: Huang YS, Lin SJ, Huang PC (eds) Essential fatty acids and eicosanoids—invited papers from the fifth international congress. AOCS, Champaign, IL, pp 115–122

Wankerl B, Hauser J, Makulska-Gertruda E, Reißmann A, Sontag TA, Tucha O, Lange KW (2014) Neurobiologische Grundlagen der Aufmerksamkeitsdefizit-/Hyperaktivitätsstörung. Fortschr Neurol Psychiatr (in press)