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Sex-specific effects of brain LC-PUFA composition on locomotor activity in rats

Beth Levant [a,d,*], Marlies K. Ozias [a], Susan E. Carlson [b,c,d]

[a] Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS 66160, USA
[b] Department of Dietetics and Nutrition, University of Kansas Medical Center, Kansas City, KS 66160, USA
[c] Department of Pediatrics, University of Kansas Medical Center, Kansas City, KS 66160, USA
[d] The Smith Mental Retardation Research Center, University of Kansas Medical Center, Kansas City, KS 66160, USA

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Abstract

Insufficient availability of $n-3$ polyunsaturated fatty acids (PUFAs) during pre- and neonatal development decreases accretion of docosahexaenoic acid (DHA, 22:6$n-3$) in the developing brain and is associated with sub-optimal sensory and cognitive function in humans, altered behavior in animals, and may contribute to neurodevelopmental disorders such as attention deficit hyperactivity disorder and schizophrenia. This study examined the effects of variation in dietary availability of $n-3$ PUFAs on brain fatty acid composition and the consequent effects on locomotor activity in male and female Long–Evans rats. Rats were raised from conception using purified diets and breeding protocols designed to produce four groups with distinct brain phospholipid compositions varying in DHA content and/or the proportion of $n-3$ and $n-6$ PUFAs. Locomotor behavior was measured for a 2-h period on postnatal days 28, 42, 56, and 70. In males, decreased brain DHA produced alterations in activity that were most pronounced post-adolescence and with the greatest decrease in DHA. However, the behavioral effects in males were not linearly related to brain DHA level. In contrast, no significant effects of variation in brain fatty acid composition were observed in females. This suggests that variation in brain DHA content produces sex-specific alterations in locomotor activity and that the neurochemical alterations underlying the observed behavioral changes vary depending on the degree of DHA depletion.

Keywords: Polyunsaturated fatty acid; Docosahexaenoic acid; Docosapentaenoic acid; Arachidonic acid; Rat; Brain; Locomotor activity; Sex; Gender; Development; Phospholipid

1. Introduction

Long-chain polyunsaturated fatty acids (LC-PUFAs), components of the phospholipids that make up cell membranes, represent a significant proportion of the lipids in brain. In neurons, variation in the LC-PUFA composition of synaptic membranes alters the microenvironment surrounding membrane-bound proteins, and thus affects the structure and function of receptors, transporters, and ion channels [1]. LC-PUFAs are also essential for normal cellular physiology since they are precursors for inter- and intracellular signaling molecules such as prostaglandins and thromboxanes. In addition, they modulate gene expression at the transcriptional level, most likely through the activation of transcription factors [2]. Docosahexaenoic acid (DHA, 22:6$n-3$), the predominant species of $n-3$ LC-PUFA, represents about 15% of total brain fatty acids [3]. Other biologically important LC-PUFAs include members of the $n-6$ family such as arachidonic acid (20:4$n-6$), which represents roughly 10% of the total fatty acids in brain, and docosapentaenoic (DPA, 22:5$n-6$), which normally represents 1–2% of brain fatty acids.

LC-PUFAs rapidly accumulate in brain during pre- and neonatal life and are important for CNS development and function [4]. In humans, decreased availability of DHA during development is associated with deficits in visual, attentional, and cognitive function (for review see: [5–7]). Animals with low brain levels of DHA during development exhibit abnormal behavior and cognitive function [7–12]. Alterations in the mesocortical and mesolimbic

* Corresponding author. Department of Pharmacology, University of Kansas Medical Center, Mail Stop 1018, 3901 Rainbow Blvd., Kansas City, KS 66160, USA. Tel.: +1 913 588 7527, fax: +1 913 588 7501.
E-mail address: blevant@kumc.edu (B. Levant).

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dopamine systems, similar to the hypofrontality associated with schizophrenia and attention deficit hyperactivity disorder (ADHD) [13–16] and the hyperactivity of the mesolimbic system proposed in schizophrenia [17,18], are also reported [19]. Furthermore, clinical and epidemiologic studies implicate altered LC-PUFA levels in several neuropsychiatric disorders including ADHD and schizophrenia [20–22].

To further extend our understanding of the role of LC-PUFAs in brain development and function, rats were raised from conception using diet and breeding protocols designed to produce four groups with distinct brain phospholipid compositions varying in DHA content and/or the proportion of $n-3$ and $n-6$ LC-PUFAs. The effects of this variation in brain fatty acids on locomotor activity were assessed across development and compared in male and females. We will show that although males and females have similar brain LC-PUFA compositions after these treatments, only males with decreased brain DHA levels exhibit altered locomotor behavior.

2. Methods

2.1. Animals and diets

All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals [23] and were approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee.

Long–Evans rats used for breeding (females >10 weeks old, male proven breeders; Harlan, Indianapolis, IN) were obtained at least 5 days prior to initiation of any treatments. Rats were housed in a temperature- and humidity-controlled animal facility with a 12-h dark–light cycle (on at 0600), food and water ad libitum, and were handled regularly.

The Control group was raised from conception on a purified diet prepared by adding pure soybean oil without partial hydrogenation (7% by weight) as a source of α-linolenic acid to a baseline diet (Harlan Teklad Basal Diet TD00235). The control diet was identical to AIN-93G and meets all current nutrient standards for rat pregnancy and growth [24]. The High $n-3$ group was raised on diet formulated with soybean oil and DHA (DHASCO™, a triacylgycerol oil produced by algae containing 42.57% DHA by weight; Martek Biosciences Corp, Columbia, MD), substituted on an equal weight basis for soybean oil, such that DHA accounted for 0.7% of total fat in the diet. The Medium Low $n-3$ and Low $n-3$ groups were the first and second generations, respectively, raised on a diet formulated with sunflower oil (7% by weight), which contains negligible α-linolenic acid compared to soybean oil. The formulation of the diets is presented in Table 1; fatty acid composition, in Table 2.

Table 1
Composition of diets

Diet composition (g per kg diet)			
	Control	High n–3	Med. Low/Low n-3
Basal mix formula[a]			
Casein	200	200	200
L-cystine	3.0	3.0	3.0
Corn starch	398	398	398
Maltodextrin	132	132	132
Sucrose	100	100	100
Cellulose	50	50	50
Mineral mix[b]	35	35	35
Vitamin mix[c]	10	10	10
Choline bitartrate	2.5	2.5	2.5
TBHQ	0.014	0.014	0.014
Soybean oil	70	68.15	–
Sunflower oil	–	–	70
DHASCO™[d]	–	1.85	–

[a]Harlan Teklad TD00235. The individual basal mix components in g/kg of diet are presented as supplied by the manufacturer. The basal mix is modified from AIN-93G (TD 94045) for use by adding 70 g/kg of the selected oil(s) to the basal mix (930 g/kg). [b]Harlan Teklad TD94046 (AIN-93G MX). [c]Harlan Teklad TD94047 (AIN-93 VX). [d]A triacylgycerol oil produced by algae containing 42.57% DHA by weight.

Table 2
Fatty acid composition of diets

Content in diet (area percent)			
Fatty acid	Control	High n-3	Med. Low/Low n-3
14:0[a]	0.1	0.4	0.3
16:0	10.89	11.18	6.3
18:0	4.4	4.3	4.4
20:0	0.3	0.3	0.2
22:0	0.4	0.3	0.2
24:0	0.1	0.08	0.1
14:1	ND	ND	0.03
16:1	0.08	0.1	0.1
18:1	22.5	22.5	20.0
20:1n-9	0.2	0.21	0.1
18:3n-3	8.0	7.7	0.5
18:2n-6	53	52	67.4
20:2n-6	0.03	0.03	0.2
20:4n-6	ND	0.01	ND
20:5n-3	ND	0.08	ND
22:5n-3	ND	0.01	ND
22:6n-3	ND	0.7	ND

Data is the total fatty acid composition of the extracted diet.
[a]Number of carbons: number of double bonds, carbons from CH_3 terminal of molecule to first double bond. ND — not detected.

Individually housed dams were placed on the respective diets at the time of initial mating. The female offspring of Medium Low $n-3$ dams (each from a different litter) used to generate Low $n-3$ offspring were maintained on the sunflower oil diet throughout their lifetime and were mated on postnatal day 70 (±5 days).

Litters were culled to 8 on postnatal day 1 (P1). Pups were weaned on P21, group-housed by sex (3 per cage), and placed on the maternal diet. At weaning, brains were collected from littermates of pups used for the locomotor studies ($n=5$ per group, each from a different litter) for determination of fatty acid composition.

2.2. Locomotor behavior

Basal locomotor activity ($n=7$–12 per group, within subject, each from a different litter) was measured for a 2-h period during the light phase using Coulbourn Tru-Scan activity monitors on P28, P42, P56, and P70. Behavior was assessed at the same time each day. Females (P56 and P70) were tested during diestrus as determined by examination of vaginal lavage specimens. Behavior was assessed for overall activity on the basis of total horizontal