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An extraordinary degree of structural specificity is required in neural phospholipids for optimal brain function: n-6 docosapentaenoic acid substitution for docosahexaenoic acid leads to a loss in spatial task performance

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Abstract

This study was conducted to determine whether provision of preformed dietary docosapentaenoic acid (DPAn-6) can replace docosahexaenoic acid (DHA) for brain function as assessed by spatial task performance. A newly modified artificial rearing method was employed to generate n-3 fatty acid-deficient rats. Newborn pups were separated from their mothers at 2 days of age and given artificial rat milk containing linoleic acid (LA), or LA supplemented with 1% DHA (DHA), 1% DPAn-6 (DPA) or 1% DHA plus 0.4% DPAn-6 (DHA/DPA). The animals were then weaned onto similar pelleted diets. At adulthood, behavioural tasks were administered and then the brains were collected for fatty acid analysis. The LA and DPA groups showed a lower (63–65%) brain DHA than the dam-reared, DHA and DHA/DPA groups and this loss was largely compensated for by an increase in brain DPAn-6. The brain fatty acid composition in the DPA group was the same as that in the LA group at adulthood. In the Morris water maze, the LA and DPA groups exhibited a longer escape latency than the dam-reared and DHA groups and had a defect in spatial retention. In conclusion, DPAn-6 could not replace DHA for brain function, indicating a highly specific structural requirement for DHA.

Keywords: artificial rearing, docosahexaenoic acid, docosapentaenoic acid, Morris water maze, n-3 essential fatty acid deficiency.

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There are now many studies in which a dietary deficiency in n-3 fatty acids has been used to induce a loss in nervous system docosahexaenoic acid (DHA) (for reviews, see (Tinoco *et al*. 1979; Neuringer *et al*. 1988; Salem 1989; Okuyama *et al*. 1996; Salem *et al*. 2001). This has most typically been accomplished through the use of a two-generational model in which the dam is deprived of n-3 fatty acid sources and her offspring are then weaned to the same deficient diet (Wheeler *et al*. 1975; Neuringer *et al*. 1984; Neuringer *et al*. 1986; Yamamoto *et al*. 1987; Bourre *et al*. 1989; Pawlosky *et al*. 1997; Wainwright *et al*. 1998; Weisinger *et al*. 1999; Moriguchi *et al*. 2000; Greiner *et al*. 2001; Catalan *et al*. 2002). More recently, artificial rearing methods have become available that allow for the more rapid induction of a neural DHA deficiency in the newborn rat (Ward *et al*. 1996; Lim *et al*. 2003; Moriguchi *et al*. 2004; Lim *et al*. 2005). When the level of DHA fell in the brain and retina, there was a concomitant increase in the n-6 polyunsaturate docosapentaenoic acid (DPAn-6) (Mohrhauer and Holman 1963; Galli *et al*. 1971; Tinoco *et al*. 1978). This 'reciprocal replacement' as it was termed by Galli *et al*. (1971) occurred in the adult animal and only recently has it been demonstrated that replacement of DHA by DPAn-6 in the cerebral cortex is incomplete in young animals (Greiner *et al*. 2003). This is an indication that linoleic acid (LA) metabolism is

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Abbreviations used: DHA, docosahexaenoic acid; DPAn-6, docosapentaenoic acid n-6; HSD, honestly significant difference; LA, linoleic acid; LNA, α-linolenic acid; PN, post-natal.

insufficient to supply an adequate amount of DPAn-6 in n-3 fatty acid-deficient rats during the explosive brain growth that occurs in the post-natal period. That is, the summation of all of the biosynthetic and transport processes are not capable of supplying the same amount of 22-carbon polyenes as are normally received by the brain of an animal where preformed DHA is included in the diet.

Thus, functional abnormalities that are associated with the n-3 fatty acid-deficient diet may be related to the deficit in total 22-carbon polyenes in the case where only LA is fed as a source of essential fatty acids. In order to rule out this factor, it is necessary to compare a diet containing preformed DPAn-6 with one containing the same amount of DHA in a behavioural or physiological measure of neural function. In order to support the proposition that optimal nervous system function requires DHA and no closely related fatty acid structure can substitute, it is necessary to evaluate n-6-based diets in which metabolic and kinetic impediments to brain supply are removed.

In the present study, an artificial rearing system was employed to produce rats with lower levels of brain DHA in the first generation by feeding diets with only LA as a source of essential fatty acids. Other groups of rats were fed diets supplemented with either preformed DPAn-6, DHA, or a mixture of DHA and DPAn-6, to determine whether preformed DPAn-6 could functionally replace brain DHA. The principal endpoint used in this study was the Morris water maze task, providing measures of spatial task performance, as this has been successfully used in several laboratories for characterization of n-3 fatty acid-deficient rats add (Frances *et al*. 1996; Wainwright *et al*. 1998; Moriguchi *et al*. 2000; Carrie *et al*. 2002; Moriguchi and Salem. 2003; Lim *et al*. 2005).

Materials and methods

Animals and study design

Time-pregnant, female Long-Evans rats of 3-d gestational age were obtained from Charles River (Portage, MI, USA) and immediately placed on an 'n-3 adequate' diet (maternal diet) containing 3.1% α-linolenic acid (LNA) (Table 1). They were maintained in our animal facility under conventional conditions with controlled temperature (23 ± 1°C) and illumination (12 h; 06.00–18.00 h) and water was provided *ad libitum*. At post-natal day (PN) 2, five male pups were selected from each litter ($n = 10$ litters) that was born within a 12-h time window and, based upon body weight, were then randomly allocated into one of five groups. In this manner, each of the groups was composed of 10 individuals from 10 different dams and each group was composed of siblings of the other groups. The first group was the control group and was allowed to suckle from dams fed on an n-3 adequate diet (dam-reared group). The remaining groups were artificially fed one of the four experimental milks: n-3 deficient milk (LA), or a similar milk supplemented with 1 wt % (DHA), 1 wt % DPAn-6 (DPA) or 1 wt % DHA : DPAn-6 (2 : 1) (DHA/DPA) (Table 2). All experimental procedures were approved by the Animal Care and Use Committee of the National Institute on Alcohol Abuse and Alcoholism, NIH.

Table 1 Nutrient and fatty acid composition of pelleted diet

Ingredient				Amount (g/100 g diet)	
Alacid 710, acid casein[a]				20	
Corn starch				15	
Sucrose				10	
Dextrose				19.9	
Maltose–dextrin				15	
Cellulose				5	
Salt-mineral mix[b]				3.5	
Vitamin mix[c]				1	
L-cystine				0.3	
Choline bitartrate				0.25	
tBHQ[d]				0.002	
Fat sources:	LA	DPA	DHA/DPA	DHA	Maternal[e]
Hydrogenated coconut oil	2.7	2.7	2.7	2.7	7.75
Safflower oil	–	–	–	–	1.77
Flaxseed oil	–	–	–	–	0.48
Medium chain triglyceride	1.3	1.3	1.3	1.3	–
18:1n-9 ethyl ester	4.5	4.4	4.37	4.4	–
18:2n-6 ethyl ester	1.5	1.5	1.5	1.5	–
DPAn-6 ethyl ester	–	0.10	–	–	–
DHA ethyl ester	–	–	–	0.10	–
DHA:DPA (2 : 1) ethyl ester	–	–	0.13	–	–
Fatty acid composition (%)					
Total saturated	27.0	27.3	26.5	22.0	77.2
18:1n-9	46.2	44.5	44.7	47.1	4.3
18:2n-6	15.4	15.1	15.3	16.3	15.3
18:3n-3	0.04	0.04	0.04	0.05	3.1
22:5n-6	–	1.04	0.49	–	–
22:6n-3	–	–	0.98	1.10	–
n-6/n-3	383.7	403.8	15.5	14.2	5.0
22:5n-6/22:6n-3	–	–	0.5	–	–

[a]NZMP North America Inc. ALACID casein; [b]Dyets Inc. catalogue no. 210025; [c]Dyets Inc. catalogue no. 310025; [d]t-butyl-hydroquinone; [e]Dyets Inc. catalogue no. 400625, vitamin-free casein was used for maternal diet.

Artificial rearing system

The artificial rearing procedure was modified from a newly developed procedure described by Hoshiba (2004) and recently adapted for essential fatty acid research (Lim *et al*. 2005). A new type of nursing bottle was employed that was composed of nipples, a milk inflow tube, a milk overflow tube and a refill syringe, custom made by Dr Junji Hoshiba (Okayama University, Okayama, Japan). The cage was placed on an electronically thermostated hot pad initially set at 33.5°C and then was slowly decreased to 30°C by PN 6, where it remained until PN 15; the pad was then maintained

Table 2 Artificial rat milk ingredients

Ingredient	Amount (mg/100 mL milk)
Casein (Alacid 710, acid casein)[a]	6275
Whey protein isolate (Alacen 895)[a]	4000
Carbohydrate (α-lactose)[b]	1893
Serine[b]	28.8
Cystine[b]	22.5
Tryptophan[b]	27.0
Mineral	
NaOH[b]	2100
KOH[b]	170
$GlyCaPO_4$[b]	800
$MgCl_2$ $6H_2O$[b]	183
$CaCl_2$ $2H_2O$[b]	210
Ca_3 $4H_2O$-citrate[b]	250
Na_2HPO_4[b]	114
KH_2PO_4[b]	51.0
$FeSO_4$[b]	3.0
$ZnSO_4$[b]	6.0
$CuSO_4$[b]	1.6
$MnSO_4$[b]	0.07
NaF[b]	0.16
KI[b]	0.18
Carnitine[b]	4.0
Picolinate[b]	2.0
Ethanolamine[b]	3.4
Taurine[b]	15.0
Vitamin mix (in dextrose)[c]	500
Tricholine citrate[b]	370
Cholesterol[b]	40

Fat sources (g/100 mL milk)	LA	DPA	DHA/DPA	DHA
MCT oil[d]	1.56	1.56	1.56	1.56
Coconut oil (hydrogenated)[e]	3.24	3.24	3.24	3.24
18:1n-9 ethyl ester[f]	5.40	5.28	5.24	5.28
18:2n-6 ethyl ester[f]	1.80	1.80	1.80	1.80
DPAn-6 ethyl ester[f]	–	0.12	–	–
DHA ethyl ester[f]	–		–	0.12
DHA/DPAn-6 ethyl ester (2 : 1)[f]	–	–	0.16	–

Component sources were as follows: [a]NZMP (North America) Inc; [b]Sigma-Aldrich Corp. (St Louis, MO, USA); [c]RX993666 Harlan, Madison, WI, USA; [d]Mead Johnson Nutritionals, Evansville, IN, USA; [e]Dyets; [f]Nu-Check Prep Inc.

at 28°C until PN 21. Fresh artificial milk was loaded into the nursing bottle by a refill syringe. There were separate cages for each of the four dietary groups LA, DHA, DPA and DHA/DPA. The pups were separated from their dams on PN 2 and fed artificial milk from a nursing bottle with a silicon nipple every 3 h by hand five times between 08.00 and 21.00 h. The nursing bottles with silicon nipples and fresh milk were kept at 4°C in a refrigerator after feeding to minimize bacterial growth. The hand feeding was performed until eye opening at PN 14–15. Milk was then provided twice per day in 40-mL aliquots in 50-mL conical tubes that were fitted with water-bottle tops so that rat pups could drink *ad libitum*. These pups were weaned to pelleted diets with a fat composition similar to that fed during the artificial rearing period (Table 1). The dam-reared group was weaned onto the same diet as their dams. When the artificially and dam-reared animals were 8 weeks old, behavioural experiments began.

Artificial rat milk

The artificial formula was modified from the method of Kanno *et al.* (1997). Table 2 shows the macronutrients and minor ingredients used, as well as their commercial sources. In order to minimize the introduction of significant levels of n-3 fatty acids, the sources for casein (Alacid 710) and whey (Alacen 895) protein were carefully chosen (NZMP North America Inc., Santa Rosa, CA, USA). Casein contained 2.9% methionine and whey protein contained 2.5% methionine. For complete dissolution, all ingredients were mixed in the order presented in Table 2, except that an alkali solution with NaOH and KOH was added to the casein solution before the three amino acids, serine, cystine and tryptophan in order to minimize precipitation. Finally, cholesterol and fat were slowly added to the above mixture with stirring and using a hand-held rotor stator homogenizer (Omni Intl., Marietta, GA, USA). The milk was then homogenized two times under high pressure (120 kg/cm^2) using a stainless steel homogenizer with a two-stage valve (model no. HP50-250 FES International, Irwindale, CA, USA) that had been cleaned by rinsing with 0.1 M NaOH and then neutralized with sterilized water. The homogenized milk was pasteurized twice at 63°C for 30 min, 6 h apart and stored at 4°C before using. The milks contained 12 wt % lipids composed of saturated fat (medium chain triglycerides and hydrogenated coconut oil) and the ethyl ester form of unsaturated fatty acids (Nu-Check Prep, Elysian, MN, USA). Our analyses indicated that the LA diet contained 18.1% linoleic acid (LA) and 0.01% LNA (Table 3). The DHA diet contained 16.1% LA, 0.01% LNA and 1.16% DHA. The DPA diet contained 16.0% LA, 0.01% LNA and 1.01% DPAn-6. The DHA/DPA diet contained 17.7% LA, 0.01% LNA, 0.42% DPAn-6 and 0.96% DHA (Table 3). The DPAn-6 fatty acid was prepared from the single cell organism, *Schizochytrium* sp. and was a kind gift of OmegaTech Inc. (Boulder, CO, USA) and purified to > 99% as the ethyl ester by Nu-Chek Prep.

Table 3 Actual fatty acid composition of artificial rat milk*

	Dietary group			
Fatty acids	LA wt %	DPA wt %	DHA/DPA wt %	DHA wt %
Total saturated	33.0	33.6	33.0	35.6
Total monounsaturated	46.9	47.5	46.8	45.5
18:2n-6	18.1	16.0	17.7	16.1
18:3n-3	0.01	0.01	0.01	0.01
22:5n-6	–	1.01	0.42	–
22:6n-3	–	–	0.96	1.16
n-6/n-3	1810	1701	18.7	13.8
22:5n-6/22:6n-3	–	–	0.44	–

*Only trace quantities of long-chain polyunsaturated fatty acids such as 20 : 4n-6, 20 : 5n-3, and 22 : 5n-3 were detected, i.e. less than 0.01%. Other minor peaks were not included.

Experimental diets

The five pelleted experimental diets used for the weaned pups were based on the AIN-93G formulation (Reeves *et al*. 1993) with several modifications in order to obtain the extremely low n-3 fatty acid level required in this study (Table 1). The custom pelleted diets were obtained commercially every 3–4 weeks and prepared by a vendor using a cold pelleting process in order to prevent lipid peroxidation associated with high heat or ageing (Dyets, Bethlehem, PA, USA). The dependent variable in the four artificially reared groups was the substitution of the ethyl esters of DHA, DPA and DHA : DPA (2 : 1) for oleate ethyl ester. The maternal diet was made up with a mixture of safflower and flax oils and contained 15.4% LA and 3.1% LNA. All five diets contained 10 wt % as fat and had a similar content of LA. The rats were maintained on these diets until they were killed.

Motor activity test

In order to measure motor activity, each rat was individually placed into a cage (25 × 45 × 20 cm) and the moving time and the moving distance were measured for 20 min using a video image analyzer after 10-min habituation session (Videomax V, Columbus Instruments, Columbus, OH, USA). The last 20-min portion of this data was used for the determination of moving time and moving distance in order to allow for an initial 10-min habituation period.

Morris water maze test

Morris water maze test has been widely used to evaluate spatial learning performance (Morris *et al*. 1982; Morris 1984), and the method as employed in our laboratory has been previously described in detail (Moriguchi *et al*. 2000; Moriguchi and Salem. 2003). In brief, a circular pool was divided into four quadrants (regions A–D) and the platform was placed in region A at 1 cm below the water surface. For swimming training, rats were allowed to freely swim for 1 min with no platform available. Thereafter, the rats were submitted to a visible trial with a black (visible) escape platform; the rat was allowed to search for the platform for up to 90 s. If a rat failed to find the platform, it was placed on the platform for 30 s. Successful rats were also allowed to remain on the platform for 30 s. The next day, a transparent, hidden platform was used in region A and daily sessions consisted of two trials with starting points 90° apart. The maximal intertrial interval was 9 min. Sessions were repeated for 4 consecutive days for a total of eight trials. The time required for a rat to reach the hidden platform (escape latency), swimming time, the duration of the floating state (resting time) were automatically recorded by computer using a VideoMax video tracking system (Columbus Instruments). On the day following the last session, the platform was removed and the rat was allowed to search for the platform for 90 s (probe trial). The number of crossings of the position where the platform had been placed (quadrant region A) and the number of crossings in the corresponding imaginary positions in the other quadrant regions (regions B–D) were recorded.

Lipid composition

After the behavioural experiments, the rats were killed by decapitation. Brains were rapidly removed and stored at −80°C. The lipid extract of brain was prepared after the method of Folch *et al*. (1957) and then was transmethylated with 14% BF_3-methanol at 100°C for 60 min by a modification of the method of Morrison and Smith (1964) with hexane addition (Salem *et al*. 1996). Gas chromatographic analysis of the fatty acid methyl esters was performed as previously described (Salem *et al*. 1996). Total lipids in milk formulas and the pelleted diets, after grinding with a homogenizer, were extracted and transmethylated by the method of Lepage and Roy (1986). The fatty acid methyl esters were identified by comparison with the retention times of a 28-component, quantitative standard mixture (Prep 462; Nu-Chek). The concentrations of individual and total fatty acids were obtained using an internal standard (22 : 3 n-3 as methyl ester, 522 μg/brain).

Statistical analysis

All data were expressed as the mean ± standard error of the mean (SEM) and significance was determined using one-way ANOVA using Statistica (Statsoft, Tulsa, OK, USA). When ANOVA analyses with repeated measures found significant differences, behavioural experiments and body weight was evaluated using Tukey's honestly significant difference (HSD) test. Fatty acid compositional differences were analyzed by one-way ANOVA with post-hoc testing using Tukey's HSD test.

Results

Body weight

The artificially reared pups showed significantly lower body weight than that of dam-reared pups from PN 2 to weaning ($F_{4,42} = 17.171$, $p < 0.001$; Fig. 1), but there were no differences among the four artificially reared groups. At 8 weeks of age, when behavioural testing began, there was no significant difference either between dam-reared and artificially reared groups or among the four artificially reared groups ($F_{4,42} = 1.522$, $p = 0.213$). The mean body weights were 356 ± 14 g for the LA, 381 ± 10 g for the DPA, 349 ± 13 g for the DHA and 382 ± 16 g for the DHA/DPA groups and 383 ± 12 g for the dam-reared group.

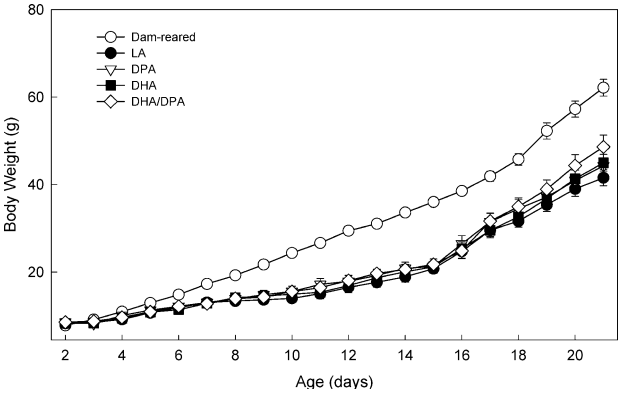

Fig. 1 Body weights of rat pups artificial reared on four different diets and a dam-reared control group.The body weight is presented as the mean ± SEM, $n = 8$–10.

Brain fatty acid composition

There were no significant differences in total saturates, total monounsaturates, long-chain polyunsaturates and total fatty acids among the five different dietary conditions (Table 4). The dam-reared group showed few differences in brain fatty acid composition with respect to the two groups that were fed DHA. However, there was significantly lower DPAn-6 and higher LA and DPAn-3 in the dam-reared group relative to all of the artificially reared groups. The LA and DPA groups showed a significantly higher percentage of total n-6 and a lower percentage of total n-3 fatty acids compared with those of the dam-reared group, DHA and DHA/DPA groups ($F_{4,26} = 329.849$, $p < 0.001$ for total n-6; $F_{4,26} = 319.4$, $p < 0.001$ for total n-3). The dam-reared group had a higher level of total n-3 with respect to the DHA/DPA group ($p < 0.05$). The artificial rearing method was useful in rapidly inducing a loss of brain DHA in rats fed on the n-3-deficient milk. There was a marked difference in brain DHA among the five groups ($F_{4,26} = 313.4$, $p < 0.001$). Rats fed the LA diet exhibited about a 65 and 63% loss in brain DHA compared with the dam-reared and DHA groups, respectively, while rats in the DPA group exhibited about a 61 and 59% loss in brain DHA compared with the dam-reared and DHA groups, respectively. The loss in brain DHA for the LA and DPA groups was largely replaced by a marked increase in the percentage of brain DPAn-6 ($F_{4,26} = 2205.7$, $p < 0.001$), but brain DHA and DPAn-6 levels were not significantly different between the LA and DPA groups.

Table 4 Effect of DHA and DPAn-6 supplementation on brain fatty acid composition*

	Dietary group				
	LA ($n = 5$)	DPA ($n = 5$)	DHA/DPA ($n = 8$)	DHA ($n = 6$)	Dam reared ($n = 8$)
Fatty acids (g/100 g)					
14:0	0.27 ± 0.01	0.28 ± 0.02	0.26 ± 0.03	0.22 ± 0.02	0.26 ± 0.02
16:0 DMA	2.23 ± 0.07	2.29 ± 0.05	2.05 ± 0.012	2.26 ± 0.08	2.16 ± 0.07
16:0	18.3 ± 0.37	18.3 ± 0.31	18.2 ± 0.21	18.2 ± 0.16	18.2 ± 0.18
18:0 DMA	3.58 ± 0.06	3.65 ± 0.08	3.42 ± 0.30	3.86 ± 0.22	3.67 ± 0.15
18:0	15.7 ± 0.18	16.0 ± 0.20	16.1 ± 0.09	16.1 ± 0.07	16.3 ± 0.13
20:0	0.64 ± 0.06^{ab}	0.68 ± 0.06^{a}	0.59 ± 0.02^{ab}	0.57 ± 0.01^{ab}	0.50 ± 0.02^{b}
22:0	0.51 ± 0.05	0.53 ± 0.04	0.48 ± 0.02	0.46 ± 0.01	0.45 ± 0.02
24:0	1.15 ± 0.09	1.17 ± 0.10	1.06 ± 0.03	1.03 ± 0.07	1.04 ± 0.07
Total saturates	42.3 ± 0.32	42.8 ± 0.27	42.2 ± 0.19	42.6 ± 0.25	42.6 ± 0.15
16:1	0.33 ± 0.01	0.33 ± 0.01	0.38 ± 0.01	0.36 ± 0.03	0.32 ± 0.03
18:1 DMA	1.64 ± 0.04	1.63 ± 0.06	1.51 ± 0.12	1.69 ± 0.09	1.54 ± 0.07
18:1n-9	15.4 ± 0.23^{b}	15.4 ± 0.19^{b}	16.4 ± 0.11^{a}	16.4 ± 0.15^{a}	16.4 ± 0.09^{a}
18:1n-7	3.81 ± 0.03	3.73 ± 0.05	3.75 ± 0.05	3.75 ± 0.07	3.64 ± 0.05
20:1n-9	2.10 ± 0.15	2.16 ± 0.15	2.10 ± 0.06	2.05 ± 0.05	1.88 ± 0.08
22:1n-9	0.19 ± 0.02	0.19 ± 0.01	0.18 ± 0.01	0.18 ± 0.003	0.16 ± 0.01
24:1n-9	3.58 ± 0.26^{a}	3.28 ± 0.21^{ab}	2.86 ± 0.17^{ab}	2.63 ± 0.14^{b}	2.74 ± 0.20^{b}
Total monounsaturates	27.0 ± 0.53	26.8 ± 0.60	27.1 ± 0.19	27.1 ± 0.25	26.6 ± 0.33
18:2n-6	0.28 ± 0.01^{c}	0.30 ± 0.01^{bc}	0.32 ± 0.01^{bc}	0.34 ± 0.02^{b}	0.43 ± 0.01^{a}
20:2n-6	0.08 ± 0.01	0.09 ± 0.003	0.08 ± 0.003	0.09 ± 0.004	0.09 ± 0.003
20:3n-6	0.22 ± 0.01^{bc}	0.23 ± 0.01^{b}	0.27 ± 0.01^{ab}	0.27 ± 0.01^{ab}	0.28 ± 0.01^{a}
20:4n-6	8.34 ± 0.23	8.04 ± 0.15	7.97 ± 0.10	7.97 ± 0.09	8.23 ± 0.15
22:4n-6	3.23 ± 0.04^{a}	3.06 ± 0.03^{a}	2.75 ± 0.04^{b}	2.67 ± 0.03^{b}	2.68 ± 0.04^{b}
22:5n-6	7.30 ± 0.14^{a}	7.40 ± 0.12^{a}	0.89 ± 0.04^{b}	0.70 ± 0.05^{b}	0.35 ± 0.02^{c}
Total n-6 PUFA	19.4 ± 0.30^{a}	19.1 ± 0.27^{a}	12.3 ± 0.14^{b}	12.0 ± 0.12^{b}	12.1 ± 0.20^{b}
22:5n-3	0.03 ± 0.00^{c}	0.02 ± 0.003^{c}	0.05 ± 0.002^{b}	0.06 ± 0.01^{b}	0.11 ± 0.004^{a}
22:6n-3	4.27 ± 0.21^{b}	4.70 ± 0.34^{b}	11.3 ± 0.10^{a}	11.61 ± 0.17^{a}	12.09 ± 0.22^{a}
Total n-3 PUFA	4.30 ± 0.21^{c}	4.72 ± 0.33^{c}	11.4 ± 0.10^{b}	11.7 ± 0.17^{ab}	12.2 ± 0.22^{a}
22:5n-6/22:6n-3	1.73 ± 0.10^{a}	1.60 ± 0.11^{a}	0.08 ± 0.004^{b}	0.06 ± 0.004^{b}	0.03 ± 0.002^{b}
22:5n-6 +22:6n-3	11.6 ± 0.25	12.1 ± 0.40	12.2 ± 0.11	12.3 ± 0.19	12.4 ± 0.23
n-6/n-3	4.57 ± 0.24^{a}	4.12 ± 0.25^{a}	1.08 ± 0.02^{b}	1.03 ± 0.02^{b}	0.99 ± 0.02^{b}
LC-PUFA	23.5 ± 0.39	23.6 ± 0.56	23.3 ± 0.16	23.4 ± 0.23	23.8 ± 0.36
Total FA (μg/mg wet brain)	36.3 ± 1.54	37.7 ± 1.24	35.0 ± 1.37	35.4 ± 1.09	34.5 ± 1.43

*Each parameter is presented as the mean (± SEM) for $n = 5$–8 rats. Different alphabetical superscripts indicate significant differences by Tukey's HSD at $p < 0.05$. LC-PUFA is defined as 20- and 22-C fatty acids with two or more double bonds.

There were statistically significant differences in the ratio of DPAn-6/DHA and n-6/n-3 between the dam-reared, DHA, DHA/DPA versus the LA and DPA groups ($F_{4,26} = 273.4$, $p < 0.001$ for DPAn-6/DHA; $F_{4,26} = 204.5$, $p < 0.001$ for n-6/n-3) but there were no statistically significant differences between the LA and DPA groups. There was generally a similar pattern in the fatty acid profiles among the dam-reared, DHA and DHA/DPA groups and no differences in fatty acid composition was observed between the DHA and DHA/DPA groups.

Behavioural tests

The motor activity test showed that there was a statistically significant difference in moving time ($F_{4,42} = 4.127$, $p < 0.01$; Fig. 2). The moving time was greater in the DPA group than the dam-reared ($p < 0.05$), DHA ($p < 0.05$) and DHA/DPA ($p < 0.01$) groups but was not different from the LA group. The LA and DPA groups tended to show higher activity in moving distance than the dam-reared, DHA and DHA/DPA groups, but the differences were not significant ($F_{4,42} = 1.814$, $p = 0.144$).

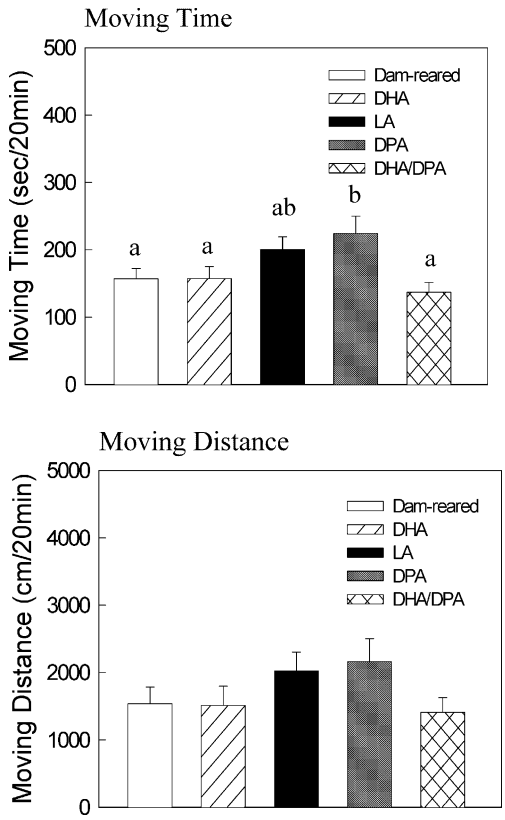

Fig. 2 Effect of DHA and DPAn-6 supplementation on moving time and moving distance in the motor activity test. The moving time and moving distance are presented as the mean ± SEM, $n = 8$–10. Different letters indicate significant differences among the five groups in the moving time ($F_{4,42} = 4.127$, $p < 0.01$). There were no differences among the five groups in the moving distance ($F_{4,42} = 1.814$, $p = 0.144$).

In the visible trial of the Morris water maze test, there were no significant differences in escape latency ($F_{4,42} = 0.941$, $p = 0.450$; Table 5) and swimming speed ($F_{4,42} = 0.744$, $p = 0.488$) among the five dietary groups. In the visible trial, six rats out of 10 in the dam-reared, DHA and DHA/DPA groups reached the platform, while six of eight in the LA group and four out of nine in the DPA group reached the platform.

In the learning trials, the escape latency of the dam-reared and DHA groups gradually decreased over the testing period (Fig. 3). However, the escape latency was significantly longer in both the LA and DPA groups compared with the dam-reared ($F_{4,42} = 7.857$, $p < 0.001$; LA, $p < 0.001$; DPA, $p < 0.001$ vs. dam-reared) and DHA groups (LA, $p < 0.05$; DPA, $p < 0.05$ vs. DHA). There was no difference in escape latency between the LA and DPA groups. The DHA/DPA group was intermediate between the LA and DHA group in escape latency. There were no significant differences in escape latency among the DHA, DHA/DPA and dam-reared groups. Also, there were no significant differences in escape latency between the DHA/DPA group and either the LA or DPA groups. Thus, the DHA/DPA group was different than the DHA group only in that the escape latency measure did not reach significance when compared with the two n-3-deficient groups, i.e. the LA and DPA groups.

To order to exclude the contribution of other factors to the escape latency, such as an increase in immobility time, the escape latency values were subdivided into swimming time and resting time. The swimming time increases were consistent with increased escape latency (Fig. 4). The swimming time in the LA and DPA groups were significantly increased ($F_{4,42} = 7.449$, $p < 0.001$; Fig. 4) relative to that of the dam-reared and DHA groups. Again, there were no differences in swimming times among the dam-reared, DHA and DHA/DPA groups although the difference between the

Table 5 Effect of DHA and DPAn-6 supplementation on the visible trial in the Morris water maze

Group	No. of rats	Escape latency[a] (s)	Swimming speed[a] (cm/ s)	No. of successful rats
LA	8	60.5 ± 11.1	23.1 ± 2.4	6
DPA	9	80.2 ± 5.8	25.2 ± 2.7	4
DHA/DPA	10	59.4 ± 9.2	27.2 ± 1.7	6
DHA	10	69.8 ± 8.6	24.2 ± 2.9	6
Dam reared	10	62.4 ± 9.0	23.2 ± 2.3	6

[a]There were no significant differences in escape latency ($F_{4,42} = 0.941$, $p = 0.450$) and swimming speed ($F_{4,42} = 0.744$, $p = 0.488$] among the five dietary groups as assessed by one-way ANOVA.

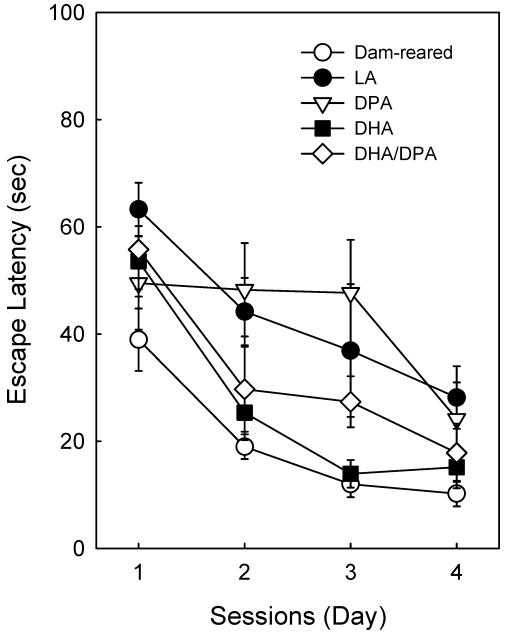

Fig. 3 Effect of DHA and DPAn-6 supplementation on escape latency in the Morris water maze. The time required (escape latency, mean of two trials) for a rat to find and climb onto a hidden platform is presented as the mean ± SEM, $n = 8$–10. Significant differences between dietary groups using a repeated measures one-way ANOVA were as follows: ($F_{4,42} = 7.857$, $p < 0.001$; LA, $p < 0.001$; DPA, $p < 0.001$ vs. dam-reared; LA, $p < 0.05$; DPA, $p < 0.05$ vs. DHA).

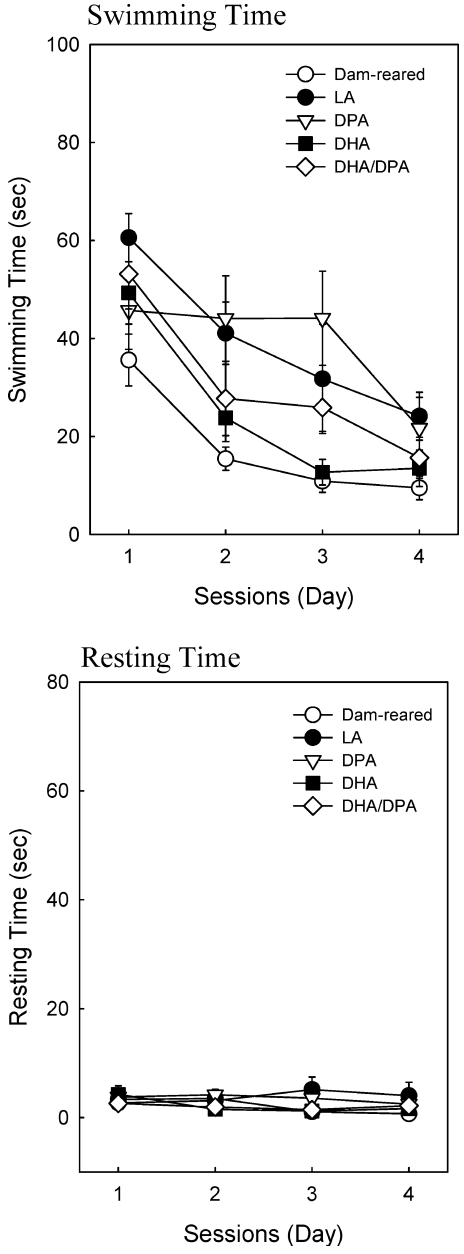

Fig. 4 Effect of DHA and DPAn-6 supplementation on swimming and resting time. The swimming and resting times are presented as the mean ± SEM, $n = 8$–10. Significant differences between dietary groups using a repeated measures one-way ANOVA were as follows: ($F_{4,42} = 7.449$, $p < 0.001$; LA, $p < 0.001$; DPA, $p < 0.001$ vs. dam-reared; LA, $p < 0.01$; DPA, $p < 0.01$ vs. DHA). The resting time was not significantly different among the three different groups ($F_{4,42} = 1.801$, $p = 0.147$).

dam-reared and DHA/DPA groups approached significance ($p = 0.06$). The resting times in all groups were similar and there were no significant differences ($F_{4,42} = 1.801$, $p = 0.147$) among the five groups (Fig. 4).

In the probe trial, the number of crossings of the platform position in region A was significantly greater than those of other regions for the dam-reared, DHA and DHA/DPA groups (dam-reared, $F_{3,36} = 6.147$, $p < 0.01$; DHA, $F_{3,36} = 5.875$, $p < 0.01$; DHA/DPA, $F_{3,36} = 2.987$, $p < 0.05$; Fig. 5). However, rats in the LA and DPA groups swam randomly without preference for any quadrant (LA, $F_{3,28} = 0.700$, $p = 0.560$; DPA, $F_{3,32} = 1.527$, $p = 0.227$). Analysis of the visiting time in region A within the area of the platform position among the diet groups indicated that the values for the dam-reared, DHA and DHA/DPA groups were different from those of the LA and DPA groups ($F_{4,42} = 3.042$, $p < 0.05$). However, there were no differences among the dam-reared, DHA and DHA/DPA groups nor when the LA and DPA groups were compared with each other.

Discussion

This study demonstrates that dietary DPAn-6 produced none of the benefit afforded by DHA addition for spatial task performance. In fact, the DPAn-6-fed animals performed no better than animals fed only LA and were, from a statistical point of view, indistinguishable from the LA group. Thus, the Morris water maze data provided a very clear answer to the question of whether the DPAn-6-fed rats would perform more like the n-3-deficient group or like those consuming DHA. This finding may be viewed as not surprising given

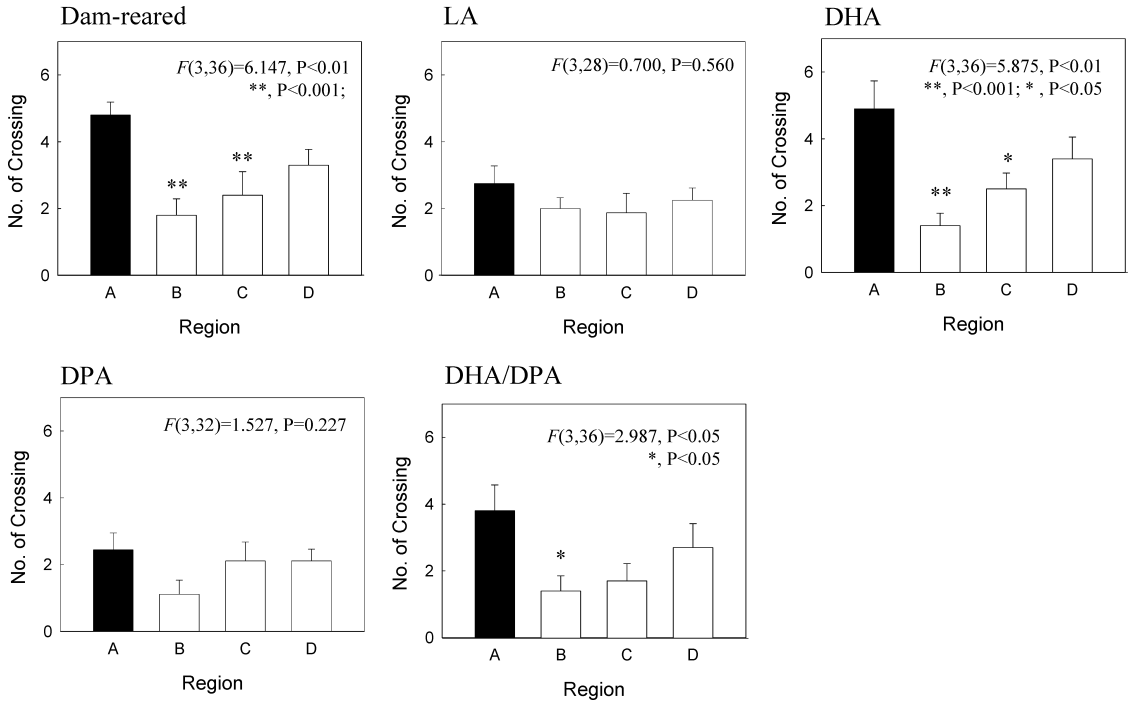

Fig. 5 Effect of DHA and DPAn-6 supplementation on the probe trial. The number of crossings of the platform position (region A; closed column) and the corresponding imaginary positions (regions B–D; open columns) are presented as the mean ± SEM, $n = 8$–10. One-way ANOVA (dam-reared, $F_{3,36} = 6.147$, $p < 0.01$; DHA, $F_{3,36} = 5.875$, $p < 0.01$; DHA/DPA, $F_{3,36} = 2.987$, $p < 0.05$). *$p < 0.05$, **$p < 0.001$ compared with region A (Tukey's HSD test).

our observation that the brain fatty acid profiles at adulthood were identical between the LA and DPA groups. However, this was not necessarily the case in the first weeks of development where the feeding of DPAn-6 may have led to a more complete reciprocal replacement of DHA. This is the period (5–20 PN) in development in which total brain DHA + DPAn-6 content declines in n-3 fatty acid deficient diets (Greiner *et al*. 2003).

The poorer performance of the LA and DPA groups relative to the DHA and dam-reared groups could not be attributed to a decrease in general arousal or locomotion, as measures of activity and swimming speed were generally the same (and even slightly higher for the DPA group in moving time). There were no obvious differences in motor ability or sensory function as evidenced by the lack of any group differences in the visible trial of the Morris water maze task. These results agreed with our previous observations with an LA (Lim *et al*. 2003, 2005) or LA-based diets (safflower oil) (Moriguchi *et al*. 2000; Moriguchi and Salem 2003) where it was suggested that poorer performance in the spatial tasks given here may relate to either cognitive capacity or emotive factors (Moriguchi and Salem 2003). It is also interesting to note that there were no significant differences in behaviour between our dam-reared group and the DHA group, even although maternal rearing can in some measures lead to improved performance.

It was interesting to consider the performance of the DHA/DPA group as the difference in escape latency observed between the two n-3 fatty acid-deficient groups (LA and DPA) and the DHA-containing groups (DHA and dam-reared) was not observed for this 'mixture' group. The difference observed in the probe trial was, however, retained as in the other DHA-containing groups, albeit somewhat weaker from a statistical standpoint. Although the brain percentage of DHA was slightly lower in the DHA/DPA group than in the DHA and dam-reared groups, this difference did not reach significance. This slightly higher brain DHA level in the DHA group may have been related to the fact that the dietary DHA level was also slightly higher (1.16% of fatty acids on analysis) in comparison with that given the DHA/DPA group (0.98%), even although the study design attempted to make these equal. One might then ask whether this small difference in DHA intake and brain DHA content could be reflected in the escape latency measure. In a previous study of the recovery of spatial task performance as DHA was repleted in the brain Moriguchi and Salem (2003) found that the escape latency became significantly different from the DHA group in a group where the brain DHA was 7.6% of total fatty acids (in their 'adult, 10 weeks' group) but not different in a group where brain DHA was 10.6% (in their 'adult, 14 weeks' group). Thus, this data would indicate that the small difference in brain DHA content found in the DHA/

DPA group relative to the DHA group is not adequate to cause an increase in escape latency. This observation then raises the possibility that the addition of the DPAn-6 to the diet led to the slightly poorer performance in the DHA/DPA group. However, closer inspection of the data indicates that only one data point (on day 3) was appreciably different than for the DHA group, and this observation should be reproduced before any conclusions are drawn on this issue.

The artificial rearing method employed here was successful in rapidly producing animals deficient in n-3 fatty acids. These animals exhibited an 95% increase in DPAn-6 and a 61–65% decrease in brain DHA, despite the fact that they were born to dams fed a diet containing 3% LNA. This approach then provides an excellent model of infant formula feeding as the experimental period of n-3 fatty acid deficiency begins after birth. The newborn rat may be considered to be at a similar developmental stage as premature human infants, those born in the third trimester. Thus, this model should prove useful for the study of infant feeding of premature infants. This technique can be used to study any fatty acid or other nutrient that can be controlled in the diet. When coupled to more rapid behavioural and physiological methods for assessment of neural function, the method can, in principle, reduce the experiment time from 5 months or more to 3–4 weeks.

This experiment clearly demonstrates for the first time that preformed DPAn-6 cannot substitute for brain DHA. Given that these molecules only differ by a single double bond at the n-3 position on the chain, it is relevant to consider what mechanisms may be responsible for the difference in brain function. Recent studies have shown that DHA is more efficient than DPAn-6 for rhodopsin signalling both *in vitro* (Mitchell *et al*. 2001; Niu *et al*. 2001; Mitchell *et al*. 2003) and *in vivo* (Niu *et al*. 2004). Rhodopsin is a G-protein coupled receptor that shares the 7 transmembrane α helical subunit structural motif with many brain receptor proteins and these would be expected to behave in a similar manner. Kim *et al*. (2000, 2003) found that DPAn-6 is not as effective as DHA in supporting the accumulation of phosphatidylserine or preventing staurosporine-induced apoptosis. NMR studies of DHA-phospholipids also indicate a highly flexible molecule with rapid transitions between a large number of conformers (Gawrisch *et al*. 2003). Eldho *et al*. (2003) compared the difference of DHA-containing phospholipids to species containing DPAn-6 in lipid matrix properties and observed that DHA in the methyl end is more flexible and isomerizes with shorter correlation times such that interactions with membrane proteins would be altered. Thus, the extraordinary specificity demonstrated in this paper for spatial task performance is supported by a similarly remarkable specificity that has been demonstrated for membrane receptor function, neuronal apoptosis and biophysical properties of these molecular species of phospholipids in biological membranes.

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References

Bourre J. M., Francois M., Youyou A., Dumont O., Piciotti M., Pascal G. and Durand G. (1989) The effects of dietary α-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning tasks in rats. *J. Nutr.* **119**, 1880–1892.

Carrie I., Smirnova M., Clement M., De J. D., Frances H. and Bourre J. M. (2002) Docosahexaenoic acid-rich phospholipid supplementation: effect on behavior, learning ability, and retinal function in control and n-3 polyunsaturated fatty acid deficient old mice. *Nutr. Neurosci.* **5**, 43–52.

Catalan J., Moriguchi T., Slotnick B., Murthy M., Greiner R. S. and Salem N. Jr (2002) Cognitive deficits in docosahexaenoic acid-deficient rats. *Behav. Neurosci.* **116**, 1022–1031.

Eldho N. V., Feller S. E., Tristram-Nagle S., Polozov I. V. and Gawrisch K. (2003) Polyunsaturated docosahexaenoic vs docosapentaenoic acid-differences in lipid matrix properties from the loss of one double bond. *J. Am. Chem. Soc.* **125**, 6409–6421.

Folch J., Lees M. and Stanley G. H. S. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**, 497–509.

Frances H., Coudereau J. P., Sandouk P., Clement M., Monier C. and Bourre J. M. (1996) Influence of a dietary α-linolenic acid deficiency on learning in the Morris water maze and on the effects of morphine. *Eur. J. Pharmacol.* **298**, 217–225.

Galli C., Trzeciak H. I. and Paoletti R. (1971) Effects of dietary fatty acids on the fatty acid composition of brain ethanolamine phosphoglyceride: reciprocal replacement of n-6 and n-3 polyunsaturated fatty acids. *Biochim. Biophys. Acta* **248**, 449–454.

Gawrisch K., Eldho N. V. and Holte L. L. (2003) The structure of DHA in phospholipid membranes. *Lipids* **38**, 445–452.

Greiner R. S., Moriguchi T., Slotnick B. M., Hutton A. and Salem N. Jr (2001) Olfactory discrimination deficits in n-3 fatty acid-deficient rats. *Physiol. Behav.* **72**, 379–385.

Greiner R. S., Catalan J. N., Moriguchi T. and Salem N. Jr (2003) Docosapentaenoic acid does not completely replace DHA in n-3 FA-deficient rats during early development. *Lipids* **38**, 431–435.

Hoshiba J. (2004) Method for hand-feeding mouse pups with nursing bottles. *Contemp. Top Lab. Anim. Sci.* **43**, 50–53.

Kanno T., Koyanagi N., Katoku Y., Yonekubo A., Yajima T., Kuwata T., Kitagawa H. and Harada E. (1997) Simplified preparation of a refined milk formula comparable to rat's milk: influence of the formula on development of the gut and brain in artificially reared rat pups. *J. Pediatr. Gastroenterol. Nutr.* **24**, 242–252.

Kim H. Y., Akbar M., Lau A. and Edsall L. (2000) Inhibition of neuronal apoptosis by docosahexaenoic acid (22 : 6n−3). Role of phosphatidylserine in anti-apoptotic effect. *J. Biol. Chem.* **275**, 35 215–35 223.

Kim H. Y., Akbar M. and Lau A. (2003) Effects of docosapentaenoic acid on neuronal apoptosis. *Lipids* **38**, 453–457.

Lepage G. and Roy C. C. (1986) Direct transesterification of all classes of lipids in a one-step reaction. *J. Lipid Res.* **27**, 114–120.

Lim S.-Y., Moriguhci T., Lefkowitz B., Loewke J., Majchrzak S., Hoshiba J. and Salem N. Jr (2003) Artificial feeding of an n-3

essential fatty acid deficient diet leads to a loss of brain function in the first generation, in *Essential Fatty Acids Eicosanoids* (Huang, Y.-S., Lin, S.-J. and Huang, P.-C., eds), pp. 122–131. AOCS Press, Champaign, IL.

Lim S.-Y., Hoshiba J., Moriguchi T. and Salem N. Jr (2005) N-3 fatty acid deficiency leads to pooer performance in spatial learning tasks using a newly modified artificial rearing method. *Pediatr Res.* in press.

Mitchell D. C., Niu S. L. and Litman B. J. (2001) Optimization of receptor-G protein coupling by bilayer lipid composition I: kinetics of rhodopsin-transducin binding. *J. Biol. Chem.* **276**, 42 801–42 806.

Mitchell D. C., Niu S. L. and Litman B. J. (2003) DHA-rich phospholipids optimize G-protein-coupled signaling. *J. Pediatr.* **143**, S80–S86.

Mohrhauer H. and Holman R. T. (1963) Alteration of the fatty acid composition of brain lipids by varying levels of dietary essential fatty acids. *J. Neurochem.* **10**, 523–530.

Moriguchi T. and Salem N. Jr (2003) Recovery of brain docosahexaenoate leads to recovery of spatial task performance. *J. Neurochem.* **87**, 297–309.

Moriguchi T., Greiner R. S. and Salem N. Jr (2000) Behavioral deficits associated with dietary induction of decreased brain docosahexaenoic acid concentration. *J. Neurochem.* **75**, 2563–2573.

Moriguchi T., Lim S. Y., Greiner R., Lefkowitz W., Loewke J., Hoshiba J. and Salem N. Jr (2004) Effects of an n-3-deficient diet on brain, retina, and liver fatty acyl composition in artificially reared rats. *J. Lipid Res.* **45**, 1437–1445.

Morris R. (1984) Developments of a water-maze procedure for studying spatial learning in the rat. *J. Neurosci. Meth.* **11**, 47–60.

Morris R. G., Garrud P., Rawlins J. N. and O'Keefe J. (1982) Place navigation impaired in rats with hippocampal lesions. *Nature* **297**, 681–683.

Morrison W. R. and Smith L. M. (1964) Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J. Lipid Res.* **5**, 600–608.

Neuringer M., Connor W. E., Van Petten C. and Barstad L. (1984) Dietary omega-3 fatty acid deficiency and visual loss in infant rhesus monkeys. *J. Clin. Invest* **73**, 272–276.

Neuringer M., Connor W. E., Lin D. S., Barstad L. and Luck S. (1986) Biochemical and functional effects of prenatal and postnatal omega 3 fatty acid deficiency on retina and brain in rhesus monkeys. *Proc. Natl Acad. Sci. USA* **83**, 4021–4025.

Neuringer M., Anderson G. J. and Connor W. E. (1988) The essentiality of n-3 fatty acids for the development and function of the retina and brain. *Annu. Rev. Nutr.* **8**, 517–541.

Niu S. L., Mitchell D. C. and Litman B. J. (2001) Optimization of receptor-G protein coupling by bilayer lipid composition II: formation of metarhodopsin II-transducin complex. *J. Biol. Chem.* **276**, 42 807–42 811.

Niu S. L., Mitchell D. C., Lim S. Y., Wen Z. M., Kim H. Y., Salem N. Jr and Litman B. J. (2004) Reduced G protein-coupled signaling efficiency in retinal rod outer segments in response to n-3 fatty acid deficiency. *J. Biol. Chem.* **279**, 31 098–31 104.

Okuyama H., Kobayashi T. and Watanabe S. (1996) Dietary fatty acids – the N-6/N-3 balance and chronic elderly diseases. Excess linoleic acid and relative N−3 deficiency syndrome seen in Japan. *Prog. Lipid Res.* **35**, 409–457.

Pawlosky R. J., Denkins Y., Ward G. and Salem N. Jr (1997) Retinal and brain accretion of long-chain polyunsaturated fatty acids in developing felines: the effects of corn oil-based maternal diets. *Am. J. Clin. Nutr.* **65**, 465–472.

Reeves P. G., Nielsen F. H. and Fahey G. C. Jr (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **123**, 1939–1951.

Salem N. Jr (1989) Omega-3 fatty acids: molecular and biochemical aspects, in *Current Topics in Nutrition and Disease: New Protective Roles for Selected Nutrients* (Spiller, G. A. and Scala, J., eds), pp. 109–228. Alan R. Liss, New York.

Salem N. Jr, Reyzer M. and Karanian J. (1996) Losses of arachidonic acid in rat liver after alcohol inhalation. *Lipids* **31**, S153–S156.

Salem N. Jr, Litman B., Kim H. Y. and Gawrisch K. (2001) Mechanisms of action of docosahexaenoic acid in the nervous system. *Lipids* **36**, 945–959.

Tinoco J., Babcock R., Hincenbergs I., Medwadowski B. and Miljanich P. (1978) Linolenic acid deficiency: changes in fatty acid patterns in female and male rats raised on a linolenic acid-deficient diet for two generations. *Lipids* **13**, 6–17.

Tinoco J., Babcock R., Hincenbergs I., Medwadowski B., Miljanich P. and Williams M. A. (1979) Linolenic acid deficiency. *Lipids* **14**, 166–173.

Wainwright P. E., Xing H. C., Girard T., Parker L. and Ward G. R. (1998) Effects of dietary n-3 fatty acid deficiency on Morris water-maze performance and amphetamine-induced conditioned place preference in rats. *Nutr. Neurosci.* **1**, 281–293.

Ward G., Woods J., Reyzer M. and Salem N. Jr (1996) Artificial rearing of infant rats on milk formula deficient in n-3 essential fatty acids: a rapid method for the production of experimental n-3 deficiency. *Lipids* **31**, 71–77.

Weisinger H. S., Vingrys A. J., Bui B. V. and Sinclair A. J. (1999) Effects of dietary n-3 fatty acid deficiency and repletion in the guinea pig retina. *Invest. Ophthalmol. Vis. Sci.* **40**, 327–338.

Wheeler T. G., Benolken R. M. and Anderson R. E. (1975) Visual membranes: specificity of fatty acid precursors for the electrical response to illumination. *Science* **188**, 1312–1314.

Yamamoto N., Saitoh M., Moriuchi A., Nomura M. and Okuyama H. (1987) Effect of dietary α-linolenate/linoleate balance on brain lipid compositions and learning ability of rats. *J. Lipid Res.* **28**, 144–151.