Specific phospholipid fatty acid composition of brain regions in mice: effects of n–3 polyunsaturated fatty acid deficiency and phospholipid supplementation

Isabelle Carrié,[1,*†] Michel Clément,[*] Dominique de Javel,[†] Henriette Francès,[*] and Jean-Marie Bourre[*]

INSERM U26,* Unite de Neuro-Pharmaco-Nutrition, 75010 Paris, France, Laboratoires Ponroy,† 78340 Les Clayes Sous Bois, France

Abstract **This study examined the effects of dietary α-linolenic acid deficiency followed or not by supplementation with phospholipids rich in n–3 polyunsaturated fatty acid (PUFA) on the fatty acid composition of total phospholipids in 11 brain regions. Three weeks before mating, mice were fed a semisynthetic diet containing both linoleic and α-linolenic acid or deficient in α-linolenic acid. Pups were fed the same diet as their dams. At the age of 7 weeks, a part of the deficient group were supplemented with n–3 polyunsaturated fatty acids (PUFA) from either egg yolk or pig brain phospholipids for 2 months. Saturated and monounsaturated fatty acid levels varied among brain regions and were not significantly affected by the diet. In control mice, the level of 22:6 n–3 was significantly higher in the frontal cortex compared to all regions. α-Linolenic acid deficiency decreased the level of 22:6 n–3 and was compensated by an increase in 22:5 n–6 in all regions. However, the brain regions were affected differently. After the pituitary gland, the frontal cortex, and the striatum were the most markedly affected with 40% reduction of 22:6 n–3. Supplementation with egg yolk or cerebral phospholipids in deficient mice restored a normal fatty acid composition in brain regions except for the frontal cortex. There was a regional distribution of the fatty acids in the brain and the impact of deficiency in α-linolenic acid was region-specific. Dietary egg yolk or cerebral phospholipids are an effective source of n–3 PUFA for the recovery of altered fatty acid composition induced by a diet deficient in n–3 PUFA.**—Carrié, I., M. Clément, D. de Javel, H. Francès, and J-M. Bourre. **Specific phospholipid fatty acid composition of brain regions in mice: effects of n–3 polyunsaturated fatty acid deficiency and phospholipid supplementation.** ***J. Lipid Res.*** **2000. 41: 465–472.**

Supplementary key words brain regions • fatty acid composition • n–3 PUFA deficiency • phospholipid supplementation

The central nervous system has the second greatest concentration of lipids, immediately after adipose tissue. These brain lipids contain a very high amount of long-chain polyunsaturated fatty acids (LCPUFA), particularly arachidonic acid (AA; 20:4 n–6) and docosahexaenoic acid (DHA; 22:6 n–3). These two LCPUFA, which are the major constituents of neural cell membrane phospholipids, are derived respectively from two dietary precursors: linoleic acid (18:2 n–6) and α-linolenic acid (18:3 n–3). Linoleic and α-linolenic acids are the only sources for LCPUFA. Vertebrates are unable to synthesize these two essential polyunsaturated fatty acids (PUFA); they must be provided by the diet. Numerous studies have reported that DHA is required during development when cellular differentiation and active synaptogenesis take place (1–4). α-Linolenic acid deficiency is associated with altered fatty acid composition of cell membranes and organelles in brain (1, 4, 5). These alterations lead to changes in physical properties of membranes, in enzyme activities, receptors, transport, and cellular interaction (4, 6). These physiological changes are accompanied by learning and behavioral deficits in rats and mice (6–8).

α-Linolenic acid deficiency reduced DHA levels and increased those of docosapentaenoic acid (22:5 n–6). Most studies have examined the alteration of fatty acid composition by analyzing the whole brain. However, Delion et al. (9) have shown that a chronic dietary α-linolenic acid deficiency alters dopaminergic and serotoninergic neurotransmission in rats. These modifications were only observed in the frontal cortex which seems to be more sensitive to n–3 PUFA deficiency than the striatum. Jumpsen et al. (10) studied the effect of diets varying in n–6 and n–3 polyunsaturated fatty acid content on brain fatty acid composition in neonatal rats. The responses to the different

Abbreviations: : PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids; AA, arachidonic acid; DHA, docosahexaenoic acid; LC, long chain; OB, olfactory bulb; FC, frontal cortex; ST, striatum; HT, hypothalamus; OC, occipital cortex; TH, thalamus; HC, hippocampus; CB, cerebellum; MB, midbrain; PM, pons medulla; PIT, pituitary; PL, phospholipid; B-PL, brain phospholipids; E-PL, egg phospholipids.

[1] To whom correspondence should be addressed.

diets differed among phosphoglycerides, cell types, and brain regions.

Our own studies have shown that mice fed a diet deficient in α-linolenic acid exhibit learning impairments on several tests (11–14). For example, on the Morris Water Maze, deficient mice performed less well than controls (13). This spatial learning test has been reported to be sensitive to hippocampal damage (15, 16). In rats with learning impairments induced by a deficiency in α-linolenic acid, the average densities of synaptical vesicles in the terminals of the hippocampus CA1 region were decreased by nearly 30% compared with controls (17). Thus, it appears more informative to investigate the fatty acid composition of specific brain regions in order to establish relationships with the observed dysfunctions.

The speed of recuperation after deficiency is very slow; many weeks are required for brain cells and organelles in rats to recover (18). The dietary α-linolenic acid requirements for obtaining and maintaining a physiological level of DHA have been determined in developing and adult rats (6, 19). Bourre, Dumont, and Durand (20) demonstrated that brain phospholipids providing n–6 and n–3 LCPUFA are useful as a source of n–3 polyunsaturated fatty acids. The requirement is 50–60 mg of n–3 PUFA/100 g of diet from brain phospholipids to restore a normal brain fatty acid composition in α-linolenic acid-deficient developing rats. Studies have reported that infants fed formulas containing only the precursors of n–3 and n–6 polyunsaturated fatty acids have lower concentrations of DHA in erythrocytes (21) and in cerebral cortex (22) compared to infants fed human milk providing both precursors and n–6 and n–3 LCPUFA. Thus, much research has been performed to investigate the effect of supplementation with DHA and AA as they are important for normal visual and brain development (23, 24).

The aim of this study was to establish the fatty acid composition of different brain regions in adult mice. We therefore investigated the effects of a diet deficient in α-linolenic acid followed or not by supplementation with n–3 LCPUFA. The effectiveness of different sources of phospholipids rich in DHA was examined by comparing purified phospholipids: those from hen egg yolks or pig brains.

The fatty acid profile of total phospholipids was determined in the olfactory bulb, frontal cortex, striatum, hypothalamus, occipital cortex, thalamus, hippocampus, cerebellum, midbrain, pons medulla and pituitary gland.

MATERIALS AND METHODS

Animals and diets

Female OF1 mice originating from IFFA-CREDO (L'Arbresle, France) and bred in our laboratory were divided into two groups 3 weeks before mating. The two groups were fed purified diets (INRA, Jouy en Josas) containing 6% lipids that were similar except for fatty acids. Control diet lipids (CTL group) were a mixture of peanut oil and rapeseed oil containing ~1200 mg of linoleic acid and ~200 mg of α-linolenic acid per 100 g of diet. α-Linolenic acid-deficient diet lipids (DEF group) were peanut oil containing ~1200 mg of linoleic acid per 100 g of diet and traces of α-linolenic acid (<6 mg per 100 g of diet). Pups were fed the same diet as their dams. At weaning, pups were separated according to sex and were housed in such a way that each home cage contained 6 pups from 6 different dams. At 7 weeks, i.e., adulthood, some of the deficient group received a diet supplemented with ~50 mg of n–3 PUFA per 100 g of diet for 2 months. This supplemented diet contained 40 mg of docosahexaenoic acid per 100 g of diet provided by either egg yolk phospholipids (E-PL group) or pig brain phospholipids (B-PL group). Hens were fed a special diet designed to increase DHA level in egg yolk phospholipid from 3% to 7%. Cerebral phospholipids were purified from pig brains and adsorbed onto an aerosyl (silica powder) matrix according to the method previously described (20). These phospholipids were prepared by Laboratoires Ponroy (Les Clayes sous Bois, France). The composition of diets and the fatty acids contents are reported in **Table 1** and **Table 2**. Diets were prepared monthly and stored at 4°C.

Mice were housed in an air-conditioned animal room illuminated from 8 am to 8 pm and maintained at 21 ± 1°C. They were given free access to their respective diets and water.

Male mice aged 4 months were killed by decapitation. The brains were quickly removed and dissected on ice into olfactory bulb (OB), frontal cortex (FC), striatum (ST), hypothalamus (HT), occipital cortex (OC), thalamus (TH), hippocampus (HC), cerebellum (CB), midbrain (MB), pons medulla (PM), and pituitary (PIT). There were 6 samples for each structure per diet group; each sample was pooled from 2 or 5 mice according to weight of the structure. Samples were lyophilized and stored at −70°C until fatty acid analysis was performed. Experimental protocols were approved and met government guidelines (Ministry of Agriculture, Authorization no. 03007; June 4 1991).

Fatty acid analyses

Lipids were extracted from the brain regions using chloroform–methanol 2:1 according to the method of Folch, Lees, and

TABLE 1. Diet composition

	Control	n–3 Deficient	Egg PL[a]	Brain PL[b]
		g/kg		
Casein	220	220	220	220
Corn starch	432.3	432.3	432.3	432.3
Saccharose	216.1	216.1	216.1	216.1
Cellulose	20	20	20	20
Mineral mixture[c]	40	40	40	40
Vitamin mixture[d]	10	10	10	10
dl methionine	1.6	1.6	1.6	1.6
Peanut oil	30	60	48.7	49.9
Rapeseed oil	30	—	—	—
Egg PL	—	—	16.2	—
Brain PL	—	—	—	20.3

[a] Egg yolk phospholipids.
[b] Cerebral phospholipids.
[c] Composition of the mineral mixture (g/kg of diet): $CaHPO_4$, $2H_2O$, 15.2; K_2HPO_4, 9.6; $CaCO_3$, 7.2; NaCl, 2.76; MgO, 0.8; $MgSO_4$, $7H_2O$, 3.6; $FeSO_4,7H_2O$, 0.344; $ZnSO_4,7H_2O$, 0.2; $MnSO_4,H_2O$, 0.2; $CuSO_4,5H_2O$, 0.04; NaF, 0.032; KI, 0.0016; $CoCO_3$, 0.0008; $Na_2SeO_3,5H_2O$, 0.0008; $(NH_4)6Mo7O_{24},4H_2O$, 0.0008; $CrK(SO_4)2$, $12H_2O$, 0.02.
[d] Composition of vitamin supplements triturated in dextrose (mg/kg of diet) United States Biochemicals Corp., Cleveland, OH, USA: l-ascorbic acid, 100; choline chlorhydrate, 750; d-calcium pantothenate, 30; inositol, 50; menadione, 1; nicotinic acid, 45; para-aminobenzoic acid, 50; pyridoxine HCl, 10; riboflavin, 10; thiamine HCl, 10; retinyl acetate, 10; cholecalciferol, 0.0625; d-biotin, 0.2; folic acid, 2; cyanocobalamin, 0.0135; dl-alpha-tocopherol acetate, 50.

TABLE 2. Fatty acid composition of dietary lipids

Fatty Acids	Control	n−3 Deficient	Egg PL	Brain PL
		mg/100 mg fatty acids		
14:0	0.5	0.4	0.4	0.3
16:0	9.0	12.5	14.0	12.2
18:0	3.2	4.0	5.6	5.4
20:0	0.9	1.5	0.7	1.3
22:0	1.3	2.5	1.9	2.2
24:0	—	—	—	0.8
Σ SFA	14.9	20.9	22.6	22.2
16:1 n−7	—	—	0.6	1.0
18:1 n−9	56.7	50.4	51.3	50.7
20:1 n−9	1.2	1.1	1.1	1.2
Σ MFA	57.9	51.5	53.0	52.9
18:2 n−6	23.6	27.8	23.5	23.5
20:4 n−6	—	—	0.3	0.7
Σ n−6 PUFA	23.6	27.8	23.8	24.2
18:3 n−3	3.6	<0.1	<0.1	<0.1
20:5 n−3	—	—	<0.1	<0.1
22:5 n−3	—	—	<0.1	<0.1
22:6 n−3	—	—	0.7	0.7
Σ n−3 PUFA	3.6	—	0.7	0.7
n−6 + n−3	27.2	27.8	24.5	24.9
n−6/n−3	6.5	—	34.0	34.6

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Egg PL, egg yolk phospholipids; Brain PL, cerebral phospholipids.

Sloane Stanley (25). Total phospholipids were separated by thin-layer chromatography using silica gel plates (Durasil-25, Macherey-Nagel, Hoerdt, France). Solvents were hexan, diethyl ether, acetic acid (90:30:1, by vol). Phospholipid fatty acids were transesterified according to the method of Lepage and Roy (26). Fatty acid methyl esters were analyzed on a Delsi gas chromatograph equipped with a flame ionization detector and a silica capillary column (length 30 m, internal diameter 0.25 mm, stationary phase SPB-PUFA, SUPELCO, France). Helium was used as the carrier gas. Analysis was performed in isothermal mode, the oven temperature was maintained at 210°C, the injector and detector temperatures were maintained respectively at 230°C and 250°C. Fatty acids were identified by comparison with standard mixtures. Areas were calculated with a Merck-Hitachi 2500 integrator, and fatty acid concentrations were reported as percent of total fatty acid content.

Statistical analyses

Two-way analysis of variance (ANOVA) with two factors, brain structures and diet (SigmaStat Software, SPSS), was used to evaluate the differences between brain structures, the effect of diet and the interaction structure and diet for each fatty acid. The Tukey test was performed for multiple comparisons. Only P values less than 0.01 were considered significant. Percentages were compared by one-way ANOVA, the Bonferroni post test was then applied with $P < 0.05$. All values are given as means ± standard deviation of the mean.

RESULTS

Saturated fatty acids

The saturated fatty acid (SFA) profile of 4 diet groups is given in **Table 3**. There was a significant difference among the different brain structures ($P < 0.001$). No effect of diet and no interaction between diet and brain structures were observed. As there was not an effect of diet, the control group was taken as reference in order to assess significant differences among brain regions. The level of total SFA was significantly higher in hippocampus (HC), occipital cortex (OC), and frontal cortex (FC) than the other structures, mainly pons medulla (PM) ($P < 0.01$). The main difference was the amount of 16:0 which represented about 26% in HC, OC, FC, and 15% in PM. On the other hand, PM contained more long-chain SFA (22:0, 24:0) than midbrain (MB) and cerebellum (CB). Thus, the level of SFA, in particular that of palmitic acid, varied according to brain structures and was not influenced by the diet.

Monounsaturated fatty acids

The level of total monounsaturated fatty acids (MUFA) differed significantly among brain structures ($P < 0.001$). As for SFA, there were no effect of diet and no region-diet interaction. The regions most rich in MUFA were pons medulla (PM), midbrain (MB) and cerebellum (CB). In comparison, frontal cortex (FC) and pituitary gland (PIT) had low levels. The difference between these regions was due to the amount of n−9 fatty acids (18:1 n−9, 20:1 n−9, 24:1 n−9) (**Table 4**). Thus, oleic acid, the major MUFA, and its derivatives were distributed differently in brain regions and were not affected by the diet.

Polyunsaturated fatty acids (PUFA)

n−6 PUFA. The concentration of n−6 PUFA varied significantly among the different brain regions ($P < 0.001$). In the control group, the region with the highest amount was the pituitary gland (PIT). Cerebellum (CB), pons medulla (PM), and midbrain (MB) had lower concentrations. The difference was mainly due to 20:4 n−6 and less to 22:4 n−6 (**Table 5**). There was a significant effect of diet ($P < 0.001$). In the deficient group there was a significant increase in 22:5 n−6 in all regions ($P < 0.001$), in 22:4 n−6 in PIT and CB, and no change in 20:4 n−6 except for PIT. Supplementation with egg yolk phospholipids (E-PL group) or cerebral phospholipids (B-PL group) decreased equally the 22:5 n−6 level to that of the control group.

n−3 PUFA. n−3 PUFA were distributed differently in brain regions ($P < 0.001$). The frontal cortex (FC) had a higher percentage of 22:6 n−3 (22%) compared with other regions ($P < 0.01$). The lowest percentage was in PIT. The level of 22:6 n−3 was significantly decreased in all regions by the deficient diet. Supplementation with egg yolk phospholipids or cerebral phospholipids restored optimal levels of DHA in all regions except FC, in which the percentage remained significantly different from the control group ($P < 0.01$).

Effects of diets in different regions of the brain

There was a significant interaction between brain regions and diet for the n−6 PUFA and n−3 PUFA ($P < 0.001$). This indicates that the effect of diet did not affect all regions similarly. The percentage of n−3 fatty acids declined 70% for pituitary gland (PIT), about 40% for fron-

TABLE 3. Saturated fatty acid composition of brain regions

Group	OB	FC	ST	HT	TH	OC	HC	CB	MB	PM	PIT
						% total fatty acids					
16:0											
CTL	21.6 ± 0.9	24.9 ± 1.3	21.6 ± 2.5	20.1 ± 1.0	21.4 ± 1.0	25.9 ± 3.0	27.5 ± 3.1	20.5 ± 1.9	17.7 ± 1.5	15.2 ± 0.6	23.8 ± 1.2
DEF	24.5 ± 3.6	25.8 ± 1.1	22.5 ± 3.8	21.2 ± 1.1	21.0 ± 1.0	26.3 ± 2.6	27.6 ± 4.0	20.4 ± 1.5	17.5 ± 1.4	15.5 ± 0.9	23.4 ± 1.0
E-PL	23.9 ± 3.0	24.1 ± 1.4	21.7 ± 2.2	20.7 ± 0.9	20.9 ± 0.6	25.4 ± 2.8	26.8 ± 4.1	19.8 ± 1.4	16.9 ± 1.5	15.1 ± 1.4	23.0 ± 0.9
B-PL	22.9 ± 2.6	24.5 ± 1.5	21.3 ± 2.7	20.6 ± 0.9	20.7 ± 0.4	24.2 ± 1.7	26.7 ± 3.9	19.9 ± 1.5	16.7 ± 1.0	15.0 ± 1.4	23.1 ± 0.5
18:0											
CTL	22.5 ± 0.4	22.0 ± 0.4	20.9 ± 0.9	22.1 ± 0.5	22.0 ± 0.5	21.5 ± 1.0	20.9 ± 0.9	19.7 ± 0.2	20.9 ± 0.2	18.0 ± 0.2	19.4 ± 0.6
DEF	21.3 ± 1.5	22.1 ± 0.5	20.4 ± 1.2	22.0 ± 0.8	22.5 ± 0.4	21.7 ± 0.7	20.8 ± 1.5	19.3 ± 0.4	20.5 ± 0.4	18.5 ± 0.7	19.8 ± 0.3
E-PL	21.8 ± 0.9	22.0 ± 0.3	21.3 ± 0.8	22.0 ± 0.2	22.5 ± 0.5	21.2 ± 0.3	21.2 ± 1.3	19.6 ± 0.2	20.3 ± 0.3	17.9 ± 0.6	19.7 ± 0.3
B-PL	22.2 ± 0.6	21.8 ± 0.2	21.0 ± 1.3	22.3 ± 0.3	22.3 ± 0.3	22.0 ± 0.7	21.8 ± 1.3	19.7 ± 0.7	20.7 ± 0.4	18.3 ± 0.6	19.6 ± 0.4
All others											
CTL	1.0 ± 0.1	0.3 ± 0.1	2.0 ± 0.4	1.3 ± 0.1	1.1 ± 0.1	0.7 ± 0.2	0.3 ± 0.1	2.3 ± 0.4	2.5 ± 0.2	4.6 ± 0.3	1.6 ± 0.2
DEF	0.8 ± 0.1	0.3 ± 0.1	2.2 ± 0.8	1.1 ± 0.1	1.1 ± 0.1	0.7 ± 0.2	0.3 ± 0.1	2.5 ± 0.3	3.0 ± 0.5	5.2 ± 0.2	1.6 ± 0.2
E-PL	0.8 ± 0.1	0.3 ± 0.1	1.5 ± 0.3	1.2 ± 0.3	1.2 ± 0.2	0.8 ± 0.1	0.5 ± 0.2	2.3 ± 0.5	2.7 ± 0.2	4.7 ± 0.6	1.6 ± 0.1
B-PL	0.9 ± 0.1	0.4 ± 0.1	1.6 ± 0.2	1.1 ± 0.1	0.9 ± 0.1	0.8 ± 0.2	0.4 ± 0.2	2.3 ± 0.4	2.7 ± 0.3	5.1 ± 0.4	1.6 ± 0.2
Σ SFA											
CTL	45.6 ± 0.6^{b}	47.1 ± 1.2^{a}	44.5 ± 2.0^{b}	43.5 ± 0.8^{b}	44.5 ± 0.8^{b}	48.1 ± 2.1^{a}	48.8 ± 2.5^{a}	42.4 ± 1.6^{b}	41.1 ± 1.3^{b}	37.7 ± 0.2^{c}	44.8 ± 1.4^{b}
DEF	46.6 ± 2.1	48.2 ± 1.2	45.1 ± 3.0	44.3 ± 0.5	44.6 ± 0.9	48.7 ± 2.1	48.7 ± 2.6	42.2 ± 1.5	40.9 ± 1.3	39.2 ± 1.3	44.7 ± 1.3
E-PL	46.5 ± 2.2	46.5 ± 1.1	44.6 ± 1.6	43.9 ± 0.8	44.3 ± 0.7	47.3 ± 2.7	48.9 ± 3.0	41.8 ± 1.2	39.9 ± 1.6	38.2 ± 1.8	44.4 ± 0.8
B-PL	46.0 ± 2.4	46.5 ± 1.3	43.9 ± 1.9	44.1 ± 0.9	44.2 ± 0.5	47.1 ± 2.3	48.4 ± 2.8	41.9 ± 1.4	37.9 ± 1.9	37.9 ± 1.9	44.3 ± 1.2

Values are mean ± SD. In control group, values with different letters are significantly different from each other ($P < 0.01$). Brain regions: OB, olfactory bulb; FC, frontal cortex; ST, striatum; HT, hypothalamus; OC, occipital cortex; TH, thalamus; HC, hippocampus; CB, cerebellum; MB, midbrain; PM, pons medulla; PIT, pituitary gland. Diet groups: CTL, control; DEF, n–3 PUFA-deficient; E-PL, egg yolk phospholipid supplemented; and B-PL, cerebral phospholipid-supplemented.

TABLE 4. Monounsaturated fatty acid composition of brain regions

Group	OB	FC	ST	HT	TH	OC	HC	CB	MB	PM	PIT
						% total fatty acids					
18:1 n–9											
CTL	14.2 ± 0.3	12.4 ± 0.4	16.5 ± 0.4	16.9 ± 0.5	15.3 ± 0.3	13.9 ± 0.4	13.9 ± 0.5	18.5 ± 0.8	19.4 ± 0.5	22.6 ± 0.9	12.7 ± 0.3
DEF	14.1 ± 0.4	12.5 ± 0.8	16.7 ± 0.5	15.9 ± 0.4	14.4 ± 0.3	12.8 ± 0.4	13.2 ± 0.2	17.7 ± 1.2	18.4 ± 0.7	21.2 ± 0.9	11.8 ± 0.5
E-PL	14.2 ± 0.3	13.7 ± 0.4	16.2 ± 0.5	16.8 ± 0.3	14.8 ± 0.3	14.0 ± 0.5	13.9 ± 0.5	18.9 ± 0.5	19.9 ± 0.9	22.5 ± 0.9	11.6 ± 0.4
B-PL	14.5 ± 0.3	13.0 ± 0.9	16.7 ± 0.4	16.6 ± 0.3	15.2 ± 0.2	13.7 ± 0.4	13.8 ± 0.5	18.2 ± 1.1	19.2 ± 0.5	22.6 ± 0.9	11.6 ± 0.5
18:1 n–7											
CTL	4.9 ± 0.1	4.4 ± 0.6	4.8 ± 0.8	4.8 ± 1.0	4.5 ± 0.7	4.0 ± 0.5	4.4 ± 0.4	5.4 ± 0.4	4.7 ± 0.7	5.2 ± 0.8	3.5 ± 0.5
DEF	4.7 ± 0.4	5.0 ± 0.5	5.2 ± 0.6	4.9 ± 0.6	5.0 ± 0.7	4.2 ± 0.5	4.8 ± 0.3	6.1 ± 0.7	5.1 ± 0.9	4.9 ± 0.5	3.5 ± 0.4
E-PL	4.6 ± 0.6	4.6 ± 0.7	5.0 ± 0.8	5.0 ± 0.4	4.7 ± 0.7	4.1 ± 0.7	4.5 ± 0.5	5.6 ± 0.6	4.9 ± 1.0	4.6 ± 0.5	3.2 ± 0.5
B-PL	4.8 ± 0.5	4.6 ± 0.5	5.4 ± 0.5	4.4 ± 0.7	4.6 ± 0.9	4.4 ± 0.6	4.5 ± 0.5	5.9 ± 0.6	5.3 ± 0.8	5.1 ± 1.1	3.4 ± 0.5
20:1 n–9											
CTL	1.2 ± 0.2	0.5 ± 0.2	2.3 ± 0.3	1.6 ± 0.1	1.6 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	3.1 ± 0.2	3.4 ± 0.4	5.9 ± 0.2	0.8 ± 0.1
DEF	1.0 ± 0.1	0.5 ± 0.1	2.3 ± 0.3	1.7 ± 0.1	1.6 ± 0.1	0.8 ± 0.2	0.6 ± 0.1	3.3 ± 0.4	3.7 ± 0.3	5.8 ± 0.4	0.7 ± 0.1
E-PL	1.0 ± 0.1	0.7 ± 0.1	2.2 ± 0.2	1.4 ± 0.1	1.5 ± 0.1	0.9 ± 0.1	0.4 ± 0.2	3.2 ± 0.1	4.0 ± 0.4	6.1 ± 0.6	0.6 ± 0.1
B-PL	1.1 ± 0.1	0.6 ± 0.1	2.2 ± 0.3	1.7 ± 0.1	1.6 ± 0.1	0.9 ± 0.1	0.6 ± 0.2	3.0 ± 0.3	3.7 ± 0.2	5.7 ± 0.1	0.7 ± 0.05
24:1 n–9											
CTL	1.2 ± 0.1	0.4 ± 0.2	3.7 ± 1.0	2.0 ± 0.3	2.3 ± 0.2	1.1 ± 0.2	0.7 ± 0.1	3.6 ± 0.2	4.9 ± 0.3	7.6 ± 0.4	0.9 ± 0.1
DEF	1.2 ± 0.3	0.4 ± 0.1	3.7 ± 0.3	1.9 ± 0.3	2.0 ± 0.2	0.7 ± 0.2	0.6 ± 0.1	3.4 ± 0.6	5.1 ± 0.3	7.8 ± 0.7	0.9 ± 0.1
E-PL	1.0 ± 0.3	0.7 ± 0.3	2.6 ± 0.5	1.6 ± 0.2	1.7 ± 0.1	1.0 ± 0.2	0.5 ± 0.1	3.3 ± 0.3	5.0 ± 0.5	7.9 ± 0.5	0.8 ± 0.2
B-PL	1.3 ± 0.2	0.6 ± 0.2	3.2 ± 0.5	2.0 ± 0.2	1.9 ± 0.2	1.0 ± 0.3	0.6 ± 0.1	3.8 ± 0.7	4.9 ± 0.3	7.5 ± 0.6	0.9 ± 0.1
All Others											
CTL	1.6 ± 0.2	1.3 ± 0.4	2.2 ± 0.2	1.7 ± 0.2	1.5 ± 0.3	2.4 ± 0.2	1.4 ± 0.2	1.9 ± 0.3	2.3 ± 0.3	2.9 ± 0.1	1.6 ± 0.1
DEF	1.7 ± 0.2	1.8 ± 0.6	2.4 ± 0.2	1.8 ± 0.3	1.7 ± 0.3	2.7 ± 0.4	1.5 ± 0.2	1.8 ± 0.3	2.8 ± 0.3	2.8 ± 0.4	1.6 ± 0.3
E-PL	1.6 ± 0.3	1.6 ± 0.3	2.2 ± 0.4	1.8 ± 0.2	1.6 ± 0.3	2.5 ± 0.4	1.3 ± 0.2	2.1 ± 0.2	2.6 ± 0.2	2.8 ± 0.4	1.6 ± 0.2
B-PL	1.6 ± 0.1	1.5 ± 0.3	2.1 ± 0.4	1.7 ± 0.2	1.5 ± 0.2	2.4 ± 0.4	1.3 ± 0.2	2.0.± 0.2	2.9 ± 0.3	2.9 ± 0.4	1.6 ± 0.2
Σ MFA											
CTL	23.2 ± 0.4^{d}	19.1 ± 1.6^{e}	29.4 ± 0.8^{c}	27.1 ± 1.3^{c}	25.0 ± 1.1^{d}	22.3 ± 0.7^{d}	20.9 ± 1.1^{d}	32.6 ± 1.1^{b}	34.8 ± 1.2^{b}	44.1 ± 0.8^{a}	19.4 ± 0.6^{e}
DEF	22.7 ± 0.6	20.2 ± 1.4	30.2 ± 1.1	26.1 ± 0.9	24.6 ± 1.0	21.2 ± 1.0	20.7 ± 0.5	32.3 ± 1.5	35.1 ± 1.7	42.5 ± 1.5	18.6 ± 1.1
E-PL	22.4 ± 0.8	21.2 ± 0.8	28.7 ± 0.7	26.3 ± 0.7	24.3 ± 0.8	22.3 ± 1.5	20.7 ± 1.3	32.9 ± 0.7	36.7 ± 0.5	44.1 ± 1.6	17.8 ± 1.1
B-PL	23.3 ± 0.8	20.4 ± 1.6	29.6 ± 1.5	26.6 ± 1.0	24.8 ± 1.1	22.5 ± 1.4	20.8 ± 1.0	33.1 ± 1.5	35.9 ± 0.7	43.8 ± 1.8	18.2 ± 1.2

Values are mean ± SD. In the control group, values with different letters are significantly different from each other ($P < 0.01$). Diet groups: CTL, control group; DEF, n–3 PUFA-deficient group; E-PL, egg yolk phospholipid supplemented group; B-PL, cerebral phospholipid-supplemented group.

TABLE 5. Polyunsaturated fatty acid composition of brain regions

Group	OB	FC	ST	HT	TH	OC	HC	CB	MB	PM	PIT
						% total fatty acids					
20:4 n−6											
CTL	9.8 ± 0.1	8.7 ± 0.1	8.7 ± 0.9	8.8 ± 0.2	8.4 ± 0.2	8.8 ± 0.9	10.5 ± 0.8	5.7 ± 0.3	6 .3 ± 0.2	4.7 ± 0.2	17.7 ± 0.5
DEF	10.6 ± 0.5	8.9 ± 1.3	8.9 ± 0.6	9.4 ± 0.3	9.2 ± 0.3	9.7 ± 0.8	11.6 ± 0.7	7.1 ± 1.0	6.8 ± 0.3	5.2 ± 0.2	19.5 ± 0.3
E-PL	9.8 ± 0.7	9.4 ± 0.5	9.3 ± 0.5	9.3 ± 0.3	8.6 ± 0.3	9.2 ± 1.0	10.6 ± 0.7	5.8 ± 0.7	6.5 ± 0.8	4.8 ± 0.1	18.4 ± 0.4
B-PL	10.1 ± 0.3	9.0 ± 0.4	8.9 ± 0.3	8.8 ± 0.1	8.9 ± 0.2	9.4 ± 0.4	11.4 ± 0.6	5.9 ± 0.4	6.5 ± 0.2	5.0 ± 0.2	19.0 ± 0.2
22:4 n−6											
CTL	3.0 ± 0.1	2.0 ± 0.2	2.7 ± 0.2	4.0 ± 0.2	3.2 ± 0.2	2.5 ± 0.3	2.3 ± 0.5	1.4 ± 0.3	2.9 ± 0.4	2.9 ± 0.1	7.0 ± 0.2
DEF	3.4 ± 0.3	2.5 ± 0.3	3.2 ± 0.5	4.5 ± 0.2	3.9 ± 0.5	2.9 ± 0.3	2.8 ± 0.4	2.4 ± 0.1*	3.7 ± 0.1	3.8 ± 0.3	9.4 ± 0.2*
E-PL	2.8 ± 0.1	2.4 ± 0.2	2.8 ± 0.1	4.0 ± 0.1	3.3 ± 0.1	2.6 ± 0.2	2.6 ± 0.3	1.6 ± 0.2	3.0 ± 0.1	2.9 ± 0.4	7.5 ± 0.5
B-PL	2.9 ± 0.3	2.2 ± 0.2	2.7 ± 0.3	4.1 ± 0.2	3.5 ± 0.3	2.6 ± 0.1	2.4 ± 0.2	1.9 ± 0.1	3.2 ± 0.3	3.2 ± 0.3	7.7 ± 0.2
22:5 n−6											
CTL	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.05	0.3 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.8 ± 0.1	0.5 ± 0.1
DEF	4.3 ± 0.4*	6.7 ± 0.6*	3.7 ± 0.4*	3.4 ± 0.5*	4.7 ± 0.5*	5.5 ± 0.4*	4.8 ± 0.5*	2.9 ± 0.4*	2.5 ± 0.4*	2.8 ± 0.3*	3.1 ± 0.1*
E-PL	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.7 ± 0.5	0.2 ± 0.1	0.1 ± 0.04	0.5 ± 0.03	0.6 ± 0.1
B-PL	0.5 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.6 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.2 ± 0.03	0.2 ± 0.1	0.7 ± 0.2	0.8 ± 0.1
All others											
CTL	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	0.6 ± 0.02	0.6 ± 0.1	0.5 ± 0.1	0.8 ± 0.2	0.5 ± 0.1	0.8 ± 0.1	3.0 ± 0.2
DEF	0.4 ± 0.1	0.6 ± 0.2	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.07	0.4 ± 0.1	0.6 ± 0.3	0.4 ± 0.1	0.5 ± 0.2	2.4 ± 0.1
E-PL	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.5 ± 0.02	0.7 ± 0.1	0.6 ± 0.2	0.7 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	2.8 ± 0.2
B-PL	0.6 ± 0.1	0.8 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.9 ± 0.2	0.6 ± 0.2	0.7 ± 0.1	3.0 ± 0.1
Σ (n−6)											
CTL	13.7 ± 0.3^b	11.7 ± 0.5^b	12.2 ± 1.2^b	13.6 ± 0.3^b	12.4 ± 0.4^b	12.2 ± 1.4^b	13.5 ± 1.4^b	8.0 ± 0.5^c	9.9 ± 0.6^c	9.2 ± 0.2^c	28.2 ± 0.8^a
DEF	18.7 ± 1.2*	18.6 ± 1.8*	16.3 ± 1.5*	17.6 ± 0.7*	18.2 ± 0.9*	18.6 ± 1.1*	19.5 ± 1.6*	13.0 ± 0.4*	13.4 ± 0.5*	12.2 ± 0.4*	34.4 ± 0.6*
E-PL	13.7 ± 0.8	12.8 ± 0.4	13.2 ± 0.7	14.2 ± 0.4	13.0 ± 0.4	13.0 ± 1.3	14.5 ± 1.5	8.5 ± 0.7	10.2 ± 0.9	8.9 ± 0.5	29.6 ± 1.0
B-PL	14.1 ± 0.7	12.5 ± 0.5	12.8 ± 0.4	14.0 ± 0.2	13.5 ± 0.5	13.2 ± 0.5	14.9 ± 0.9	8.7 ± 0.5	10.5 ± 0.4	9.5 ± 0.6	30.3 ± 0.3
22:6 n−3											
CTL	18.1 ± 0.1^b	22.1 ± 0.8^a	13.9 ± 0.9^c	15.8 ± 0.6^b	18.1 ± 0.6^b	17.4 ± 0.7^b	16.8 ± 0.5^b	17.0 ± 0.7^b	14.2 ± 0.3^c	9.0 ± 0.8^d	6.6 ± 0.2^d
DEF	12.0 ± 1.2*	13.0 ± 1.0‡	8.4 ± 0.9*	12.0 ± 0.5*	12.6 ± 0.6*	11.5 ± 0.9*	11.1 ± 1.1*	12.6 ± 0.5*	10.6 ± 0.9*	6.1 ± 0.8*	2.2 ± 0.2*
E-PL	17.4 ± 0.7	19.5 ± 0.7‡	14.0 ± 0.5	15.6 ± 0.3	18.4 ± 0.4	17.4 ± 0.9	15.9 ± 1.5	16.8 ± 0.6	13.2 ± 0.7	8.8 ± 0.3	7.7 ± 0.2
B-PL	16.6 ± 1.1	20.6 ± 0.8‡	13.7 ± 0.6	15.3 ± 0.3	17.5 ± 0.6	17.2 ± 0.9	15.9 ± 1.3	16.3 ± 0.7	13.5 ± 0.3	8.8 ± 0.6	6.9 ± 0.2

Values are mean ± SD. Differences between regions: values with different letters are significantly different from each other ($P < 0.01$). Diet groups: CTL, control; DEF, n−3 PUFA-deficient; E-PL, egg yolk phospholipid-supplemented; B-PL, cerebral phospholipid-supplemented.

Effect of diet: * $P < 0.01$ versus CTL, E-PL, and B-PL groups; ‡ $P < 0.01$ versus control group.

tal cortex (FC) and striatum (ST), and 25% for cerebellum (CB) in the deficient group (**Fig. 1**). After PIT, regions the most marked by a diet deficient in n−3 PUFA were CF and ST. The percentage of increase in 22:5 n−6 in the deficient group varied from region to region. The greatest increase was in HC, the lowest in PIT and PM.

In order to visualize the effect of n−3 PUFA supplementation, the average of 22:5 n−6 and 22:6 n−3 levels of two supplemented groups was calculated for each brain region. Then, the percentages of increase in 22:5 n−6 and decrease in 22:6 n−3 versus controls was represented. Phospholipid supplementation cancels the effect of deficiency in all brain regions. Although the recovery appears not to be complete in several regions, there was no significant difference between egg or brain phospholipid-supplemented groups and controls, except for DHA level in the frontal cortex.

DISCUSSION

This study examined the effects of a diet deficient in or supplemented with n−3 PUFA on the fatty acid composition of different brain regions in adult mice. First of all, the fatty acid composition of total phospholipids in different brain regions in control mice was established. Saturated fatty acid (SFA) levels were higher in the hippocampus, frontal and occipital cortex than in the midbrain or pons medulla. In contrast, the latter two regions together with the cerebellum were rich in monounsaturated fatty acids (MUFA). The differences between regions depend on their amount of gray or white matter. It is known that the cerebral white matter is rich in SFA and particularly in (n−9) MUFA and poor in PUFA (27). The level of n−6 PUFA varied, it was very high in pituitary gland as already reported (28). The region with the highest DHA level was the frontal cortex. These results agree with those observed in rats by Delion et al. (9) who found more DHA in frontal cortex than striatum.

There was no effect of diet on SFA or MUFA levels. In all brain regions studied in the α-linolenic acid deficient mice, our results showed a reduced 22:6 n−3 (DHA) level and compensatory higher n−6 levels, especifically 22:5 n−6. There were no variations of 20:4 n−6 or 22:4 n−6, except for pituitary gland. These results are in agreement with those for whole brain in this mouse strain (11). Nevertheless, the α-linolenic acid deficiency did not affect the levels of DHA in brain regions to the same extent. The most affected structure was the pituitary gland with a 70% reduction of DHA. However, n−3 PUFA levels in pituitary gland were low, the major PUFA being 20:4 n−6 (AA).

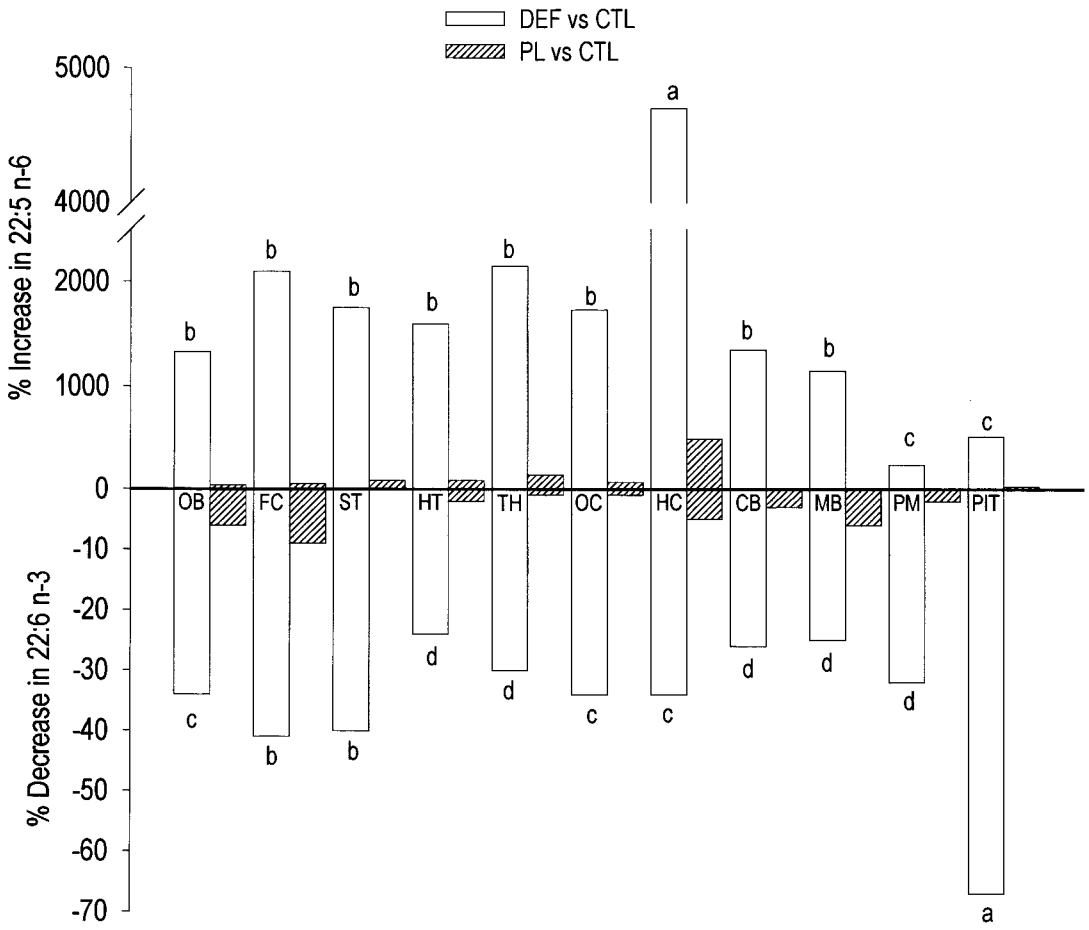

Fig. 1. Percentage of increase in 22:5 n−6 and decrease in 22:6 n−3 in n−3 PUFA-deficient mice compared with control. Different letters were assigned when percentages differed significantly with $P < 0.05$ among regions. The effect of n−3 PUFA supplementation is represented.

The only two studies on the pituitary gland have investigated the effect of both linoleic and α-linolenic acid deficiency in rats (29, 30). The authors observed changes mainly in n−6 PUFA related to modifications in growth hormone. Owing to the low level of DHA and the involvement of AA in pituitary hormonal regulation, it can be supposed that α-linolenic acid deficiency has no profound effects on pituitary function. In regions with greater amounts of gray matter, the level of DHA was lower, about 40% in frontal cortex or striatum, and 34% in olfactory bulb, occipital cortex, and hippocampus. A chronic α-linolenic acid deficiency in rats led to a greater decrease in the level of DHA in plasmenylethanolamine in frontal cortex than striatum (31). Moreover, the specific markers of dopaminergic and serotoninergic neurotransmission are only modified in frontal cortex in deficient rats (9). Thus, frontal cortex, which contains a higher level of DHA, appears very sensitive to n−3 PUFA deficiency. In hippocampus, no effect of α-linolenic acid deficiency was observed on noradrenaline or serotoninergic levels in rats (32). Nevertheless, Yoshida et al. (17) have reported that a deficiency in α-linolenic acid affects synaptic vesicle turnover in the hippocampal CA1 region that is associated with reduced brightness-discrimination learning in rats. It has been shown in CA1 neurons isolated from rat hippocampus that n−3 PUFA modulates sodium and calcium currents (33). As the hippocampus is involved in learning processes via the cholinergic system, studies of the effects of n−3 PUFA deficiency on cholinergic activity will be interesting (34, 35). In rats, olfactory bulbectomy induces behavioral changes such as hyperactivity and deficits in avoidance learning associated with alterations of the serotoninergic and dopaminergic systems (36, 37). For the same reason as for frontal cortex, a modification of fatty acid composition might lead to neurochemical alterations in the olfactory bulb.

On the other hand, it is important to note that the decrease in 22:6 n−3 is not mirrored by the increase in 22:5 n−6 in the deficient group. Indeed, the increase in 22:5 n−6 was the greatest in the hippocampus and the lowest in pons medulla. Thus, the highest decrease in 22:6 n−3 was not compensated by the highest increase in 22:5 n−6 and vice versa. There was no correspondence between the decrease of 22:6 n−3 and the increase of 22:5 n−6 in brain regions.

Supplementation with egg yolk phospholipids (E-PL) or cerebral phospholipids (B-PL), in α-linolenic acid-deficient mice increased DHA concentration to control levels in all brain regions except frontal cortex, where the difference remained significant. Thus, a 2-month diet with phospho-

lipids as source of n−3 PUFA restored a normal fatty acid composition of brain regions in deficient mice. 22:5 n−6 levels were decreased in all brain regions. Although B-PL provided 2-fold more arachidonic acid than E-PL, arachidonic acid levels did not differ between the two supplemented groups. Thus, either brain phospholipids or egg phospholipids can be used as an effective source of n−3 PUFA.

In conclusion, our results show that the distribution of fatty acids in the brain is region-specific. The cerebral cortex regions, such as the frontal cortex where the DHA level is very high, were particularly affected by n−3 PUFA deficiency. In α-linolenic acid-deficient mice, supplementation for 2 months with egg yolk phospholipids or cerebral phospholipids proved effective for restoring normal fatty acid composition in brain. These conclusive biochemical results now warrant investigation of whether supplementation for 2 months with egg yolk phospholipids or cerebral phospholipids can correct the behavioral deficits induced by n−3 PUFA deficiency in OF1 mice.

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REFERENCES

1. Bourre, J. M., G. Pascal, G. Durand, M. Masson, O. Dumont, and M. Piciotti. 1984. Alterations in the fatty acid composition of rat brain cells (neurons, astrocytes, and oligodendrocytes) and of subcellular fractions (myelin and synaptosomes) induced by a diet devoid of n−3 fatty acids. *J. Neurochem.* **43:** 342−348.
2. Neuringer, M., J. Anderson, and W. E. Connor. 1988. The essentiality of n-3 fatty acids for the development and function of the retina and brain. *Annu. Rev. Nutr.* **8:** 517−541.
3. Scott, B. L., and N. Bazan. 1989. Membrane docosahexaenoate is supplied to the developing brain and retina by the liver. *Proc. Natl. Acad. Sci. USA.* **86:** 2903−2907.
4. Clandinin, M. T., and J. Jumpsen. 1997. Fatty acid metabolism in brain in relation to development, membrane structure, and signaling. *In* Handbook of Essential Fatty Acid Biology. S. Yehuda and D I. Mostofsky, editors. Humana Press, Totowa, NJ. 15−65.
5. Foot, M., T. F. Cruz, and M. T. Clandinin. 1982. Influence of dietary fat on the lipid composition of rat brain synaptosomal and microsomal membranes. *Biochem. J.* **208:** 631−640
6. Bourre, J. M., M. François, A. Youyou, O. Dumont, M. Piciotti, G. Pascal, and G. Durand. 1989. The effects of dietary alpha-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.
7. Yamamoto, N., A. Hashimoto, Y. Takemoto, H. Okuyama, M. Nomura, R. Kitajima, T. Togashi, and Y. Tamai. 1988. Effect of the dietary alpha-linolenate/linoleate balance on lipid compositions and learning ability of rats. Discrimination process, extinction process, and glycolipid compositions. *J. Lipid Res.* **29:** 1013−1021.
8. Nakashima, Y., S. Yuasa, Y. Hukamisu, H. Okuyama, T. Ohhara, T. Kameyama, and T. Nabeshima. 1993. Effect of a high linoleate and a high alpha-linolenate diet on general behavior and drug sensitivity in mice. *J. Lipid Res.* **34:** 239−247.
9. Delion, S., S. Chalon, J. Hérault, D. Guilloteau, J. C. Besnard, and G. Durand. 1994. Chronic dietary alpha-linolenic acid deficiency alters dopaminergic and serotoninergic neurotransmission in rats. *J. Nutr.* **124:** 2466−2476.
10. Jumpsen, J., E. Elien, Y. Goh, and M. Clandinin. 1997. Small changes of dietary (n-6) and (n-3)/fatty acid content ratio alter phosphatidylethanolamine and phosphatidylcholine fatty acid composition during development of neuronal and glial cells in rats. *J. Nutr.* **127:** 724−731.
11. Francès, H., C. Monier, and J. M. Bourre. 1995. Effects of dietary alpha-linolenic acid deficiency on neuromuscular and cognitive functions in mice. *Life Sci.* **57:** 1935−1947.
12. Francès, H., C. Monier, M. Clément, A. Lecorsier, M. Debray, and J. M. Bourre. 1996. Effect of dietary alpha-linolenic acid deficiency on habituation. *Life Sci.* **58:** 1805−1816.
13. Francès, H., J. P. Coudereau., P. Sandouk, M. Clément, C. Monier, and J. M. Bourre. 1996. Influence of a dietary alpha-linolenic acid deficiency on learning in the Morris water maze and on the effects of morphine. *Eur. J. Pharmacol.* **298:** 217−225.
14. Carrié, I., M. Clément, D. de Javel, H. Francès, and J. M. Bourre. 1999. Learning deficits in first generation OF1 mice deficient in (n-3) polyunsaturated fatty acids do not result from visual alteration. *Neurosci. Lett.* **266:** 69−72.
15. Morris, R. G., P. Garrud, J. N. Rawlins, and J. O'Keefe. 1982. Place navigation impaired in rats with hippocampal lesions. *Nature.* **297:** 681−683.
16. Brandeis, R., Y. Brandys, and S. Yehuda. 1989. The use of the Morris water maze in the study of memory and learning. *Int. J. Neurosci.* **48:** 29−69.
17. Yoshida, S., A. Yasuda, H. Kawasato, K. Sakai, T. Shimada, M. Takeshita, S. Yuasa, T. Kobayashi, S. Watanabe, and H. Okuyama. 1997. Synaptic vesicle ultrastructural changes in the rat hippocampus induced by a combination of alpha-linolenate deficiency and a learning task. *J. Neurochem.* **68:** 1261−1268.
18. Youyou, A., G. Durand, G. Pascal, M. Piciotti, O. Dumont, and J. M. Bourre. 1986. Recovery of altered fatty acid composition induced by a diet devoid of n-3 fatty acids in myelin, synaptosomes, mitochondria, and microsomes of developing rat brain. *J. Neurochem.* **46:** 224−228.
19. Bourre, J. M., O. Dumont, G. Pascal, and G. Durand. 1993. Dietary alpha-linolenic acid at 1.3 g/kg maintains maximal docosahexaenoic acid concentration in brain, heart and liver of adult rats. *J. Nutr.* **123:** 1313−1319.
20. Bourre, J. M., O. Dumont, and G. Durand. 1993. Brain phospholipids as dietary source of (n-3) polyunsaturated fatty acids for nervous tissue in the rat. *J. Neurochem.* **60:** 2018−2028.
21. Carlson, S. E., P. G. Rhodes, and M. G. Ferguson. 1986. Docosahexaenoic acid status of preterm infants at birth and following feeding with human milk or formula. *Am. J. Clin. Nutr.* **44:** 798−804.
22. Farquharson, J., F. Cockburn, W. A. Patrick, E. C. Jamieson, and R. W. Logan. 1992. Infant cerebral cortex phospholipid fatty acid composition and diet. Lancet. **340:** 810−813.
23. Hamosh, M., and N. J. Salem. 1998. Long-chain polyunsaturated fatty acids. *Biol. Neonate.* **74:** 106−120.
24. Dobbing, J. 1997. Developing Brain and Behaviour. The Role of Lipids in Infant Formula. Academic Press, San Diego, CA.
25. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497−509.
26. Lepage, G., and C. Roy., 1986. Direct transesterification of all classes of lipids in a one-step reaction. *J. Lipid Res.* **27**: 114−120.
27. Kishimoto, Y., B. W. Agranoff, N. S. Radin, and R. M. Burton. 1969. Comparison of the fatty acids of lipids of subcellular brain fractions. *J. Neurochem.* **16:** 397−404.
28. Marteinsdottir, I., D. F. Horrobin, C. Stenfors, E. Theodorsson, and A. A. Mathé. 1998. Changes in dietary fatty acids alter phospholipid fatty acid composition in selected regions of rat brain. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry.* **22:** 1007−1021.
29. Alessio, M. L., D. E. Wandscheer, M. C. F. Soares, H. Clauser, A. Enjalbert, C. Kordon, and C. L. Léger. 1992. Effect of an essential fatty acid deficiency on the phospholipid composition in anterior pituitary membranes. *Biochem. Biophys. Res. Commun.* **183**: 1047−1055.
30. Soares, M. C., M. L. Aléssio, C. L. Léger, M. T. Bluet-Pajot, H. Clauser, A. Enjalbert, C. Kordon, and D. E. Wandscheer. 1995. Effect of an essential fatty acids deficiency on membrane fatty acid

content and growth hormone stimulation of rat pituitaries during postnatal development. *J. Lipid Res.* **36:** 1401–1406.
31. Favrelière, S., L. Barrier, G. Durand, S. Chalon, and C. Tallineau. 1998. Chronic dietary n-3 polyunsaturated fatty acids deficiency affects the fatty acid composition of plasmenylethanolamine and phosphatidylethanolamine differently in rat frontal cortex, striatum, and cerebellum. *Lipids.* **33:** 401–407.
32. Delion, S., S. Chalon, D. Guilloteau, B. Lejeune, J. C. Besnard, and G. Durand. 1997. Age-related changes in phospholipid fatty acid composition and monoaminergic neurotransmission in the hippocampus of rats fed a balanced or an n-3 polyunsaturated fatty acid-deficient diet. *J. Lipid Res.* **38:** 680–689.
33. Vreugdhenil, M., C. Bruehl, R. A. Voskuyl, J. X. Kang, A. Leaf, and W. J. Wadman. 1996. Polyunsaturated fatty acids modulate sodium and calcium currents in CA1 neurons. *Proc. Natl. Acad. Sci. USA.* **93:** 12559–12563.
34. Muir, J. L. 1997. Acetylcholine, aging, and Alzheimer's disease. *Pharmacol. Biochem. Behav.* **56:** 687–696.
35. Felix, R., and E. D. Levin. 1997. Nicotinic antagonist administration into the ventral hippocampus and spatial working memory in rats. *Neuroscience.* **81:** 1009–1017.
36. Van Riezen, H., and B. E. Leonard. 1990. Effects of psychotropic drugs on the behavior and neurochemistry of olfactory bulbectomized rats. *Pharmacol. Ther.* **47:** 21–34.
37. Redmond, A. M., J. P. Kelly, and B. E. Leonard. 1997. Behavioural and neurochemical effects of dizocilpine in the olfactory bulbectomized rat model of depression. *Pharmacol. Biochem. Behav.* **58:** 355–359.