Reversibility of n-3 fatty acid deficiency-induced changes in dopaminergic neurotransmission in rats: critical role of developmental stage

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Abstract Previous investigations have shown that the lipid composition of cerebral membranes and dopaminergic neurotransmission are changed under chronic α-linolenic acid diet deficiency in the rat. This study investigated whether these changes could be reversed and if the stage of brain maturation might play a role in the recovery process. The effects of reversion on the fatty acid (FA) composition and dopaminergic neurotransmission were studied in brain regions known to be affected by such deficiency (i.e., the prefrontal cortex and nucleus accumbens) in 2-month-old animals. Dopamine release under pharmacological stimulation was studied using a dual-probe microdialysis method. Vesicular monoamine transporters were studied using quantitative autoradiography. The reversal diet, with adequate levels of n-6 and n-3 polyunsaturated fatty acids (PUFAs), was given to deficient rats at different stages of development (0, 7, 14, or 21 days of age). The results showed that when given during the lactating period, this diet was able to restore both the FA composition of brain membranes and the parameters of dopaminergic neurotransmission studied. However, when given from weaning, it allowed partial recovery of biochemical parameters but no recovery of neurochemical factors. The occurrence of profound n-3 PUFA deficiency during the lactating period could therefore be an environmental insult leading to irreversible damage to specific brain functions.—Kodas, E., S. Vancassel, B. Lejeune, D. Guilloteau, and S. Chalon. **Reversibility of n-3 FA deficiency-induced changes in dopaminergic neurotransmission in rats: critical role of developmental stage. *J. Lipid Res.* 2002.** 43: **1209–1219.**

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The biochemical composition of brain membranes is characterized by large amounts of long chain polyunsaturated fatty acids (LC-PUFAs), mainly arachidonic acid (AA, 20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3) (1, 2). These LC-PUFAs and linoleic (18:2n-6) and α-linolenic acid (18:3n-3), the precursors from which they are derived, respectively, must be supplied from exogenous sources in mammals (3). During the prenatal and early postnatal periods, these LC-PUFAs, especially DHA, are actively accumulated in high amounts in the brain, where they are involved in neurogenesis and synaptogenesis (4–7). However, although AA and DHA are present in human milk, contradictory findings have been reported on the beneficial effects of addition of these LC-PUFAs to infant formula on visual acuity (8–10) and mental development (11–13). The precise need for LC-PUFAs during the developmental period for optimal brain function remains therefore to be clarified, and animal models can supply valuable information for this purpose. Numerous studies performed in such models have reported that chronic dietary deficiency in α-linolenic acid greatly affects the fatty acid (FA) composition of cerebral membrane phospholipids (14–17). More recent studies showed that the composition of PUFAs in cerebral membranes was not homogenous throughout the brain and was affected differently in response to n-3 PUFA deficiency according to cerebral region (18, 19). In addition to these biochemical changes, numerous studies have demonstrated that α-linolenic acid deficiency in rodents impairs performance in a variety of learning tasks (16, 20–23), and alters several sensory processes such as olfaction (24) and audition (25). We have

Abbreviations: AA, arachidonic acid; DA, dopamine; DHA, docosahexaenoic acid; FA, fatty acid; LC-PUFA, long chain polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; NAcc, nucleus accumbens; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PFCx, prefrontal cortex; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; $VMAT_2$, vesicular monoamine transporter.

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proposed that impaired behavioral responses could involve monoaminergic neurotransmission processes (18). Recent investigations on adult rats chronically deficient in α-linolenic acid showed abnormal functioning of the mesocortical and mesolimbic dopaminergic pathways (26–28). These changes could be related to the effects of n-3 PUFA deficiency on motivation, response to reward, and learning ability (29, 30). Thus, it has now been established that profound n-3 PUFA deficiency acts both on brain composition and brain functions such as neurotransmission and behavior. In order to understand mechanisms relating biochemical and functional events, it is of major interest to study the reversibility of the deleterious effects of n-3 PUFA deficiency. Although little evidence is available, it seems possible to obtain full recovery of brain DHA levels after dietary supply of n-3 PUFAs in n-3 PUFA-deficient animals. This recovery was shown to be slow, as it required at least 6–12 weeks of repletion diet (31–33) and seemed not to be homogenous throughout the different brain regions (19). In addition, it must be emphasized that this biochemical recovery was not always correlated with functional recovery as assessed by behavioral tests (19, 33, 34). In agreement with this, Weisinger et al. (35) showed the slowness of recovery of retinal DHA after repletion and failure to restore all aspects of retinal function. It was also demonstrated a critical period in the perinatal window for n-3 FAs to permanently affect blood pressure in rats (36).

In order to establish the links between biochemical and functional parameters, we studied the effects of reversion of α-linolenic acid diet deficiency on the FA composition and dopaminergic neurotransmission in two dopaminergic brain regions known to be affected by such deficiency, i.e., the prefrontal cortex (PFCx) and the nucleus accumbens (NAcc), in 2-month-old animals. Dopamine (DA) release under pharmacological stimulation induced by tyramine was studied in both cerebral regions using a dual-probe microdialysis method in awake animals. The vesicular monoamine transporter ($VMAT_2$) binding sites were studied using quantitative autoradiography with [^{3}H]dihydrotetrabenazine in the NAcc. We began to supply the reversal diet to chronically deficient rats at different stages of development, i.e., 0, 7, 14, and 21 days of age. Our hypothesis was that the degree of brain maturation might play a critical role in the ability to recover the neurochemical functions affected by n-3 PUFA deficiency in rats.

MATERIALS AND METHODS

Animals and diets

Two generations of female Wistar rats originating from the Laboratoire de Nutrition et Sécurité Alimentaire (INRA, Jouy-en-Josas, France) were fed with a diet containing 6% fat in the form of African peanut oil specifically deficient in α-linolenic acid as already described (18). This deficient diet provided 1,200 mg of linoleic acid but less than 6 mg of α-linolenic acid per 100 g of diet. Two weeks before mating, female rats from the second generation of α-linolenic acid-deficient rats were divided into two groups. The first group received the deficient diet (deficient) and the second group received a diet in which peanut oil was replaced by a mixture of 60% peanut oil and 40% rapeseed oil (control). This control diet provided the same amount of linoleic acid as the deficient diet and in addition 200 mg of α-linolenic acid per 100 g of diet (n-6/n-3 = 6), an amount that has previously been shown to restore the maternal level of DHA (37). The overall composition of diets and the FA composition of dietary lipids are summarized in **Tables 1** and **2**. A number of deficient females were divided into four dietary groups, each receiving the control diet instead of the deficient diet at different stages: the day of parturition or 7, 14, and 21 days after parturition. Dietary groups were named D_0, D_7, D_{14}, and D_{21} respectively, as shown in **Fig. 1**. At weaning, the male progeny of each group received the same diet as their respective dams. All diets were available ad libitum. Experiments were performed on 2 month old male rats from the six dietary groups. Four litters were used for each dietary group, i.e., a total of 24 litters for the study. Each litter provided a mean of five male rats, which were mixed at weaning. For the overall study, an average of 18 male rats was used for each dietary group, i.e., a total of 108 animals for the study. The experimental procedures were in compliance with guidelines from the European Community Commission directives 86/609/EEC.

Lipid analysis

Five to six male rats from each dietary group were sacrificed by decapitation. PFCx and NAcc were rapidly dissected on ice, weighed, frozen in liquid nitrogen, and stored at −80°C until use. Tissue was homogenized using a Polytron Kinematica PT 1200 (Bioblock Scientific, Strasbourg, France) in 5 ml of chloroform-methanol solution 2:1 (v/v) in the presence of butylhydroxy-toluene (0.002 g/l). Total lipids were extracted according to the procedure of Folch et al. (38). To assess the effectiveness of the procedure and quantify total FAs, known amounts of diheptadecanoyl (17:0) phosphatidylcholine (Sigma, St Quentin Fallavier, France) were added as an internal standard prior to extraction and represented approximately 10% of estimated total FAs. Aliquots of total lipids were used for the analytical quantification of total phospholipids and phospholipid classes. The three main

TABLE 1. Diet composition

	Control	n-3 Deficient
	g/kg	
Casein	220	220
DL methionine	1.6	1.6
Corn starch	432.4	432.4
Saccharose	216	216
Cellulose	20	20
Mineral mixture[a]	40	40
Vitamin mixture[b]	10	10
Oils[c]		
Peanut	23.6	60
Rapeseed	36.4	–

[a] Composition (g/kg of mineral mixture): $CaHPO_4{\cdot}2H_2O$, 380; K_2HPO_4, 240; $CaCO_3$, 180; NaCl, 69; MgO, 20; $MgSO_4{\cdot}7H_2O$, 90; $FeSO_4{\cdot}7H_2O$, 8.6; $ZnSO_4{\cdot}H_2O$, 5; $MnSO_4{\cdot}H_2O$, 5; $CuSO_4{\cdot}5H_2O$, 1; NaF, 0.8; $CrK(SO_4)_2{\cdot}12H_2O$, 0.5; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.02; KI, 0.04; $CoCO_3$, 0.02; Na_2SeO_3, 0.02.

[b] Composition of vitamin supplement triturated in dextrose (mg/kg of vitamin mixture): retinyl acetate (UI), 500,000; cholecalciferol (UI), 250,000; acetate dl-alpha-tocopherol (UI), 5,000; menadione (UI), 100; thiamine HCl (UI), 1,000; riboflavine, 1,000; nicotinic acid, 4,500; D-calcium panthotenate, 3,000; pyridoxine HCl, 1,000; inositol, 5,000; D-biotin, 20; folic acid, 200; cyanocobalamin, 1.35; L-ascorbic acid, 10,000; paraamino-benzoic acid, 5,000; choline chlorhydrate, 75,000.

[c] Total dietary lipids: 6 g/100 g of diet.

TABLE 2. Fatty acid composition of dietary lipids

Fatty Acids[a]	Control[b]	n-3 Deficient[c]
	mg/100 mg fatty acids	
16:0	8.1	9.9
18:0	2.4	3.1
20:0	0.9	1.2
22:0	1.2	1.8
24:0	0.6	0.8
SFA	13.2	16.8
16:1n-7	1.1	0.0
18:1n-9	60.9	60.8
18:1n-7	0.0	0.0
20:1n-9	1.1	1.1
MUFA	63.1	61.9
18:2n-6	21.2	21.3
n-6 PUFA	21.2	21.3
18:3n-3	3.6	<0.1
n-3 PUFA	3.6	<0.1
n-6 + n-3	24.8	21.3
n-6/n-3	5.9	–
PUFA, mg/100 g diet		
18:2n-6	1196	1201
18:3n-3	203	<6

Oils were kindly supplied by Lesieur-Alimentaire (Coudekerque; France).

[a] Abbreviations used: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

[b] African peanut oil rapeseed oil mixture (60.5%:39.5%).

[c] African peanut oil.

phospholipid classes (phosphatidylcholine, PC; phosphatidylethanolamine, PE; and phosphatidylserine, PS) were separated from total lipids on a silica gel cartridge (BAKERBOND spe™ Amino, Baker, Phillipsburg, NJ) adapted from the procedure of Pietsch and Lorentz (39). Briefly, an isopropanol/chloroform mixture (1:2) eluted neutral lipids from the total lipid extract deposited beforehand on the silica cartridge. A mixture of diethylether/acetic acid (98:2) eluted free FAs, and acetonitrile/n-propanol (4:1) eluted PC. PE was eluted by acetonitrile/n-propanol (1:1) and acetone. PS was eluted by isopropanol/methanol HCl (4:1). Phospholipid classes were then transmethylated with 10% boron trifluoride (Fluka, Socolab, Paris, France) at 90°C for 20 min according to the procedure of Morisson and Smith (40). FA composition of each phospholipid class was determined by GLC (41), and results were expressed as percentage of total FAs (wt %).

Surgery and dual-probe implantation

Rats (n = 7–8 per dietary group) were anesthetized with ketamine at a dose of 150 mg/kg i.p. (Imalgène, Rhône Mérieux, France) and placed in a stereotaxic apparatus (Stoelting, Wood Dale, IL). Body temperature was maintained at 37 ± 1°C throughout the surgery time using a thermostatically controlled heating blanket (CMA 150, CMA/Microdialysis, Stockholm, Sweden). The skull was exposed and two holes were drilled. Guide cannulas were implanted according to the atlas of Paxinos and Watson (42) into the left PFCx at coordinates antero-posterior = 5.2, lateral = −0.6, dorso-ventral = −1.8 mm, tilt 26° angle (MAB 2 14 G, CMA/Microdialysis, Stockholm, Sweden) and in the left NAcc at coordinates antero-posterior = 1.7, lateral = −1.2, dorso-ventral = −6.4 mm (MAB 2 20 G, CMA/Microdialysis) from Bregma. Guide cannulas were anchored to the skull with a stainless-steel screw and dental cement. Microdialysis probes with a 3 mm membrane length (MAB 6 14 3, 15 kDa molecular mass cut-off) in the PFCx and a 1 mm membrane length (MAB 6 20 1, 15 kDa molecular mass cut-off) in the NAcc were slowly lowered through the guide cannula. Animals were housed in cylindrical Plexiglas cages (diameter 40 cm, height 32 cm), which served as home cages during the microdialysis experiments, with a counterbalance arm holding a liquid swivel. They were allowed to recover postoperatively overnight and given ad libitum access to water and to their respective diets. After implantation, probes were immediately and continuously perfused with Dulbecco's buffer modified liquid (ICN, Costa Mesa, CA) supplemented with 2.2 mM $CaCl_2$ and 1.1 mM $MgCl_2$ (pH 7.4) at 0.8 μl/min using a microsyringe pump (Harvard Apparatus, South Natick, MA).

Microdialysis procedure

After a postoperative recovery period (22 h), the flow rate was increased to 1.2 μl/min for 1 h before experiment until equilibrium was reached. Dialysates were collected at 20 min intervals into vials that were preloaded with 5 μl 0.1 M perchloric acid, resulting in a total sampling volume of 29 μl. The microinjection pump was mounted with four syringes, two containing perfusion buffer alone (for PFCx and NAcc) and two containing perfusion buffer supplemented with tyramine (Sigma, St. Louis, MO). Tyramine was freshly dissolved in Dulbecco's buffer before use and infused locally via the probes. During the first 80 min the dialysis probes were infused with perfusion buffer alone, and then perfused with the tyramine solution for 40 min by switching syringes in the PFCx and NAcc at the same time. The syringes for the PFCx and NAcc contained 1.2 mM and 600 μM of tyramine, respectively. Perfusion was then continued with buffer alone until the end of experiment. The baseline value of released DA was obtained by averaging the first four samples, and values obtained in subsequent samples were expressed as percentages of this baseline.

The animals were sacrificed after the experiment by a pentobarbital bolus (Sanofi, Libourne, France), and the localization of dialysis probes was macroscopically checked on brain sections.

Separation and quantification of DA

DA was measured in dialysates by high performance liquid chromatography (HPLC) with electrochemical detection on a Concorde apparatus (Waters, St. Quentin-Yvelines, France). Samples were injected using a Rheodyne 7725i injector valve with a 20 μl injection loop. The mobile phase consisting of 7% acetonitrile, 3% methanol, and 90% citric acid 20 mM, 10 mM monobasic phosphate sodium, 3.25 mM octanesulfonic acid, 3 mM heptanesulfonic acid, 0.1 mM EDTA, 2 mM KCl, 6 ml/liter o-phosphoric acid, and 2 ml/liter diethylamine with pH 3 adjusted using HCl was pumped at 0.3 ml/min with a Gold 118 system (Beckman, Fullerton, CA). Separation was performed with a 5 μm C18, 3.2 × 100 mm reversed phase column (LC-22C, BAS, West Lafayette, IN). A glassy carbon working electrode set at 800 mV with reference to an in situ Ag/AgCl reference electrode was used to detect compounds. Signals were recorded and quantified with a Beckman Gold 118 integrator. Amounts of DA were calculated by comparing peak levels from the microdialysis samples with those of external standards. Under these conditions, the limit of detection of DA was 1 fmol/μl.

In vitro autoradiographic study of $VMAT_2$

Rats were sacrificed after microdialysis experiments. Brains were rapidly removed on ice and then frozen (−35°C) in dry ice-cooled isopentane before storage at −80° until use. Twenty-micron thick coronal sections were cut at −20°C on a cryostat microtome (Reichter-Jung Cryocut 1800, Leica, France), thaw mounted on gelatin microscope slides, and kept at −80°C until autoradiographic experiments. Labeling of $VMAT_2$ with [^{3}H]dihydrotetrabenazine (specific activity 20 Ci/mmole, American Radiolabeled Chemicals, St Louis, MO) was carried out using the

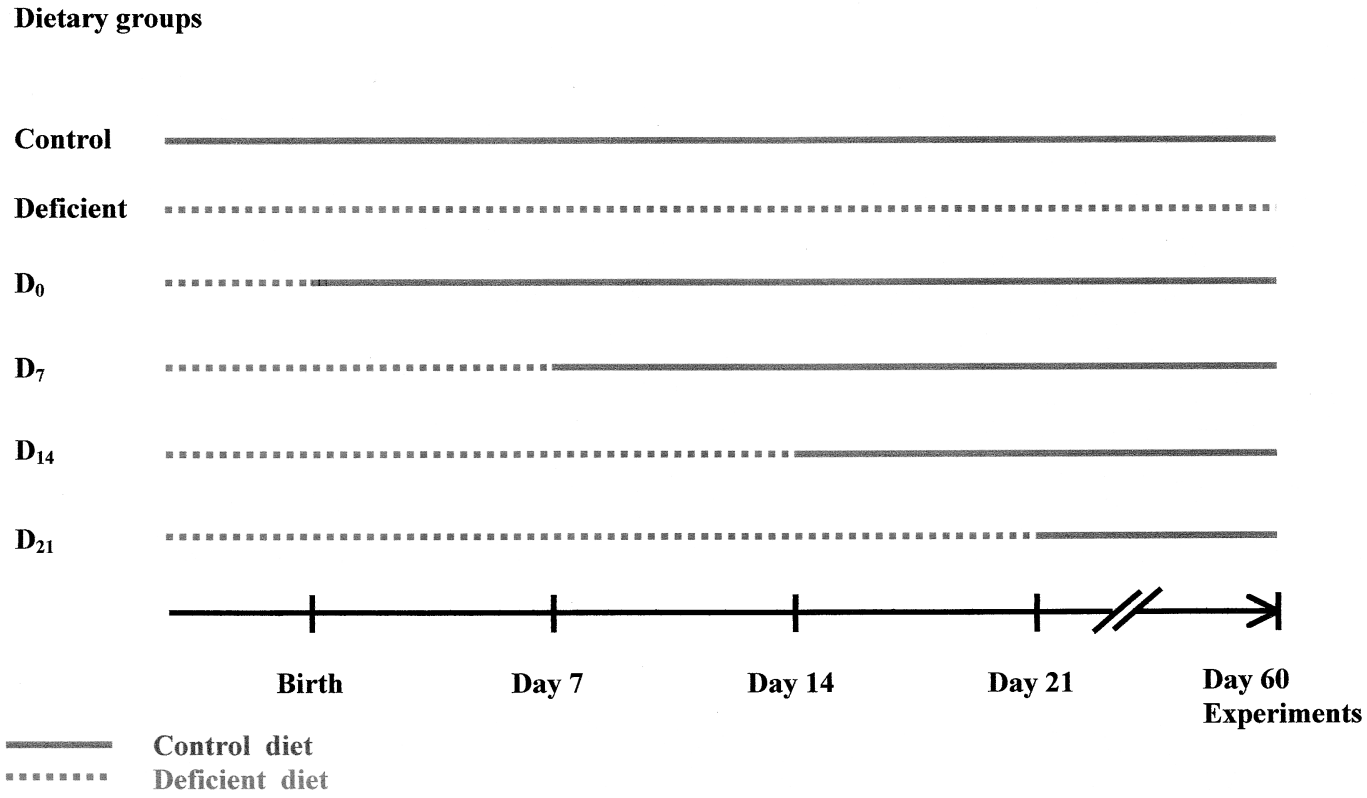

Fig. 1. Study design. Composition of control and deficient diets are detailed in Tables 1 and 2. Females from the deficient group were divided into four dietary groups (n = 4 females/group), each receiving the control diet instead of the deficient diet at different stages after parturition, i.e., at birth (D_0) and 7, 14, or 21 days after parturition (D_7, D_{14}, and D_{21} respectively). At weaning, the male progeny of each group received the same diet as their respective dams until experimentation.

procedure of Wilson and Kish (43) with minor modifications. Briefly, the sections were prewashed in sodium phosphate buffer (50 mM, pH 7.7) for 1 h at room temperature in order to remove endogenous competing substances. Sections were then incubated in sodium phosphate buffer containing 7.5 nM [^{3}H]dihydrotetrabenazine for 1 h. Nonspecific binding was defined from adjacent sections incubated in the presence of 2 μM reserpine. Following incubation, slices were washed in sodium phosphate buffer at 4°C for 3 min and rinsed in cold distilled water before drying. Dried sections were exposed on tritium-sensitive films (Biomax MR, Kodak, France) with tritium-calibrated standards (Microscales, Amersham) for 12 weeks. Films were then developed and fixed. Optical density measurements were performed on autoradiograms to determine the density of [^{3}H]dihydrotetrabenazine binding in the right intact NAcc. A computerized video-assisted densitometer (Biocom, Siemens Nixdorf, France) was used. Optical densities were converted into apparent tissue ligand concentrations with reference to tritiated standards and specific activity of the radioligand. The intensity of [^{3}H]dihydrotetrabenazine binding was thus expressed in pmol/g equivalent tissue.

Statistical analysis

The results of FA composition in PC, PE, and PS obtained for each reversal group D_0, D_7, D_{14}, and D_{21} were compared with the results of the control group (a) and those of the deficient group (b) using one-way ANOVA followed by post hoc Dunnett's test ($P < 0.01$).

The maximal release of DA and the specific binding of [^{3}H]dihydrotetrabenazine to $VMAT_2$ for each reversal group were compared with the control (a) and the deficient group (b) using a one-way ANOVA followed by the Dunnett's test ($P < 0.05$).

RESULTS

The mean body weight of animals was not significantly different between dietary groups (control, D_0, D_7, D_{14}, D_{21}, and deficient).

FA composition of PC, PE, and PS in the PFCx

As shown in **Table 3**, the amounts of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) in the three phospholipid classes PC, PE, and PS were identical in the six dietary groups.

The total amount of n-6 PUFAs was always significantly increased in the deficient compared with the control group, reaching 32% for PC (10.8 ± 0.5 vs. 8.2 ± 0.2% of total FA, $P < 0.01$), 77% for PE (46.1 ± 1.2 vs. 26.0 ± 0.5% of total FA, $P < 0.01$), and 252% for PS (29.9 ± 1.0 vs. 8.5 ± 0.5% of total FA, $P < 0.01$). This increase was mainly due to increased amounts of 22:5n-6 in deficient rats, whereas the amount of 20:4n-6 did not significantly change between deficient and control groups for each phospholipid class.

The amount of total n-6 PUFAs was similar to control group values in all shifted dietary groups (D_0, D_7, D_{14}, and D_{21}) for PC and PE as well as in D_0, D_7, and D_{14} for PS, whereas a slight increase of 34% ($P < 0.01$) was observed for PS in D_{21} (11.4 ± 0.2 vs. 8.5 ± 0.5% total FA, $P < 0.01$). Amounts of 20:4n-6 measured in each dietary group were similar to controls for all phospholipid classes.

TABLE 3. Effect of a shift of deficient diet to control diet at different times after birth on the fatty acid composition of PC, PE, and PS in the prefrontal cortex of 2-month-old rats

	Control	D_0	D_7	D_{14}	D_{21}	Deficient
PC						
SFA	60.0 ± 0.3	59.3 ± 0.7	59.1 ± 0.8	59.9 ± 0.3	60.2 ± 0.7	61.2 ± 0.4
MUFA	27.1 ± 0.2	27.0 ± 0.4	27.9 ± 0.5	26.8 ± 0.3	27.4 ± 0.3	26.6 ± 0.6
20:4n-6	6.0 ± 0.2	6.5 ± 0.2	6.4 ± 0.1	6.4 ± 0.3	6.1 ± 0.2	6.1 ± 0.4
22:5n-6	0.5 ± 0.1[b]	0.2 ± 0.0[b]	0.3 ± 0.0[b]	0.3 ± 0.0[b]	0.3 ± 0.0[b]	2.5 ± 0.2[a]
n-6 PUFA	8.2 ± 0.2[b]	8.7 ± 0.3[b]	8.4 ± 0.2[b]	8.5 ± 0.4[b]	8.4 ± 0.3[b]	10.8 ± 0.5[a]
22:6n-3	4.5 ± 0.1[b]	4.6 ± 0.1[b]	4.4 ± 0.1[b]	4.3 ± 0.2[b]	4.0 ± 0.2[b]	1.2 ± 0.1[a]
n-3 PUFA	4.9 ± 0.1[b]	4.9 ± 0.1[b]	4.7 ± 0.1[b]	4.7 ± 0.2[b]	4.4 ± 0.2[b]	1.5 ± 0.2[a]
n-6/n-3	1.7 ± 0.1[b]	1.8 ± 0.1[b]	1.8 ± 0.1[b]	1.8 ± 0.1[b]	1.9 ± 0.1[b]	7.6 ± 1.1[a]
PE						
SFA	29.3 ± 0.6	31.1 ± 1.6	35.7 ± 2.1	29.8 ± 1.1	31.9 ± 0.9	32.7 ± 0.7
MUFA	13.5 ± 0.2	13.4 ± 0.4	15.9 ± 1.1	13.8 ± 0.7	13.6 ± 0.8	12.6 ± 0.2
20:4n-6	17.8 ± 0.3	17.9 ± 0.8	17.5 ± 0.3	18.4 ± 0.6	17.5 ± 0.2	19.7 ± 1.1
22:5n-6	1.5 ± 0.3[b]	1.9 ± 0.3[b]	1.4 ± 0.1[b]	1.7 ± 0.2[b]	2.6 ± 0.2[a,b]	17.9 ± 0.4[a]
n-6 PUFA	26.0 ± 0.5[b]	26.6 ± 0.7[b]	24.7 ± 0.4[b]	27.0 ± 1.0[b]	26.8 ± 0.6[b]	46.1 ± 1.2[a]
22:6n-3	30.2 ± 0.3[b]	28.3 ± 1.0[b]	26.3 ± 1.8[a,b]	28.7 ± 0.8[b]	27.3 ± 0.5[a,b]	8.7 ± 0.5[a]
n-3 PUFA	31.0 ± 0.4[b]	28.6 ± 1.0[b]	26.3 ± 1.8[a,b]	29.2 ± 0.9[b]	27.9 ± 0.6[a,b]	9.0 ± 0.6[a]
n-6/n-3	0.8 ± 0.0[b]	0.9 ± 0.0[b]	0.9 ± 0.0[b]	0.9 ± 0.0[b]	1.0 ± 0.0[b]	5.3 ± 0.5[a]
PS						
SFA	44.8 ± 0.6	44.8 ± 0.1	47.9 ± 1.2	44.5 ± 0.4	46.0 ± 0.7	46.8 ± 1.2
MUFA	14.8 ± 1.0	13.2 ± 0.2	12.8 ± 0.5	12.9 ± 0.3	12.6 ± 0.3	13.9 ± 0.5
20:4n-6	3.1 ± 0.3	2.6 ± 0.1	2.9 ± 0.1	3.0 ± 0.1	2.9 ± 0.1	3.3 ± 0.3
22:5n-6	1.5 ± 0.2[b]	1.6 ± 0.0[b]	1.9 ± 0.2[b]	3.2 ± 0.2[b]	5.0 ± 0.1[a,b]	21.6 ± 1.1[a]
n-6 PUFA	8.5 ± 0.5[b]	7.9 ± 0.1[b]	8.3 ± 0.3[b]	10.0 ± 0.2[b]	11.4 ± 0.2[a,b]	29.9 ± 1.0[a]
22:6n-3	32.1 ± 0.6[b]	32.9 ± 0.3[b]	30.7 ± 1.3[b]	31.6 ± 0.4[b]	29.3 ± 0.6[b]	8.4 ± 0.6[a]
n-3 PUFA	32.5 ± 0.6[b]	33.5 ± 0.4[b]	31.1 ± 1.3[b]	32.3 ± 0.4[b]	29.9 ± 0.6[b]	8.9 ± 0.6[a]
n-6/n-3	0.3 ± 0.0[b]	0.2 ± 0.0[b]	0.3 ± 0.0[b]	0.3 ± 0.0[b]	0.4 ± 0.0[b]	3.5 ± 0.2[a]

Male rats (n = 5–6 per dietary group) received the control diet from birth (D_0) or from 7, 14, or 21 days of life (D_7, D_{14}, D_{21}, respectively). Results are expressed as mean percentage of total fatty acid ± SD. Abbreviations used: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine.

[a] Results obtained for each diet were compared to those of the control group ($P < 0.01$) using a one-way ANOVA followed by a Dunnett's test.

[b] Results obtained for each diet were compared to those of the deficient group ($P < 0.01$) using a one-way ANOVA followed by a Dunnett's test.

Amounts of 22:5n-6 were similar to control values in all shifted dietary groups for PC, and in D_0, D_7, and D_{14} for PE and PS. The increase in the amount of 22:5n-6 reached 73% in D_{21} for PE (2.6 ± 0.2 vs. 1.5 ± 0.3% total FA, $P <$ 0.01), whereas it reached 233% (5.0 ± 0.1 vs. 1.5 ± 0.2% total FA, $P < 0.01$) for PS.

The amount of total n-3 PUFAs was significantly decreased in the deficient compared with the control group for all three phospholipid classes. This decrease reached around −69%, −71%, and −73% for PC, PE, and PS, respectively (PC: 1.5 ± 0.2 vs. 4.9 ± 0.1% of total FA, $P <$ 0.01; PE: 9.0 ± 0.6 vs. 31.0 ± 0.4% of total FA, $P < 0.01$; PS: 8.9 ± 0.6 vs. 32.5 ± 0.6% of total FA, $P < 0.01$). This decrease was of the same order of magnitude (around −72%) for 22:6n-3 for all three phospholipid classes.

The total n-3 PUFAs and 22:6n-3 values obtained in the four shifted dietary groups (D_0, D_7, D_{14}, and D_{21}) were identical to those of the control group for PC, PS, and PE, except in D_7 and D_{21} for PE where a slight decreases of around 13% and 10%, respectively, were observed both for total n-3 PUFAs and 22:6n-3 (for D_7: 26.3 ± 1.8 vs. 31 ± 0.4% and 26.3 ± 1.8 vs. 30.2 ± 0.3% of total FA, respectively, $P < 0.01$; for D_{21}: 27.9 ± 0.6 vs. 31 ± 0.4% and 27.3 ± 0.5 vs. 30.2 ± 0.3% of total FA, respectively, $P < 0.01$).

The n-6-n-3 ratio was greatly increased in the deficient compared with the control group. It was 5, 6, and 11 times higher for PC, PE, and PS, respectively. This ratio was similar in the D_0, D_7, D_{14}, and D_{21} groups in comparison to controls.

FA composition of PC, PE, and PS in the NAcc

As shown in **Table 4**, the amounts of SFAs and MUFAs were identical in the six dietary groups for the three phospholipid classes.

The total amount of n-6 PUFAs was always significantly increased in the deficient compared with control group. This increase reached 44% for PC (10.1 ± 0.6 vs. 7.0 ± 0.6% of total FA, $P < 0.01$), 57% for PE (42.3 ± 1.8 vs. 26.9 ± 0.5% of total FA, $P < 0.01$), and 106% for PS (20.2 ± 2.7 vs. 9.8 ± 0.5% of total FA, $P < 0.01$). This increase could be mainly ascribed to a considerable increase in 22:5n-6 in deficient rats, estimated at 950%, 1,500%, and 1,070% for PC, PE, and PS, respectively, compared with controls (PC: 2.1 ± 0.2 vs. 0.2 ± 0.1% of total FA, $P <$ 0.01; PE: 14.5 ± 0.8 vs. 0.9 ± 0.1% of total FA, $P < 0.01$; PS: 11.7 ± 1.7 vs. 1.0 ± 0.1% of total FA, $P < 0.01$). The amount of 20:4n-6 was not significantly different in either group for any phospholipid class.

The values obtained for n-6 PUFAs in the four shifted dietary groups (D_0, D_7, D_{14}, and D_{21}) were identical to those measured in the control group for PC, PE, and PS. The amounts of 20:4n-6 were similar to controls for each phospholipid class for all dietary groups. The amount of 22:5n-6 was similar to controls in D_0, D_7, D_{14}, and D_{21}, except

TABLE 4. Effect of a shift of deficient diet to control diet at different times after birth on the fatty acid composition of PC, PE, and PS in the nucleus accumbens of 2 month old rats

	Control	D_0	D_7	D_{14}	D_{21}	Deficient
PC						
SFA	60.0 ± 1.0	60.8 ± 1.8	65.2 ± 3.5	61.4 ± 1.8	60.5 ± 0.9	59.4 ± 1.4
MUFA	29.3 ± 0.3	28.4 ± 1.0	24.8 ± 2.7	28.6 ± 1.2	27.9 ± 0.9	28.9 ± 1.0
20:4n-6	5.1 ± 0.4	5.2 ± 0.3	4.5 ± 0.4	5.0 ± 0.3	5.3 ± 0.3	6.1 ± 0.3
22:5n-6	0.2 ± 0.1[b]	0.1 ± 0.0[b]	0.2 ± 0.0[b]	0.2 ± 0.0[b]	0.4 ± 0.1[b]	2.1 ± 0.2[a]
n-6 PUFA	7.0 ± 0.6[b]	7.1 ± 0.4[b]	6.6 ± 0.6[b]	6.9 ± 0.5[b]	8.1 ± 0.1[b]	10.1 ± 0.6[a]
22:6n-3	2.8 ± 0.2[b]	2.9 ± 0.3[b]	2.3 ± 0.3[b]	2.5 ± 0.3[b]	2.4 ± 0.2[b]	1.0 ± 0.1[a]
n-3 PUFA	3.2 ± 0.2[b]	3.5 ± 0.3[b]	3.3 ± 0.4[b]	3.1 ± 0.2[b]	2.9 ± 0.2[b]	1.4 ± 0.1[a]
n-6/n-3	2.2 ± 0.1[b]	2.1 ± 0.1[b]	2.1 ± 0.2[b]	2.2 ± 0.1[b]	2.8 ± 0.5[a,b]	7.2 ± 0.4[a]
PE						
SFA	29.9 ± 1.4	32.4 ± 3.6	30.7 ± 1.2	33.5 ± 3.8	31.0 ± 1.9	31.5 ± 1.6
MUFA	21.1 ± 0.7	21.6 ± 1.3	22.4 ± 1.2	24.7 ± 2.9	20.1 ± 0.9	21.2 ± 1.5
20:4n-6	17.2 ± 0.5	17.1 ± 0.6	16.8 ± 0.7	16.1 ± 0.7	18.2 ± 0.4	17.7 ± 1.0
22:5n-6	0.9 ± 0.1[b]	0.8 ± 0.0[b]	1.1 ± 0.1[b]	1.2 ± 0.2[b]	2.9 ± 0.4[a,b]	14.5 ± 0.8[a]
n-6 PUFA	26.9 ± 0.5[b]	26.1 ± 0.6[b]	26.5 ± 0.8[b]	25.2 ± 1.8[b]	29.7 ± 0.6[b]	42.3 ± 1.8[a]
22:6n-3	21.6 ± 0.9[b]	22.0 ± 0.6[b]	20.0 ± 1.2[b]	20.3 ± 1.1[b]	19.3 ± 0.6[b]	6.1 ± 0.3[a]
n-3 PUFA	22.4 ± 0.7[b]	22.7 ± 0.6[b]	20.8 ± 1.1[b]	21.1 ± 0.9[b]	20.1 ± 0.4[a,b]	6.4 ± 0.3[a]
n-6/n-3	1.2 ± 0.0[b]	1.2 ± 0.1[b]	1.3 ± 0.1[b]	1.3 ± 0.1[b]	1.5 ± 0.1[b]	6.6 ± 0.4[a]
PS						
SFA	51.8 ± 3.0	52.0 ± 2.7	57.5 ± 3.8	55.7 ± 3.3	51.6 ± 3.1	53.2 ± 2.7
MUFA	24.3 ± 1.1	23.3 ± 1.2	23.9 ± 1.3	23.8 ± 1.4	23.3 ± 1.0	22.0 ± 0.9
20:4n-6	4.5 ± 0.3	4.8 ± 0.3	3.8 ± 0.6	3.9 ± 0.4	5.1 ± 0.2	4.3 ± 0.6
22:5n-6	1.0 ± 0.1[b]	1.1 ± 0.1[b]	1.4 ± 0.4[b]	1.4 ± 0.1[b]	2.8 ± 0.3[b]	11.7 ± 1.7[a]
n-6 PUFA	9.8 ± 0.5[b]	9.7 ± 0.7[b]	8.1 ± 1.4[b]	8.5 ± 0.8[b]	12.1 ± 0.6[b]	20.2 ± 2.7[a]
22:6n-3	15.0 ± 0.6[b]	13.7 ± 1.3[b]	12.6 ± 1.6[b]	11.3 ± 1.2[b]	11.2 ± 1.8[a,b]	3.6 ± 0.5[a]
n-3 PUFA	16.3 ± 0.6[b]	14.4 ± 1.4[b]	13.3 ± 1.6[b]	12.4 ± 1.4[b]	12.0 ± 1.7[a,b]	4.4 ± 0.5[a]
n-6/n-3	0.6 ± 0.0[b]	0.7 ± 0.1[b]	0.6 ± 0.1[b]	0.7 ± 0.1[b]	1.0 ± 0.2[b]	4.6 ± 0.4[a]

Male rats (n = 5–6 per dietary group) received the control diet from birth (D_0) or from 7, 14, or 21 days of life (D_7, D_{14}, D_{21} respectively). Results are expressed as mean percent of total fatty acid ± SD.

[a] Results obtained for each diet were compared to those of the control group ($P < 0.01$) using a one-way ANOVA followed by a Dunnett's test.

[b] Results obtained for each diet were compared to those of the deficient group ($P < 0.01$) using a one-way ANOVA followed by a Dunnett's test.

for PE in D_{21}, in which a significant increase of 222% was measured (2.9 ± 0.4 vs. 0.9 ± 0.1% of total FA, $P < 0.01$).

The total amount of n-3 PUFAs was significantly decreased in the deficient group compared with the control group for all phospholipid classes, reaching −56%, −71%, and −73% for PC, PE, and PS, respectively (PC: 1.4 ± 0.1% vs. 3.2 ± 0.2% of total FA, $P < 0.01$; PE: 6.4 ± 0.3 vs. 22.4 ± 0.7% of total FA, $P < 0.01$; PS: 4.4 ± 0.5% vs. 16.3 ± 0.6% of total FA, $P < 0.01$).

The total amounts of n-3 PUFAs and 22:6n-3 in the four shifted dietary groups (D_0, D_7, D_{14}, and D_{21}) were identical to those of the control group for PC, PE, and PS, except in D_{21}, where a slight decrease around 10–11% was observed for PS both in terms of total n-3 PUFAs and 22:6n-3 (12 ± 0.7 vs. 16.3 ± 0.6% and 11.2 ± 1.8 vs. 15 ± 0.6% of total FA, respectively, $P < 0.01$). The n-6/n-3 ratio was greatly increased in the deficient compared with the control group. It was 3, 5, and 7 times higher for PC, PE, and PS, respectively. The ratio was similar in D_0, D_7, D_{14}, and D_{21} compared with controls, except for PC in D_{21}, where a slight increase was observed (2.8 ± 0.5 vs. 2.2 ± 0.1%, $P < 0.01$).

DA release under tyramine stimulation

Tyramine infusion induced release of DA in all the dietary groups studied both in the PFCx (**Fig. 2A**) and the NAcc (Fig. 2B).

In the PFCx, DA levels at the maximal effect were 480 ± 85% and 970 ± 150% higher than the basal values for the deficient and the control groups, respectively. Stimulated release of DA in the PFCx was therefore dramatically and significantly lower in deficient than in control rats ($P < 0.05$). The stimulated release of DA in D_0, D_7, and D_{14} dietary groups was similar to that obtained in controls (952 ± 107%, 983 ± 160%, and 918 ± 149% of basal level, respectively). In contrast, the stimulated effect obtained in the D_{21} group (504 ± 106% of basal level) was significantly lower than in controls ($P < 0.05$) and not statistically different from the effect measured in the deficient group ($P > 0.05$).

In the NAcc, DA levels at the maximal effect were 426 ± 81% and 798 ± 96% higher than the basal values for deficient and control groups, respectively. Stimulated release of DA was then significantly lower in deficient than in control rats ($P < 0.05$). As in the PFCx, the maximal DA-stimulated increases were similar in D_0, D_7, and D_{14} groups and controls (839 ± 104%, 818 ± 103%, and 713 ± 88%, respectively, vs. 798 ± 96% of basal level), whereas the effect in the D_{21} group (492 ± 90% of basal level) was significantly lower than in controls ($P < 0.05$) and not statistically different from the deficient group ($P > 0.05$).

Autoradiographic study of $VMAT_2$ in the NAcc

The density of $VMAT_2$ in the NAcc evaluated by the specific binding of [^{3}H]dihydrotetrabenazine is shown in **Figs. 3** and **4**. The density of [^{3}H]dihydrotetrabenazine binding

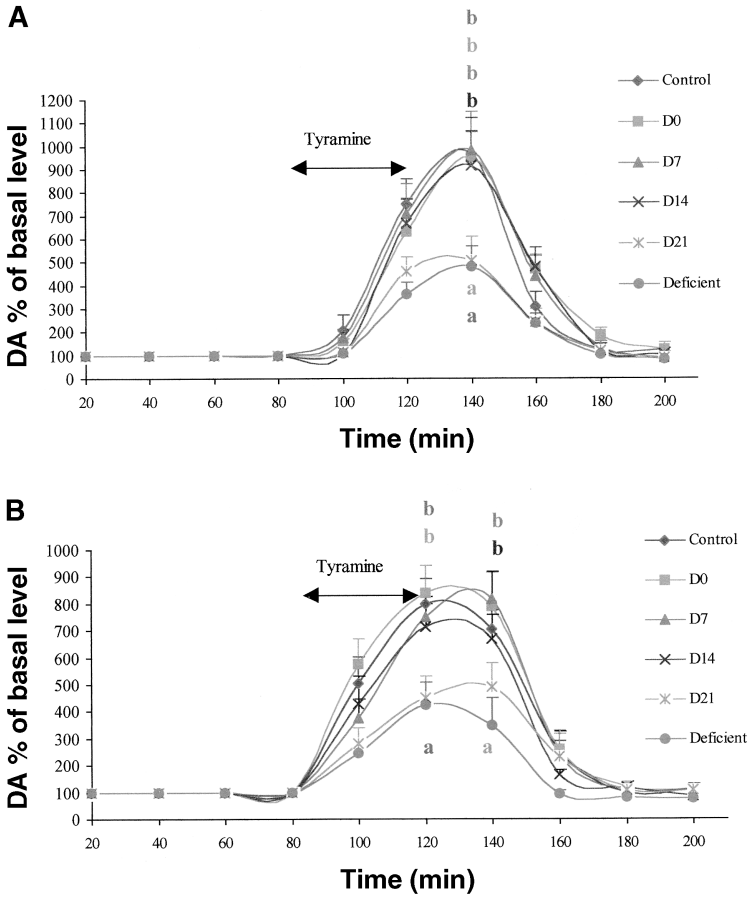

Fig. 2. Effect of a shift from deficient diet to control diet at different times after birth on dopamine levels in the prefrontal cortex (A) and the nucleus accumbens (B) of 2 month old awake animals under tyramine stimulation. Arrows indicate the period of tyramine infusion through probes implantated both in the PFCx and NAcc of each rat. Male rats (n = 7–8 per dietary group) received the control diet from birth (D_0) or from 7, 14, or 21 days of life (D_7, D_{14}, and D_{21}, respectively). Results were expressed as mean percentages of basal levels and compared with values obtained in controls (a, $P < 0.05$) and deficient (b, $P < 0.05$) rats using a one-way ANOVA followed by a Dunnett's test.

sites was significantly decreased in deficient rats compared with controls (−23%, 331 ± 16 vs. 408 ± 13 pmol/g equivalent tissue, $P < 0.05$). In D_0, D_7, and D_{14} groups, the intensity of specific [^{3}H]dihydrotetrabenazine binding did not differ from the values obtained in the control group (432 ± 31; 414 ± 33; 407 ± 23 vs. 408 ± 13 pmol/g equivalent tissue). In the D_{21} group, the intensity of specific [^{3}H]dihydrotetrabenazine binding was not significantly different from the intensity quantified in controls despite a 19% reduction (343 ± 43 vs. 408 ± 13 pmol/g equivalent tissue). Furthermore, this intensity was very close to that measured in the deficient group (331 ± 16 vs. 343 ± 43 pmol/g equivalent tissue).

DISCUSSION

The main aim of this study was to examine the effects of a shift from an α-linolenic acid-deficient diet to a balanced diet applied at different stages of the postnatal period on the FA composition, pharmacologically-stimulated release of DA, and density of $VMAT_2$ in the PFCx and the NAcc of adult rats. Both these cerebral regions have previously been described as changed in FA composition and in dopaminergic function under chronic α-linolenic acid diet deficiency (18, 26, 28, 44). It was therefore important to study the reversibility of these biochemical and neurochemical changes under α-linolenic acid repletion. As may be expected, chronic α-linolenic acid diet deficiency induced a strong reduction in total n-3 PUFA and DHA content of the three phospholipid classes, PC, PE, and PS, both in the PFCx and NAcc. This decrease was accompanied by a compensatory increase in total amount of n-6 PUFAs, and especially, in docosapentaenoic acid (22:5n-6), as already observed (18, 44, 45). This substitution of DHA by docosapentaenoic acid was particularly high for PE and PS in that these phospholipid classes contain the highest amounts of DHA (7). It can also be seen that in control rats, the content of DHA was higher in the PFCx than in the NAcc, thus confirming the particular abundance of DHA in this cerebral region, as previously described (18, 19). A return to control levels of total n-3 PUFA and DHA was obtained in the brains of adult rats receiving the reversal diet at 0, 7, 14, and 21 days of age, although slight reductions were still observed for the PE of the PFCx and for the PS of the NAcc of the latest dietary group. This last result could be related to the shorter interval between α-linolenic acid supply and time of experiment for the D_{21} group (6 weeks) than for other dietary groups (9, 8, and 7 weeks for D_0, D_7, and D_{14}, respectively). This interval might in fact have a role, as it has been shown that recovery of normal cerebral DHA content requires at least 8–9 weeks after initiation of repletion with a diet supplemented with α-linolenic acid plus DHA (32) or DHA alone (33). In addition, another experimental feature could be involved, as the rats from D_0, D_7, and

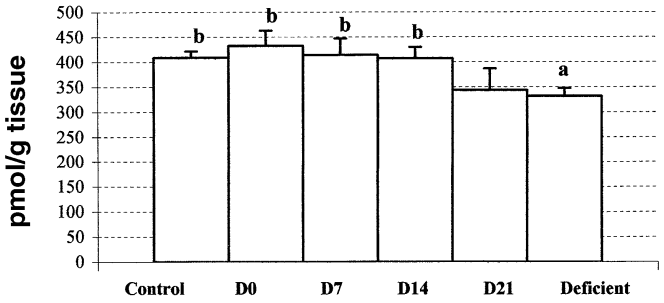

Fig. 3. Effect of a shift from deficient diet to control diet at different times after birth on the $VMAT_2$ binding sites in the NAcc studied by autoradiography with [^{3}H]dihydrotetrabenazine. Six rats per group and eight brain sections per animal were studied. Brain sections of each dietary group (control, D_0, D_7, D_{14}, D_{21}, and deficient) were each exposed on same films. Specific binding of [^{3}H]dihydrotetrabenazine to $VMAT_2$ was expressed as mean concentration ± SD. Values obtained for each group were compared with those of the control group ($^aP < 0.05$) and to those of the deficient group ($^bP < 0.05$) using a one-way ANOVA followed by a Dunnett's test.

D_{14} groups began to receive the reversal diet through maternal milk, whereas the rats from the D_{21} group received this diet at the time of weaning. In the first three groups, the maternal milk provided not only α-linolenic acid but also LC-PUFAs, which are mainly incorporated in neuronal phospholipids (7, 41). By contrast, animals from the D_{21} group were fed a milk during the suckling period that contained only traces of n-3 PUFAs, thus resulting in poor accumulation of DHA in the brain, as previously demonstrated in this type of diet deficiency (41). At weaning, α-linolenic acid was supplied to rats from the D_{21} group directly through their food and had therefore to be converted by a series of chain elongation-desaturation enzymatic reactions before incorporation in cerebral membranes. These enzymatic activities are in normal diet conditions maximal during the brain maturation processes, i.e., the prenatal and early postnatal periods (46). The incomplete biochemical reversibility observed in the D_{21} group could therefore be related to abnormal enzymatic activities during this period. This finding highlighted the importance of PUFA intake during the lactation period on the FA composition of cerebral membranes. Our biochemical analysis also showed that the compensatory increase in 22:5n-6 was not totally reversed in the D_{21} group and that this remaining modification was more marked than the other remaining difference in DHA amounts between this group and the control group. This finding is in agreement with the already reported slower reciprocal decrease in n-6 PUFAs than the increase in n-3 PUFAs under reversal diet (32, 33). This could be related to the concomitant restoration of competition between α-linolenic acid and linoleic acid for delta-6 desaturase known to be in favor of n-3 PUFA biosynthesis, and higher incorporation of newly synthesized n-3 PUFAs than elimination of n-6 PUFAs (47, 48).

Our neurochemistry results confirmed that dopaminergic neurotransmission was affected by chronic α-linolenic acid deficiency. In deficient and control rats, pharmacological stimulation with tyramine induced a significant release of DA stored in synaptic secretory vesicles. However, at the time of maximal response, the intensity of release in the deficient group was about half that of the control group in the PFCx and NAcc. These results corroborated our previous findings in which the increase in DA release under tyramine stimulation was higher than measured here, both in the PFCx (26) and NAcc (28). These differences in the magnitude of DA release were unexpected, although there were several differences in experimental conditions used, such as the use of awake (present study) or anesthetized (previous studies) animals, the sites of probe localization chosen here taking into account the feasibility of dual probe implantation in the same animal, the perfusion flow-rate, and the doses of tyramine provid-

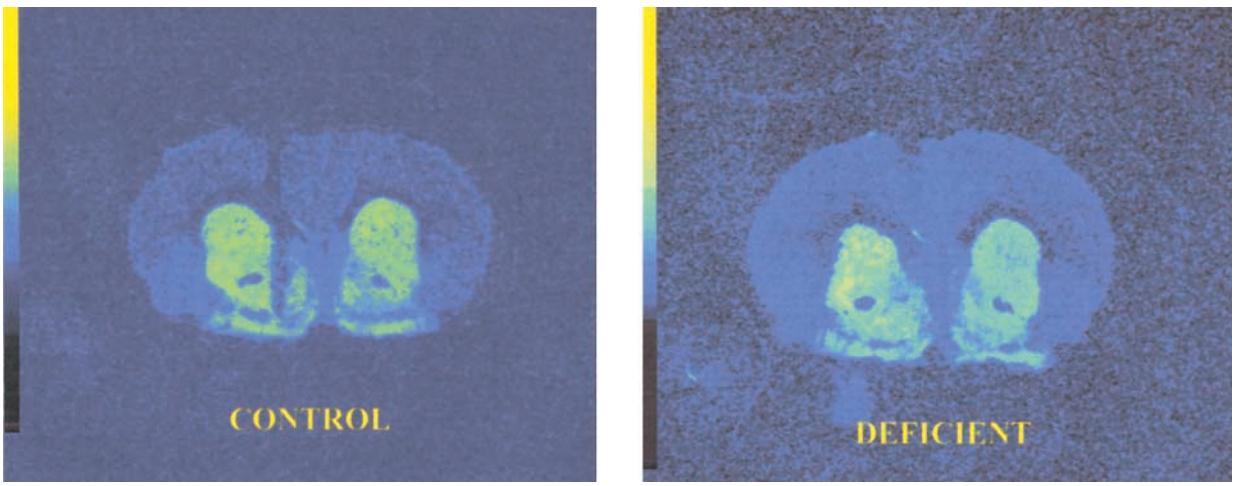

Fig. 4. Representative colored autoradiographies showing the [^{3}H]dihydrotetrabenazine binding on $VMAT_2$ on brain sections of control (left) and n-3 PUFA-deficient rats (right). Quantification was performed on the right hemisphere as microdialysis probes were implanted on the left side. Note the high labeling in the nucleus accumbens and the striatum. Color scale: from blue (low radioactivity) to green and yellow (highest radioactivity).

ing strong measurable responses. Further investigations allowed us to demonstrate that this decrease in DA-stimulated release in deficient rats probably resulted from a significantly diminished number of storage vesicles in dopaminergic terminals (27, 28). In accordance with this hypothesis, we also observed in this study that the $VMAT_2$ binding sites were significantly reduced in the deficient rats.

Rats shifted to the control reversal diet at 0, 7, or 14 days of life responded to tyramine stimulation in a similar way to control animals in both the PFCx and NAcc, and the level of $VMAT_2$ binding sites in the NAcc was similar to that observed in the control group. It seemed therefore that the control diet given at these developmental stages was able to reverse both biochemical and neurochemical changes induced by α-linolenic acid deficiency. This is in accordance with previously reported findings in a piglet model of n-3 PUFA deficiency in which supplementation with DHA provided during the lactation period restored both the n-3 PUFA composition of neuronal phospholipids and DA levels in the frontal cortex (49). The main outcome of the present study is that the tyramine-stimulated release of DA and $VMAT_2$ binding sites was similar in the D_{21} group and the deficient group, thus demonstrating that the shift from an α-linolenic acid-deficient diet to an equilibrated diet at weaning did not allow the recovery of these neurochemical factors. This lack of neurochemical recovery could be related to brain FA composition in this dietary group. Such a relationship had already been revealed between behavior and brain DHA status in rodent models of n-3 PUFA deficiency (50, 51). However, it has more recently been proposed that not only DHA but also n-6 PUFAs might be involved in learning and cognitive performance, as these processes could not be restored in animals with normal brain DHA recovery accompanied by an n-6/n-3 PUFA ratio that was higher than in controls (32, 33). These findings indicate that an adequate balance between n-6 and n-3 PUFAs is necessary for a normal behavioral response. In the D_{21} dietary group, we observed that the level of DHA was slightly reduced in the PE and PS of the PFCx and NAcc, whereas 22:5n-6 levels were generally increased, with greater differences in comparison to normal values for 22:5n-6 than for DHA. It can therefore be hypothesized that this incomplete biochemical recovery could be related to neurochemical changes, as already described in chronic α-linolenic acid-deficient rats (18, 26, 28). The neurochemical changes in this group might be ascribed to a low dopaminergic synaptic vesicle density, previously observed in the deficient rats (27, 28). The reduced level of DHA in cerebral membranes could probably affect their architecture and consequently the recycling of these synaptic vesicles. In agreement with this, Kitajka et al. (52) recently speculated that among numerous genes, brain genes encoding for endocytosis and formation of synaptic vesicles could be underexpressed in n-3 PUFA diet-deficient animals. As animals from the D_0, D_7, and D_{14} groups had tyramine-stimulated release of DA and $VMAT_2$ binding site levels close to those of the control group, whereas animals from the D_{21} group did not, it can be assumed that the neurochemical functions were influenced not only by the diet fed after weaning, but also by the essential PUFAs received during the lactating period. Inadequate supply of n-3 PUFAs or n-6/n-3 PUFA balance during this period in the D_{21} group could affect the neurochemical processes in adulthood, *i*) because the interval was too short between the dietary shift (weaning) and neurochemical studies for complete recovery and/or *ii*) because the dietary deficiency occurred during a critical stage in early nervous system development. The first hypothesis could be tested by studying animals from the D_{21} group after a longer period of dietary reversal. The second hypothesis agrees with a recent finding showing that restoration of n-3 PUFAs in deficient mice through different exogenous sources completely restored rearing activity and learning deficits, but provided only partial recovery of anxiety level. This suggests that profound n-3 PUFA deficiency occurring during early development can induce long term abnormalities of brain functioning that could not be reversed. This hypothesis has already been proposed for various environmental agents such as early exposure to neurotoxic agents (53) or to stress (54).

In conclusion, we demonstrated that *i*) a reversal diet with adequate n-6 and n-3 PUFAs given during the lactating period to rats originating from α-linolenic acid-deficient dams was able to restore both the FA composition of brain membranes and several parameters of dopaminergic neurotransmission, and *ii*) when given from weaning, this reversal diet allowed partial recovery of biochemical parameters, but no recovery of neurochemical factors. The neurochemical recovery could be a consequence of biochemical recovery, but the occurrence of profound n-3 PUFA deficiency during the lactating period could be an environmental insult leading to irreversible damage to specific brain functions. This could be linked to the emergence of critical neurodevelopmental processes during this period.

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