

## The dopamine mesocorticolimbic pathway is affected by deficiency in n-3 polyunsaturated fatty acids<sup>1-3</sup>

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### ABSTRACT

**Background:** Several findings in humans support the hypothesis of links between n-3 polyunsaturated fatty acid (PUFA) status and psychiatric diseases.

**Objective:** The involvement of PUFAs in central nervous system function can be assessed with the use of dietary manipulation in animal models. We studied the effects of chronic dietary n-3 PUFA deficiency on mesocorticolimbic dopamine neurotransmission in rats.

**Design:** Using dual-probe microdialysis, we analyzed dopamine release under amphetamine stimulation simultaneously in the frontal cortex and the nucleus accumbens. The messenger RNA (mRNA) expression of vesicular monoamine transporter<sub>2</sub> and dopamine D<sub>2</sub> receptor was studied with the use of in situ hybridization. The protein expression of the synthesis-limiting enzyme tyrosine 3-monooxygenase (tyrosine 3-hydroxylase) was studied with the use of immunocytochemistry.

**Results:** Dopamine release was significantly lower in both cerebral areas in n-3 PUFA-deficient rats than in control rats, but this effect was abolished in the frontal cortex and reversed in the nucleus accumbens by reserpine pretreatment, which depletes the dopamine vesicular storage pool. The mRNA expression of vesicular monoamine transporter<sub>2</sub> was lower in both cerebral areas in n-3 PUFA-deficient rats than in control rats, whereas the mRNA expression of D<sub>2</sub> receptor was lower in the frontal cortex and higher in the nucleus accumbens in n-3 PUFA-deficient rats than in control rats. Finally, tyrosine 3-monooxygenase immunoreactivity was higher in the ventral tegmental area in n-3 PUFA-deficient rats than in control rats.

**Conclusions:** Our results suggest that the mesolimbic dopamine pathway is more active whereas the mesocortical pathway is less active in n-3 PUFA-deficient rats than in control rats. This provides new neurochemical evidence supporting the effects of n-3 PUFA deficiency on behavior. *Am J Clin Nutr* 2002;75:662-7.

**KEY WORDS** Dopamine, mesocorticolimbic pathway, frontal cortex, microdialysis, n-3 polyunsaturated fatty acids, nucleus accumbens, rats

### INTRODUCTION

The presence in the brain of large amounts of n-3 polyunsaturated fatty acids (n-3 PUFAs) is indicative of the major role

that these compounds play in the structure and function of this organ (1). These PUFAs are provided exclusively by the diet in the form of a precursor ( $\alpha$ -linolenic acid) and long-chain derivatives. Several findings in humans support the hypothesis of links between PUFA status and psychiatric diseases such as schizophrenia (2). The involvement of PUFAs in central nervous system function can be assessed with the use of dietary manipulation in animal models. Chronic dietary deficiency in  $\alpha$ -linolenic acid in rodents greatly affects the fatty acid composition of cerebral membrane phospholipids (1) and impairs performance in learning ability and motivational processes (3).

Although behavioral findings cannot be precisely related to specific neurochemical pathways, we proposed that the behavioral effects of n-3 PUFA deficiency could be mediated through dopaminergic systems (4). This hypothesis was based mainly on the known role of dopamine as a major modulator of attention and motivation (5). In support of our proposal, we showed that long-term dietary deficiency in n-3 PUFAs induces a significant reduction in the amount of dopamine and dopamine D<sub>2</sub> receptors, specifically in the frontal cortex (6). In addition, using microdialysis, we found a decrease in cortical dopamine release accompanied by an increase in metabolite release, suggesting modifications in dopamine turnover and metabolism in n-3 PUFA-deficient rats (7, 8). Because of functional links between the frontal cortex and the limbic system (5, 9), we next studied several variables of dopaminergic neurotransmission in the nucleus accumbens. Because this cerebral region is involved in reinforcement processes (10), behavioral perturbations in n-3 PUFA-deficient rats might be related to a modified dopaminergic function in the nucleus accumbens (11).

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**TABLE 1**  
Fatty acid composition of the study diets<sup>1</sup>

Fatty acid	Control <sup>2</sup>	n-3 PUFA-deficient <sup>3</sup>
% by wt of fatty acids		
SFA		
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
Total	13.2	16.8
MUFA		
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
Total	63.1	61.9
n-6 PUFA		
18:2n-6	21.2	21.3
Total	21.2	21.3
n-3 PUFA		
18:3n-3	3.6	<0.1
Total	3.6	<0.1
(n-6) + (n-3)	24.8	21.3
(n-6)/(n-3)	5.9	—

<sup>1</sup>SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

<sup>2</sup>African peanut oil-rapeseed oil mixture (60.5%:39.5%).

<sup>3</sup>African peanut oil.

In agreement with this hypothesis, we showed that extracellular dopamine was increased in the nucleus accumbens of awake n-3 PUFA-deficient rats (12). Our hypothesis is that this result might be related to the decrease in cortical dopamine, thus removing the inhibitory effect exerted by the frontal cortex efferents on the dopamine concentration in the nucleus accumbens.

In the present study, we used dual-probe microdialysis to simultaneously evaluate the effects of n-3 PUFA deficiency on dopamine release under pharmacologic stimulation in the frontal cortex and the nucleus accumbens. We measured amphetamine-stimulated dopamine release both with and without pretreatment with reserpine, which depletes the dopamine vesicular storage pool. Because dopamine release is partly dependent on the synthesis rate, we also evaluated the immunoreactivity of the synthesis-limiting enzyme tyrosine 3-monooxygenase (TM; tyrosine 3-hydroxylase) in the ventral tegmental area (VTA) containing the dopaminergic cell bodies of the mesocortical and mesolimbic pathways. In addition, we measured the concentration of messenger RNA (mRNA) for vesicular monoamine transporter<sub>2</sub> (VMAT<sub>2</sub>) and dopamine D<sub>2</sub> receptor, both of which are main protein targets of dopamine, in the frontal cortex and the nucleus accumbens.

## 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 a diet containing 6% fat by weight in the form of African peanut oil specifically deficient in  $\alpha$ -linolenic acid as previously described (4, 6, 7). This n-3 PUFA-deficient

diet provided 12 mg linoleic acid/g but <0.06 mg  $\alpha$ -linolenic acid/g. Two weeks before mating, female rats originating from the second generation of  $\alpha$ -linolenic acid-deficient rats were divided into 2 groups. The first group received the n-3 PUFA-deficient diet. The second group received a control diet in which peanut oil was replaced by a mixture of 60% peanut oil and 40% rapeseed oil but that provided the same amount of linoleic acid as did the deficient diet plus 2 mg  $\alpha$ -linolenic acid/g [(n-6)/(n-3) = 6]. The diets were consumed ad libitum by both groups. At weaning, the male progeny of these 2 groups of female rats received the same diets as did their respective dams. The fatty acid composition of the study diets is shown in **Table 1**. Experiments were performed on 250–300-g male rats (2–3 mo of age) from both dietary groups. The experimental procedures were in compliance with guidelines from the European Communities Council directories 86/609/EEC.

### Dual-probe microdialysis

The rats were anesthetized with urethane [1.5 g/kg body wt intraperitoneally (ip)] and placed in a stereotaxic apparatus under body-temperature control (7). Microdialysis was performed with 2 vertical probes (MAB, Stockholm). One probe (1 mm) was implanted into the nucleus accumbens shell, and the second probe (4 mm) was implanted into the ipsilateral frontal cortex. The coordinates of implantation were as follows: anteroposterior 1.7, mediolateral 1.0, and dorsomedial -6.0 in the nucleus accumbens and anteroposterior 3.2, mediolateral 1.2, and dorsomedial -6.0 in the frontal cortex, according to the atlas of Paxinos and Watson (13). The probes were immediately perfused at 5  $\mu$ L/min as previously described (7, 12). Two hours after implantation of the probes, dialysates were collected at 20-min intervals and kept at -80°C until analyzed. The rats remained under anesthesia throughout the microdialysis measurements. Different groups of rats were used for experiments 1 and 2.

#### Experiment 1: effects of amphetamine on dopamine release

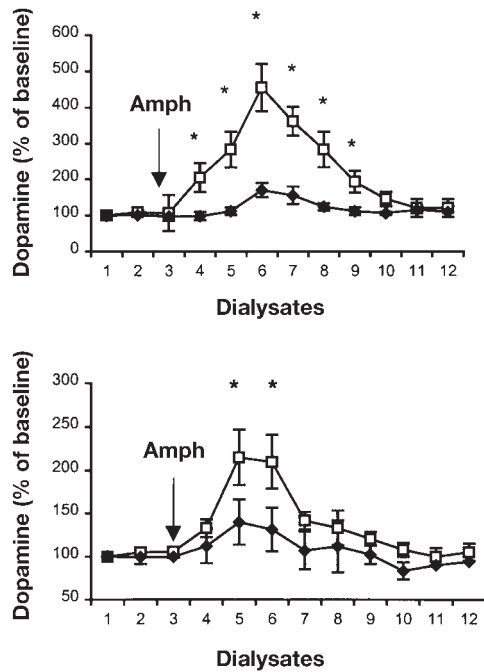
Two hours after probe implantation, baseline dopamine measurements were taken from both areas for  $\geq 1$  h. Eight n-3 PUFA-deficient and 7 control rats then received amphetamine sulfate dissolved in saline (1.5 mg/kg body wt ip). Samples were collected for 3 h after amphetamine injection.

#### Experiment 2: effects of reserpine pretreatment on amphetamine-stimulated dopamine release

The procedure was the same as in experiment 1 except for drug administration. Six rats from each dietary group were pretreated with reserpine (5 mg/kg body wt ip), which was dissolved in a solution of glacial acetic acid (3%) and glucose and injected 3 h before amphetamine treatment. Samples were collected for 3 h after reserpine injection and then for 2 h after amphetamine injection. After the experiments, each rat was given an overdose of pentobarbital, and probe placements were atlas-matched. Dopamine was quantified with the use of electrochemical HPLC as previously described (7); the limit of detection for dopamine was 0.1 nmol/L.

### In situ hybridization experiments

Five rats from each dietary group were decapitated, and their brains were quickly removed, frozen in isopentane at -35°C, and stored at -80°C. Coronal tissue sections (20  $\mu$ m thick) were cut in a cryostat at -20°C from throughout the nucleus



**FIGURE 1.** Effect of amphetamine (Amph) injection (1.5 mg/kg body wt intraperitoneally) on dopamine release ( $\bar{x} \pm \text{SEM}$ ) in dual-probe dialysates collected simultaneously from the frontal cortex (top) and the nucleus accumbens (bottom) of anesthetized control ( $\square$ ;  $n = 7$ ) and  $n-3$  polyunsaturated fatty acid (PUFA)-deficient ( $\blacklozenge$ ;  $n = 8$ ) rats. Each dialysate corresponds to a 20-min interval. The arrow indicates the time of amphetamine injection. \*Significantly different from  $n-3$  PUFA-deficient rats,  $P < 0.05$  (Scheffe's test).

accumbens and the frontal cortex and thaw-mounted onto gelatin-coated glass slides. For  $D_2$  receptors, 3 mRNA-complementary oligonucleotide probes were used according to Le Moine and Bloch (14). For VMAT<sub>2</sub>, 2 mRNA-complementary oligonucleotide probes were used according to Lu and Wolf (15). The probes were radiolabeled at the 3'-terminus with the use of dATP-<sup>35</sup>S and terminal transferase and purified by chromatographic separation. The brain sections were fixed in 4% paraformaldehyde, washed in phosphate-buffered saline, and dehydrated in ethanol baths at increasing concentrations. Hybridization was performed with a labeling probe mix (50% formamide, 10% dextran sulfate, 1% Denhardt solution, 5% Sarkosyl, 4× sodium saline citrate (SSC), 500 g DNA/L, 200 mmol dithiothreitol/L, 20 mmol Na<sub>2</sub>HPO<sub>4</sub>/L, 250 g transfer RNA/L, and 0.1% diethyl pyrocarbonate; probe corresponding to a radioactivity of 100 000 counts per minute/section) after slides were incubated overnight at 42°C. Slides were then washed twice in 1× SSC at room temperature (for 30 min and then for 15 min), twice in 1× SSC at 40°C (for 30 min and then for 15 min), and twice in 0.1× SSC at 40°C (for 30 min and then for 15 min). After ethanol dehydration, the slides were exposed on βmax Hyperfilm (Amersham, Saclay, France) for 5 d. Regional absorbances were measured at 400–800 nm with the use of an image analyzer (Biocom, Les Ulis, France).

#### Immunocytochemistry experiments

Eight rats from each dietary group were anesthetized with sodium pentobarbital (50 mg/kg body wt ip), and 40 mL heparin

( $1 \times 10^6$  U heparin/L in 0.15 mol NaCl/L) and 50 mL 2% paraformaldehyde in phosphate buffer (PB), pH 7.4, were perfused through the ascending aorta. After fixation, the brains were removed and fixed again for 30 min in 2% paraformaldehyde and maintained in a solution of PB containing 25% sucrose until processed. Coronal sections (30 μm thick) were cut through the midbrain and maintained in a solution of 25% sucrose in PB. The free-floating tissue sections were incubated overnight at room temperature in a solution of bovine serum albumin (0.1%) and trisaline (0.9% NaCl in 0.1 mol tris/L), pH 7.6, to which a 1:6000 dilution of a mouse monoclonal TM antiserum (Sigma, Saint-Quentin-Fallavier, France) was added. The bound TM antiserum was identified with the use of the avidin-biotin-peroxidase complex method previously described (16). The absorbance of the peroxidase product at 400–800 nm was measured as pixels with the use of an image analyzer (Biocom). The VTA from both sides of 5 matched sections were measured for each rat, and the 10 readings were averaged to provide 1 pixel value for each animal.

#### Statistical analyses

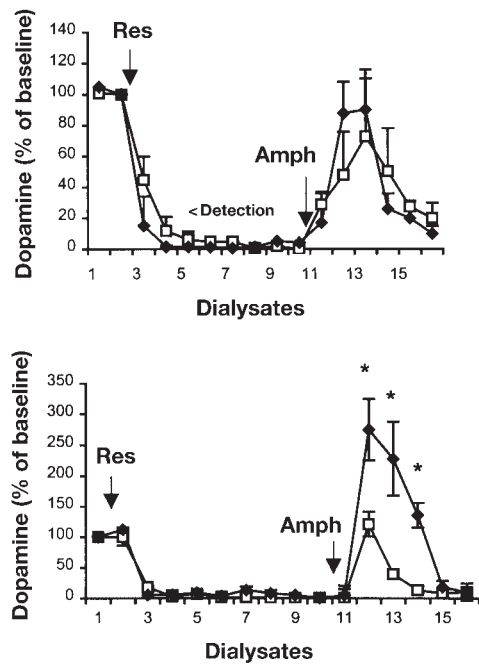
Microdialysis results for  $n-3$  PUFA-deficient and control rats were compared with the use of one-way analysis of variance (diet factor) with repeated measures over time. Comparisons at individual time points were made with the use of a post hoc Scheffe's test. In situ hybridization and immunocytochemistry experiments were analyzed with the use of Student's *t* test for unpaired values. Differences in values were considered significant when  $P < 0.05$ . Data were analyzed with the use of Microsoft EXCEL (version 97; Microsoft, Saint-Ouen, France).

#### RESULTS

Changes in dopamine release in the frontal cortex and the nucleus accumbens of anesthetized rats after amphetamine injection are shown in **Figure 1**. The baseline dopamine concentrations did not differ significantly between the  $n-3$  PUFA-deficient and control rats either in the frontal cortex ( $0.40 \pm 0.14$  and  $0.42 \pm 0.13$  nmol/L dialysate, respectively) or in the nucleus accumbens ( $1.54 \pm 0.31$  and  $1.34 \pm 0.28$  nmol/L dialysate, respectively). The increase in dopamine release after amphetamine injection was significantly different between the  $n-3$  PUFA-deficient and control rats both in the frontal cortex and in the nucleus accumbens. The highest dopamine concentrations in the frontal cortex were 1.7- and 4.5-fold and in the nucleus accumbens were 1.4- and 2.1-fold baseline in the  $n-3$  PUFA-deficient and control groups, respectively. The stimulated release of dopamine in the frontal cortex and the nucleus accumbens was therefore significantly lower in the  $n-3$  PUFA-deficient rats than in the control rats.

The effects of reserpine pretreatment on changes in dopamine release in the frontal cortex and the nucleus accumbens of anesthetized rats after amphetamine stimulation are shown in **Figure 2**. The baseline dopamine concentrations did not differ significantly between the  $n-3$  PUFA-deficient and control rats either in the frontal cortex ( $0.44 \pm 0.18$  and  $0.52 \pm 0.19$  nmol/L dialysate, respectively) or in the nucleus accumbens ( $1.56 \pm 0.32$  and  $1.39 \pm 0.24$  nmol/L dialysate, respectively). The extracellular dopamine concentration observed in the frontal cortex after reserpine treatment was lower than our detection limit ( $<0.1$  nmol/L) and became detectable again after the amphetamine injection. The cortical dopamine concentration in both dietary groups





**FIGURE 2.** Effect of reserpine (Res) injection (5 mg/kg body wt intraperitoneally) 3 h before amphetamine (Amph) injection (1.5 mg/kg body wt intraperitoneally) on dopamine release ( $\bar{x} \pm \text{SEM}$ ) in dual-probe dialysates collected simultaneously from the frontal cortex (top) and the nucleus accumbens (bottom) of anesthetized control ( $\square$ ;  $n = 6$ ) and n-3 polyunsaturated fatty acid (PUFA)-deficient ( $\blacklozenge$ ;  $n = 6$ ) rats. Each dialysate corresponds to a 20-min interval. The arrows indicate the time of reserpine and amphetamine injection. <Detection, values  $< 0.1$  nmol/L. \*Significantly different from control rats,  $P < 0.05$  (Scheffe's test).

increased to a similar extent under amphetamine stimulation ( $\approx 0.8$ -fold the basal concentrations). In contrast, the amphetamine-stimulated dopamine concentration in the nucleus accumbens was higher in the n-3 PUFA-deficient rats than in the control rats (2.7- compared with 1.2-fold baseline).

As shown in **Table 2**, mRNA expression of VMAT<sub>2</sub> was significantly lower (28%) in the n-3 PUFA-deficient rats than in the control rats, both in the frontal cortex and in the nucleus accumbens. As shown in **Table 3**, mRNA expression of D<sub>2</sub> receptor was significantly lower (32%) in the frontal cortex and significantly higher (19%) in the nucleus accumbens in the n-3 PUFA-deficient rats than in the control rats.

The semiquantification of TM immunoreactivity in the VTA of control and n-3 PUFA-deficient rats is shown in **Figure 3**. Localization of TM by light microscopy showed numerous labeled cell bodies. A total of 360 TM-labeled bodies were observed and semiquantified for each dietary group. TM immunoreactivity was significantly higher (15%) in the n-3 PUFA-deficient rats than in the control rats.

## DISCUSSION

We previously showed that n-3 PUFA deficiency in rats affects several aspects of dopaminergic neurotransmission in the frontal cortex (4, 6–8) and in the nucleus accumbens (12). These studies considered each cerebral area separately rather than the mesocorticolimbic pathway as a whole. It was therefore valuable

**TABLE 2**  
Messenger RNA expression of vesicular monoamine transporter<sub>2</sub><sup>1</sup>

	Control rats ( $n = 5$ )	n-3 PUFA-deficient rats ( $n = 5$ )
	<i>arbitrary absorbance units</i>	
Frontal cortex	12.9 $\pm$ 1.0	9.3 $\pm$ 0.4 <sup>2</sup>
Nucleus accumbens	13.7 $\pm$ 0.9	9.8 $\pm$ 0.8 <sup>2</sup>

<sup>1</sup> $\bar{x} \pm \text{SEM}$ . Wavelength = 400–800 nm. PUFA, polyunsaturated fatty acid.

<sup>2</sup>Significantly different from control rats,  $P < 0.05$  (Student's *t* test).

to explore dopaminergic neurotransmission in the same animal in both the mesocortical and mesolimbic pathways to show how interactions between the 2 pathways could be altered by the deficiency. The results of the present study show that chronic deficiency in n-3 PUFAs acts strongly at several levels: amphetamine-stimulated dopamine release, mRNA expression of VMAT<sub>2</sub> and D<sub>2</sub> receptor, and TM immunoreactivity.

In contrast with our previous findings (7, 12), we observed no significant differences between the 2 dietary groups in baseline concentrations of dopamine in either the frontal cortex or the nucleus accumbens. This discrepancy could be explained by different experimental conditions; ie, in the previous studies the rats were awake, whereas the rats in the present study were anesthetized. Indeed, we used microdialysis on anesthetized animals because handling during drug injection and responses to environmental stimuli may modify cortical and limbic dopaminergic release (5). Under these conditions, amphetamine-stimulated dopamine release was lower both in the frontal cortex and in the nucleus accumbens of n-3 PUFA-deficient rats than in control rats. Amphetamine releases dopamine, which can come from either the newly synthesized pool or the vesicular storage pool (17, 18). We previously found that [<sup>3</sup>H]dihydrotetrabenazine binding, which reflects the expression or functional state of the VMAT<sub>2</sub>, was markedly decreased in the frontal cortex (8) and the nucleus accumbens (12) of n-3 PUFA-deficient rats. In the present study, we observed significantly (30%) lower mRNA expression of this transporter in both regions in the n-3 PUFA-deficient rats than in the control rats, indicating that the decrease in [<sup>3</sup>H]dihydrotetrabenazine binding probably corresponds to a reduced expression of VMAT<sub>2</sub>. This finding suggests that the lower dopamine release in response to amphetamine in the n-3 PUFA-deficient rats than in the control rats was due to a reduction in the dopamine vesicular storage pool as previously suggested (8, 12).

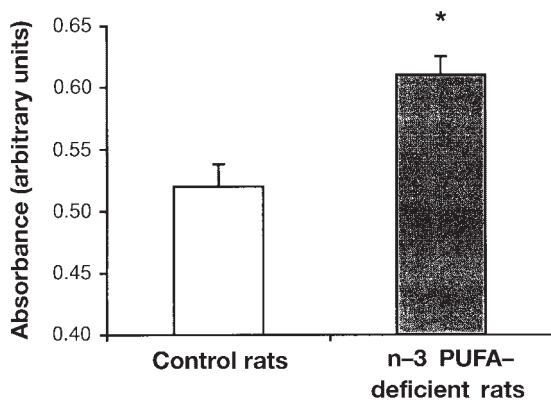
To eliminate the response of the dopamine vesicular storage pool, we performed the same pharmacologic stimulation after reserpine pretreatment. Reserpine is known to deplete dopamine vesicles and has also been shown to increase dopamine synthesis (18, 19). Interestingly, although the concentrations of dopamine released by amphetamine were lower in n-3 PUFA-deficient

**TABLE 3**  
Messenger RNA expression of dopamine D<sub>2</sub> receptors<sup>1</sup>

	Control rats ( $n = 5$ )	n-3 PUFA-deficient rats ( $n = 5$ )
	<i>arbitrary absorbance units</i>	
Frontal cortex	6.9 $\pm$ 0.4	4.7 $\pm$ 0.4 <sup>2</sup>
Nucleus accumbens	12.1 $\pm$ 0.3	14.4 $\pm$ 0.8 <sup>2</sup>

<sup>1</sup> $\bar{x} \pm \text{SEM}$ . Wavelength = 400–800 nm. PUFA, polyunsaturated fatty acid.

<sup>2</sup>Significantly different from control rats,  $P < 0.05$  (Student's *t* test).



**FIGURE 3.** Semiquantification of tyrosine 3-monooxygenase immunoreactivity in the ventral tegmental area in control ( $n = 8$ ) and  $n-3$  polyunsaturated fatty acid (PUFA)-deficient ( $n = 8$ ) rats at a wavelength of 400–800 nm. \*Significantly different from control rats,  $P < 0.05$  (Student's  $t$  test).

rats than in control rats in both cerebral regions, the response after reserpine pretreatment differed between the frontal cortex and the nucleus accumbens. Dopamine-stimulated release was similar in both dietary groups in the frontal cortex, whereas it was significantly higher in the nucleus accumbens in the  $n-3$  PUFA-deficient rats than in control rats. This finding may have been related to an increase in the rate of dopamine synthesis in the  $n-3$  PUFA-deficient rats. To check this, we explored TM immunoreactivity in the VTA, which contains dopamine neuron cell bodies, and found that it was significantly higher in the  $n-3$  PUFA-deficient rats than in the control rats. This higher TM immunoreactivity might appear as a compensatory mechanism occurring in response to the deficit of vesicle-stored dopamine in  $n-3$  PUFA-deficient rats. However, the different response to amphetamine-stimulated dopamine release after reserpine pretreatment between the nucleus accumbens and the frontal cortex remains intriguing. It is known that there are anatomic differences in ascending pathways originating from the VTA (20) and that VTA projections of dopamine neurons are denser in subcortical than in cortical regions (21). Moreover, several characteristics such as responsiveness to dopamine antagonists and agonists, dopamine turnover, and changes in the pattern and rate of cell firing can be distinguished between the mesocortical and mesolimbic dopamine systems (5).

Because  $D_2$  receptors are major targets of dopamine, it was of great value to compare them in the frontal cortex and the nucleus accumbens of  $n-3$  PUFA-deficient and control rats. We previously found that expression of these receptors was lower in the frontal cortex (4, 6) but higher in the nucleus accumbens (12) in  $n-3$  PUFA-deficient rats than in control rats. The present results showed that the expression of  $D_2$  receptor mRNA was lower in the cortical region and higher in the nucleus accumbens in the  $n-3$  PUFA-deficient rats than in the control rats. This is in agreement with changes in the number rather than the affinity of  $D_2$  receptors induced by  $n-3$  PUFA deficiency. The increased density of  $D_2$  receptors in the nucleus accumbens of  $n-3$  PUFA-deficient animals could be a presynaptic response mechanism to the rise in basal dopamine concentrations that we found in this cerebral region in awake rats (12). These results may also

suggest that changes in dietary lipid content that affect the lipid composition of cerebral membranes may also affect the regulation of gene transcription.

Finally, the present findings are compatible with those of several studies that report behavioral effects of  $n-3$  PUFA deficiency (3). Mesolimbic dopamine neurons play a strong role in motivational behavior and emotional functions, and mesocortical dopamine neurons are involved in cognitive functions such as working memory (22). In addition, the dopamine storage pool is mobilized during cognitive tasks (23). The deficit in this pool that we found both in the frontal cortex and in the nucleus accumbens could therefore contribute significantly to the poorer performance of  $n-3$  PUFA-deficient rats than of control rats on various cognitive tasks.

In conclusion, the present study shows that  $n-3$  PUFA deficiency induces changes at several levels of the dopaminergic mesocorticolimbic pathway. First, the main finding is that the mesolimbic pathway functions more and the mesocortical pathway functions less in  $n-3$  PUFA-deficient rats than in control rats. Second, the vesicular storage pool of dopamine is impaired in the frontal cortex and the nucleus accumbens of  $n-3$  PUFA-deficient rats. Further studies are necessary to explain these regional regulations. Finally, our results show that the amount of  $n-3$  PUFAs in the diet might act on the regulation of cerebral gene expression. This direct involvement of dietary PUFAs in the regulation of gene expression described previously (24) opens a new research field in nutritional neuroscience. 🌱

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