The dopamine mesocorticolimbic pathway is affected by deficiency in n–3 polyunsaturated fatty acids¹⁻³

Luc Zimmer, Sylvie Vancassel, Sylvain Cantagrel, Patrick Breton, Séraphin Delamanche, Denis Guilloteau, Georges Durand, and Sylvie Chalon

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 transporter₂ and dopamine D₂ 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 transporter₂ was lower in both cerebral areas in n–3 PUFA–deficient rats than in control rats, whereas the mRNA expression of D₂ 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.

INTRODUCTION
The presence in the brain of large amounts of n–3 polyunsaturated fatty acids (n–3 PUFA) 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 (α-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 α-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 D₂ 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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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 α-linolenic acid as previously described (4, 6, 7). The n−3 PUFA–deficient diet provided 12 mg linoleic acid/g but <0.06 mg α-linolenic acid/g. Two weeks before mating, female rats originating from the second generation of α-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 α-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.

### MATERIALS AND METHODS

#### 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 μL/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 ≥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 μm thick) were cut in a cryostat at −20°C from throughout the nucleus.

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**Table 1**

Fatty acid composition of the study diets

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control</th>
<th>n−3 PUFA–deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% by wt of fatty acids</td>
<td>% by wt of fatty acids</td>
</tr>
<tr>
<td>SFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>8.1</td>
<td>9.9</td>
</tr>
<tr>
<td>18:0</td>
<td>2.4</td>
<td>3.1</td>
</tr>
<tr>
<td>20:0</td>
<td>0.9</td>
<td>1.2</td>
</tr>
<tr>
<td>22:0</td>
<td>1.2</td>
<td>1.8</td>
</tr>
<tr>
<td>24:0</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Total</td>
<td>13.2</td>
<td>16.8</td>
</tr>
<tr>
<td>MUFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:1n−7</td>
<td>1.1</td>
<td>0.0</td>
</tr>
<tr>
<td>18:1n−9</td>
<td>60.9</td>
<td>60.8</td>
</tr>
<tr>
<td>18:1n−7</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>20:1n−9</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Total</td>
<td>63.1</td>
<td>61.9</td>
</tr>
<tr>
<td>n−6 PUFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:2n−6</td>
<td>21.2</td>
<td>21.3</td>
</tr>
<tr>
<td>Total</td>
<td>21.2</td>
<td>21.3</td>
</tr>
<tr>
<td>n−3 PUFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:3n−3</td>
<td>3.6</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Total</td>
<td>3.6</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>(n−6) + (n−3)</td>
<td>24.8</td>
<td>21.3</td>
</tr>
<tr>
<td>(n−6)/(n−3)</td>
<td>5.9</td>
<td>—</td>
</tr>
</tbody>
</table>

1. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
2. African peanut oil–rapeseed oil mixture (60.5%:39.5%).
3. African peanut oil.
acccumbens and the frontal cortex and thaw-mounted onto gelatin-coated glass slides. For D2 receptors, 3 mRNA-complementary oligonucleotide probes were used according to Le Moine and Bloch (14). For VMAT2, 2 mRNA-complementary oligonucleotidic probes were used according to Le Moine and Bloch (14). For VMAT2, 2 mRNA-complementary oligonucleotidic probes were used according to Lu and Wolf (15). The probes were radiolabeled at the 3’-terminus with the use of dATP-35S 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, 4X sodium saline citrate (SSC), 500 g DNA/L, 200 mmol dithiothreitol/L, 20 mmol Na2HPO4/L, 250 g transfer RNA/L, and 0.1% diethyl pyrocarbonate; probe corresponding to a radioactivity of 100000 counts per minute/section) after slides were incubated overnight at 42°C. Slides were then washed twice in 1X SSC at room temperature (for 30 min and then for 15 min), twice in 1X SSC at 40°C (for 30 min and then for 15 min), twice in 0.1X 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 × 106 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 tris-saline (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 ± 0.14 and 0.42 ± 0.13 nmol/L dialysate, respectively) or in the nucleus accumbens (1.54 ± 0.31 and 1.34 ± 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 rats, respectively. The stimulated 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

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**FIGURE 1.** Effect of amphetamine (Amph) injection (1.5 mg/kg body wt intraperitoneally) on dopamine release (T ± SEM) in dual-probe dialyses collected simultaneously from the frontal cortex (top) and the nucleus accumbens (bottom) of anesthetized control (□; n = 7) and n–3 polyunsaturated fatty acid (PUFA)–deficient (●; 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).
studies considered each cerebral area separately rather than the frontal cortex (4, 6–8) and in the nucleus accumbens (12). These effects several aspects of dopaminergic neurotransmission in the Discussion

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 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 VMAT2 and D2 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; i.e., 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 [3H]dihydrotetrabenazine binding, which reflects the expression or functional state of the VMAT2, 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 lower (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 [3H]dihydrotetrabenazine binding probably corresponds to a reduced expression of VMAT2. 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 2

<table>
<thead>
<tr>
<th></th>
<th>Control rats (n = 5)</th>
<th>n–3 PUFA–deficient rats (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex</td>
<td>12.9 ± 1.0</td>
<td>9.3 ± 0.4&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td>13.7 ± 0.9</td>
<td>9.8 ± 0.8&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> t ± SEM. Wavelength = 400–800 nm. PUFA, polyunsaturated fatty acid.

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

TABLE 3

<table>
<thead>
<tr>
<th></th>
<th>Control rats (n = 5)</th>
<th>n–3 PUFA–deficient rats (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex</td>
<td>6.9 ± 0.4</td>
<td>4.7 ± 0.4&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td>12.1 ± 0.3</td>
<td>14.4 ± 0.8&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> t ± SEM. Wavelength = 400–800 nm. PUFA, polyunsaturated fatty acid.

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


