Modification of dopamine neurotransmission in the nucleus accumbens of rats deficient in n–3 polyunsaturated fatty acids

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Abstract **We studied the effects of a diet chronically deficient in α-linolenic acid, the precursor of long-chain n–3 polyunsaturated fatty acids, on dopaminergic neurotransmission in the shell region of the nucleus accumbens of rats. In vivo microdialysis experiments showed increased basal levels of dopamine and decreased basal levels of metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in awake rats from the deficient group compared to controls. The release of dopamine under KCl stimulation was similar in both dietary groups. By contrast, the release of dopamine from the vesicular storage pool under tyramine stimulation was 90% lower in the deficient than in the control rats. Autoradiographic studies in the same cerebral region revealed a 60% reduction in the vesicular monoamine transporter sites in the deficient group. Dopamine D_2 receptors were 35% increased in these rats compared to controls, whereas no change occurred for D_1 receptors and membrane dopamine transporters. These results demonstrated that chronic n–3 polyunsaturated fatty acid deficiency modifies several factors of dopaminergic neurotransmission in the nucleus accumbens. These findings are in agreement with the changes in dopaminergic neurotransmission already observed in the frontal cortex, and with the behavioral disturbances described in these deficient rats.**—Zimmer, L., S. Delion-Vancassel, G. Durand, D. Guilloteau, S. Bodard, J-C. Besnard, and S. Chalon. **Modification of dopamine neurotransmission in the nucleus accumbens of rats deficient in n–3 polyunsaturated fatty acids.** ***J. Lipid Res.*** **2000.** 41: **32–40.**

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The presence in the brain of large amounts of polyunsaturated fatty acids (PUFA) from the n–3 and n–6 families is in agreement with their major role in the structure and function of this organ. These PUFA are exclusively provided by the diet, in the form of precursors (α-linolenic acid and linoleic acid) and long-chain derivatives. Their functional role can therefore be assessed using dietary manipulation in animal models. Deprivation of n–3 PUFA in the rat over several generations results in overall changes in performance on learning tasks as reviewed by Wainwright (1). However, these modified responses can often associate with differences in learning abilities and differences in sensorial, motor, or motivational abilities (2). In particular, increased response rates of several reinforcement factors and slower extinction have been shown in n–3 PUFA-deficient rats (3–5), which can be interpreted as changes in motivation. Although it is difficult to link responses to behavioral tests and specific neurochemical pathways, we have recently proposed that the effects of n–3 PUFA deficiency on the rat's behavior could be mediated through dopaminergic systems (6). This hypothesis was mainly based on the known role of dopamine (DA) as a major modulator of attention, motivation, and emotion (7). Supporting our proposal, we have shown that dietary provision of n–3 PUFA is able to act on dopaminergic neurotransmission in the case of chronic deficiency (6, 8) and also in the case of overload (9). In the frontal cortex, several elements of this neurotransmission, i.e., the D_2 receptors and the overall amount of DA, were decreased in rats with long-term n–3 PUFA deficiency. By contrast, we observed no change in the striatum, in agreement with the lack of motor effects previously found in such deficient animals (10–12). In addition, microdialysis studies performed in the frontal cortex of deficient rats showed reduction of the intra-neuronal vesicular compartment of DA (13).

In order to clarify the relationships between the supply of n–3 PUFA and neurochemical processes, we studied several aspects of dopaminergic neurotransmission in one termination of the mesolimbic dopaminergic pathway, the nucleus accumbens (nAcc), in n–3 PUFA-deficient rats.

Abbreviations: DTBZ, dihydrotetrabenazine; nAcc, nucleus accumbens; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; DA, dopamine; DAT, DA transporter; $VMAT_2$, vesicular monoamine transporter.

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This cerebral region is of great interest for several reasons: *i*) the mesolimbic dopaminergic pathway has a major role in attentional, motivational, and emotional processes (7, 14), *ii*) several anatomical and functional findings demonstrate that the activity of the nAcc is partly regulated by cortical input (15–17). This can be expressed as an inhibitory effect of the prefrontal cortex on the dopaminergic activity in the nAcc (18, 19). In addition, it has recently been proposed that the reduced levels of DA and D_2 receptors measured in the frontal cortex of n–3 PUFA-deficient rats (6, 8) could lead to reduction of the inhibition of the nAcc, and this would be in agreement with the behavioral disturbances described in these animals (20, 21).

Several findings of the present study demonstrate the strong effects of n–3 PUFA deficiency on dopaminergic functions in the nAcc. First, using a dynamic approach to neurotransmission processes with the microdialysis method, we studied basal DA release in awake rats and stimulated cytoplasmic and vesicular DA release in anesthetized rats. In addition, autoradiographic studies were performed in order to evaluate several elements of dopaminergic transmission such as the DA transporter (DAT), D_1 and D_2 receptors, and the vesicular monoamine transporter ($VMAT_2$).

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 g/100 g fat in the form of African peanut oil specifically deficient in α-linolenic acid, as described previously (6, 8). This deficient diet provided 1200 mg of linoleic acid but less than 6 mg of α-linolenic acid per 100 g of diet. Two weeks before mating, female rats originating from the second generation of α-linolenic acid-deficient rats were divided into two groups. The first group received the deficient diet, and the second group received a diet in which peanut oil was replaced by a mixture of 60% peanut oil and 40% rapeseed oil. This diet (control) 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). Diets were consumed ad libitum by both groups. At weaning, the male progeny of these two groups of female rats received the same diets as their respective dams. The overall composition of diets and the fatty acid composition of dietary lipids are reported in **Table 1** and **Table 2**. Experiments were performed on 250–300 g male rats (2–3 months of age) from both dietary groups. A total of 8 litters receiving each diet were used; each litter provided a mean of 5 male rats which were mixed at weaning. The experimental procedures were in compliance with guidelines from the European Communities Council directories 86/609/EEC.

Microdialysis in awake rats

Each animal was placed in a stereotaxic apparatus (Stoelting, Wood Dale, IL) under ketamine anesthesia at a dose of 1.5 mg/kg i.p. (Imalgène, Rhône Mérieux, France). A probe guide (CMA/Microdialysis, Sweden) was implanted in the shell region of the left nAcc (coordinates: antero-posterior 1.7; medio-lateral 1.0; dorso-ventral −6, from Bregma) according to the atlas of Paxinos and Watson (22) (**Fig. 1**). Burr holes were drilled in the skull directly above the point of insertion. Two anchor screws were inserted at least 3 mm away from the implantation site. After fixation of the probe guide with dental cement and removing the animal from the stereotaxic frame, a microdialysis probe with 10% recovery (polycarbonate, 15 kDA cut-off, 1 mm length, MAB 14-1, Sweden) was slowly lowered into the nAcc shell under stereotaxic guidance. The probe was immediately and continuously perfused with perfusion buffer (Dulbecco modified liquid, ICN, USA + 2.2 mmol/L $CaCl_2$) at 0.8 μl/min using a microsyringe pump (Harvard Appa-

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): $CaHPO_4 \cdot 2H_20$, 380; K_2HPO_4, 240; $CaCO_3$, 180; NaCl, 69; MgO, 20; $MgSO_4 \cdot 7H_2O$, 90; $FeSO_4.7H_2O$, 8.6; $ZnSO_4 \cdot H_2O$, 5; $MnSO_4 \cdot H_2O$, 5; $CuSO_4 \cdot 5H_2O$, 1; NaF, 0.8; $CrK(SO_4)_2.12H_2O$, 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), 250000; acetate dl-α-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	Control[a]	n–3-Deficient[b]
	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	13.1	16.8
16:1(n–7)	1.1	0.0
18:1(n–9)	60.9	60.8
18:1(n–7)	0.0	0.0
20:1(n–9)	1.1	1.1
MUFA	60.2	61.9
18:2(n–6)	21.2	21.3
(n–6) PUFA	21.2	21.3
18:3(n–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		
18:2(n–6)	1196	1201
18:3(n–3)	203	<6

Abbreviations used: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Oils were kindly supplied by Lesieur-Alimentaire (Coudekerque; France).

[a] African peanut oil–rapeseed oil mixture (60.5%–39.5%).

[b] African peanut oil.

ratus, South Natick, MA). The operated rats were allowed 24 h recovery with this perfusion before experimentation. After this equilibrium period, the rate was modified to 2 μl/min and dialysates were collected in 20-min fractions (40 μl) and kept at −80°C until analysis. The animals were killed after the experiments by a pentobarbital bolus (Sanofi, France) and the probe site was macroscopically checked on sections.

Microdialysis in anesthetized rats

Under urethane anesthesia (Sigma, St. Louis, MO) at a dose of 1.5 g/kg i.p. in a single injection, each rat was placed in the stereotaxic apparatus. After drilling the skull on the previously described coordinates, the microdialysis probe (without guide) was implanted in the shell region of the left nAcc as described above. The probe was immediately and continuously perfused with the perfusion buffer at 2 μl/min. Body temperature was maintained at 37 ± 1°C using a thermostatically controlled heating blanket (CMA 150, CMA/Microdialysis Sweden). Three or four 20-min dialysate samples were collected after pre-perfusion for 1 h and were defined as basal level before drug infusion. Potassium chloride (KCl 200 mmol/L, Sigma) or tyramine (200 μmol/L, RBI Bioblock, Illkirch, France) was added to the buffer perfusing the dialysis probe for 20 min. After this stimulation (separate animals for each drug), the basal level was recovered for 1 h 40 (5 dialysates). Samples of dialysate were kept at −80°C until analysis.

HPLC analysis

Dialysate levels of catecholamines were measured using HPLC with electrochemical detection according to the previously described procedure (13). Briefly, an isocratic mobile phase (consisting of 7% acetonitrile, 3% methanol and 90% citric acid 0.02 mol/L + monobasic phosphate sodium 0.01 mol/L + octanesulfonic acid 0.0045 mol/L + heptanesulfonic acid 0.003 mol/L + EDTA 0.0001 mol/L + o-phosphoric acid and diethylamine with pH 2.5 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 column (LC-22C, Bioanalytical Systems, West Lafayette, IN). A glassy carbon working set at + 0.8 V with reference to an Ag/AgCl electrode was used to detect compounds of interest. Signals were recorded and quantified by the Beckman Gold 118 integrator, calibrated with a standard aqueous solution of DA, 3,4 dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Under these conditions, the limit of detection of DA was 0.1 fmol/μl.

Autoradiographic studies

S(−)-sulpiride (+)-butaclamol hydrochloride, desipramine hydrochloride, and reserpine were purchased from RBI, pargyline was obtained from Sigma (France), and mazindol was a gift from Sandoz (France). [^{3}H]-SCH-23390 (specific activity, 71.1 Ci/mmol), [^{3}H]-YM-09151-2 (specific activity, 81.4 Ci/mmol), and [^{3}H]mazindol (specific activity, 17 Ci/mmol) were obtained from NEN (France) and [^{3}H]dihydrotetrabenazine ([^{3}H]-DTBZ, specific activity, 186 Ci/mmol) was obtained from Amersham (Netherlands).

Rats were killed by decapitation, the brains were rapidly removed and divided on the coronal plane anterior to the cerebellum. The forebrain portion was frozen in a mixture of isopentane/dry ice (at −35°C) and stored at −80°C until use. Twenty micrometer-thick sequential coronal sections through the nAcc (antero-posterior coordinates 1.7 from Bregma according to the atlas of Paxinos and Watson) were cut at −20°C (Jung Cryocut 1800, Leica, France), thaw mounted onto gelatin-coated slides and stored at −80°C until assay.

Binding to DA D_1 receptors, DA D_2 receptors, and DAT was measured with the specific tritiated ligands, [^{3}H]-SCH-23390, [^{3}H]-YM-09151-2, and [^{3}H]mazindol, respectively, according to previously described procedures (8).

Binding of [^{3}H]-DTBZ to the vesicular monoamine transporter ($VMAT_2$) was assessed using the procedure of Wilson and Kish (23) with minor modifications. Briefly, 20 μm-thick coronal sections were pre-washed for 1 h at room temperature in 50 mmol/L sodium phosphate buffer, pH 7.7, in order to remove endogenous competing substances. Sections were then incubated at room temperature for 1 h in sodium phosphate buffer containing 7.5 nmol/L [^{3}H]-DTBZ. Non-specific binding was defined in the presence of 2 μmol/L reserpine in the sodium phosphate buffer. After incuba-

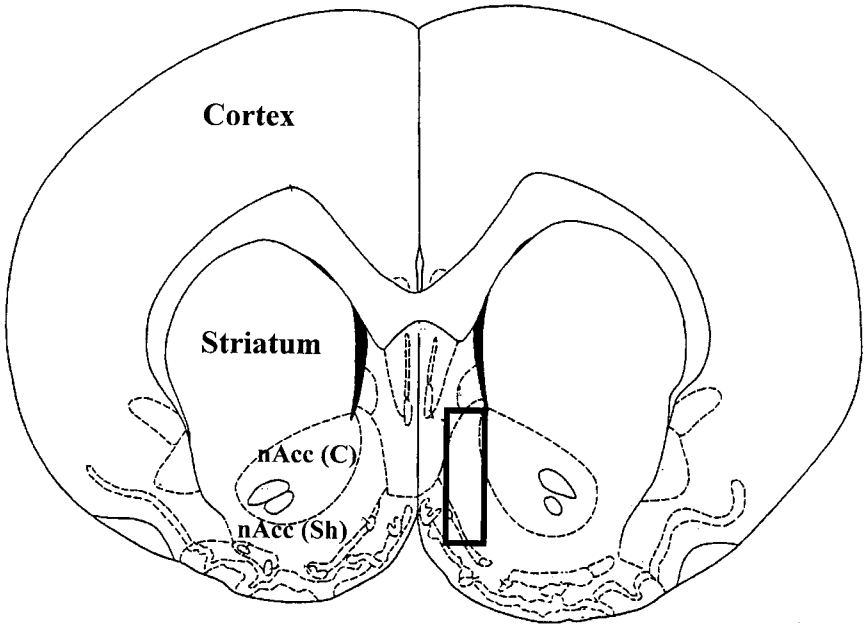

Fig. 1. Coronal section illustrating the location of the microdialysis probe and the analyzed area in autoradiographic studies (open rectangle) in the nucleus accumbens shell, according to the atlas of Paxinos and Watson (22); nAcc(Sh), nucleus accumbens shell; nAcc(C), nucleus accumbens core.

tion, slices were washed in sodium phosphate buffer at 4°C for 3 min and rinsed in distilled water before drying.

Dried sections were exposed on tritium-sensitive film ([^{3}H]-Hyperfilm, Amersham, France) in the presence of tritium-calibrated standards (Microscales, Amersham) for appropriate periods, i.e., 9 days for D_1 receptors, 5 weeks for D_2 receptors, 5 weeks at 4°C for DAT and 3 weeks for $VMAT_2$. Films were developed and densitometric analysis of autoradiograms in the nAcc shell (Fig. 1) was performed by computer-assisted video densitometry (Biocom, Siemens Nixdorf). Optical densities were converted to apparent tissue ligand concentrations with reference to radioactive standards and specific activity of the radioligands.

Statistical analyses

Microdialysis results were compared between deficient and control rats by one-way ANOVA (diet-factor) with repeated measures over time. Comparisons at individual time points were made by a post hoc F Scheffe test.

Autoradiographic data were analyzed by Student's *t*-test for unpaired values. Differences in values were considered significant when $P < 0.05$.

RESULTS

Figure 2 shows the extracellular basal levels of DA, DOPAC, and HVA in the nAcc shell of awake rats. The basal level of released DA was significantly higher in deficient rats compared to control rats (2.95 ± 0.70 versus 0.74 ± 0.17 fmol/μl of dialysate, respectively, $P < 0.05$). Moreover, the basal level of DOPAC was significantly lower in deficient rats (44.2 ± 10.6 fmol/μl versus 248.9 ± 48.8 fmol/μl in the control group, $P < 0.05$). The basal level of HVA was also significantly lower in deficient rats (48.4 ± 7.6 fmol/μl versus 76.5 ± 8.1 fmol/μl in the control group, $P < 0.05$).

Figure 3 shows the changes in DA, DOPAC, and HVA release in anesthetized rats after KCl stimulation (200 mmol/L). There was no significant difference between dietary groups in terms of basal levels of DA and metabolites (0.97 ± 0.19 versus 1.29 ± 0.32 fmol/μl of dialysate for DA, 87.5 ± 10.2 versus 92.3 ± 16.3 fmol/μl for DOPAC and 70.6 ± 6.9 versus 89.3 ± 15.9 fmol/μl for HVA, in deficient and control rats, respectively). Arrows on the figures indicate the duration of KCl infusion. This led to an increase in the dialysate DA content: the maximum response was 5.5 times higher than basal values for both deficient and control rats. This increase in DA level was accompanied by a reduction in the levels of DOPAC and HVA, without significant difference between dietary groups.

Figure 4 shows the changes in DA, DOPAC, and HVA release in anesthetized rats after tyramine stimulation (200 μmol/L). Basal levels of DA and metabolites were similar for both dietary groups (1.44 ± 0.39 versus 1.24 ± 0.41 fmol/μl of dialysate for DA, 99.4 ± 30.2 versus 100.0 ± 26.6 fmol/μl for DOPAC and 84.5 ± 8.4 versus 109.0 ± 16.6 fmol/μl for HVA for deficient and control rats, respectively). Arrows on the figures indicate the duration of tyramine infusion. This resulted in significantly different increases in DA release between the deficient and control rats. At the maximum effect, the DA levels were 12- and 113-fold the basal values for the deficient and control groups, respectively. Stimulated release of DA was therefore dramatically and significantly lower in deficient rats than in control rats ($P < 0.05$). DOPAC levels were similarly enhanced under tyramine infusion in both dietary groups, whereas HVA levels were unchanged.

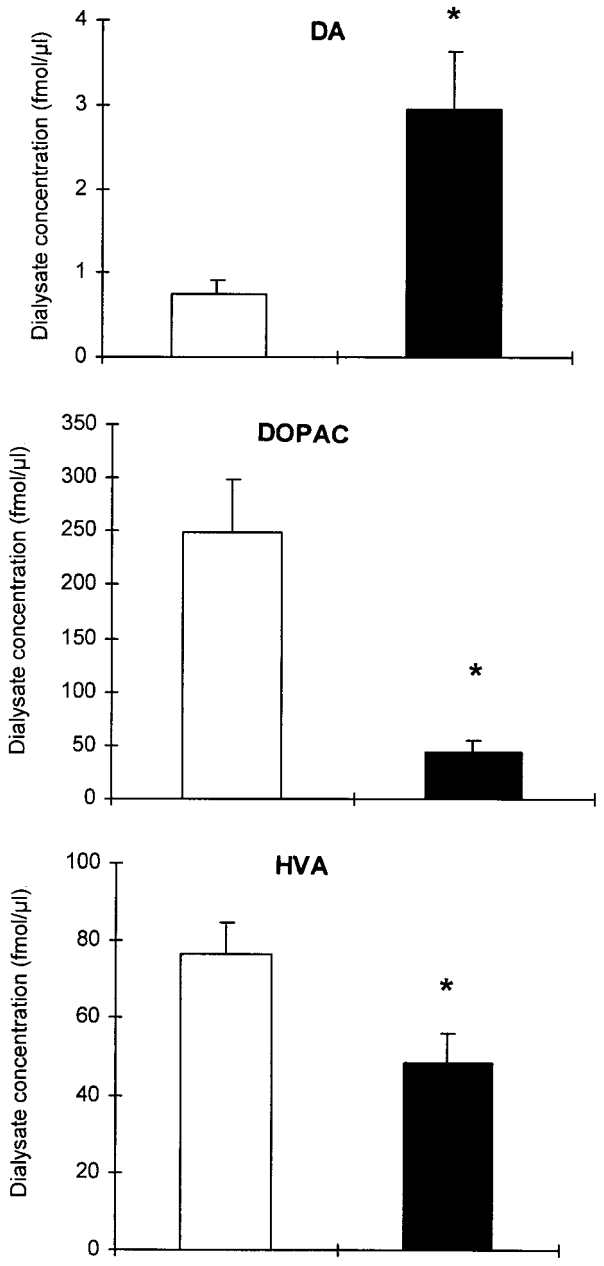

Fig. 2. Basal levels of DA and metabolites (DOPAC, HVA) obtained by microdialysis in the shell part of the nAcc of awake animals (n = 6 for each dietary group). Data are the mean ± SEM and are expressed in fmol/μl of dialysate (without probe correction). * Significant difference between control (□) and deficient (■) rats ($P < 0.05$, ANOVA).

Figure 5 shows representative false-color coded microphotographs demonstrating the location of dopaminergic receptors (D_1, D_2) and the transporters (DAT, $VMAT_2$) in the nucleus accumbens shell of n–3 PUFA-deficient rats.

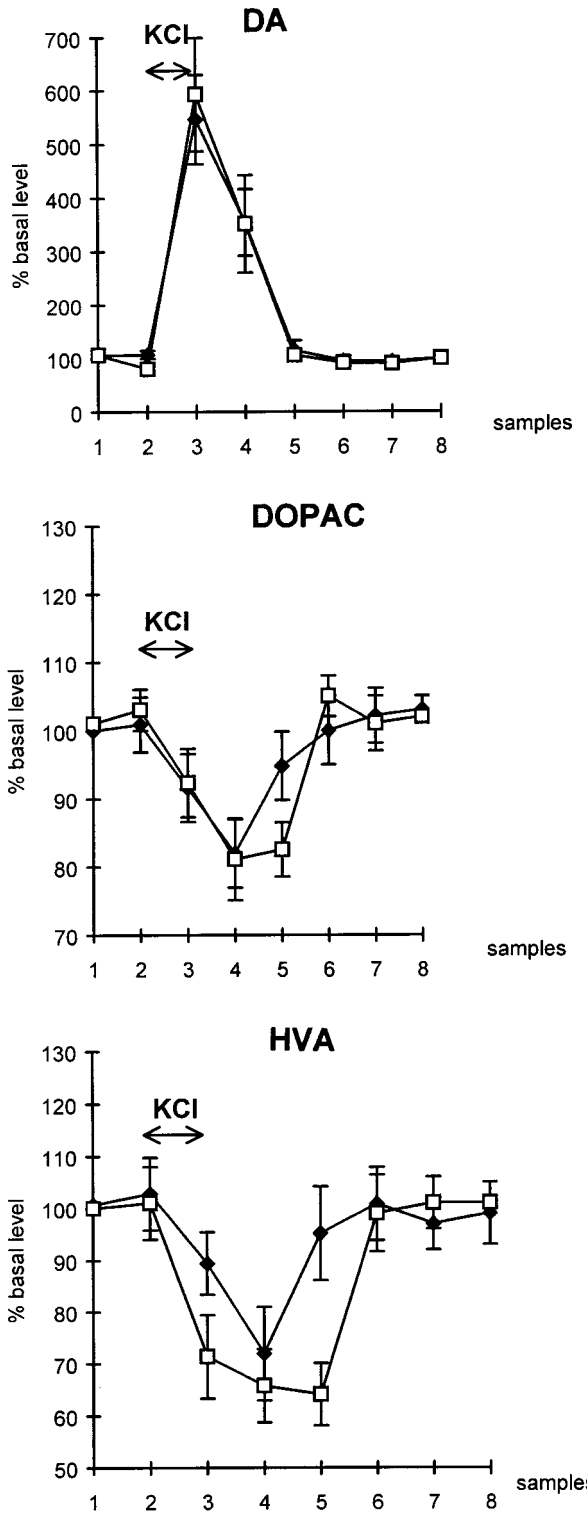

Fig. 3. Effect of in situ KCl infusion (200 mmol/L) on DA and metabolite levels in dialysates collected from the nAcc shell of anesthetized control (□) and deficient rats (◆). Double arrows indicate the KCl infusion period. Data are the mean percentages of basal levels ± SEM (n = 7 for each dietary group).

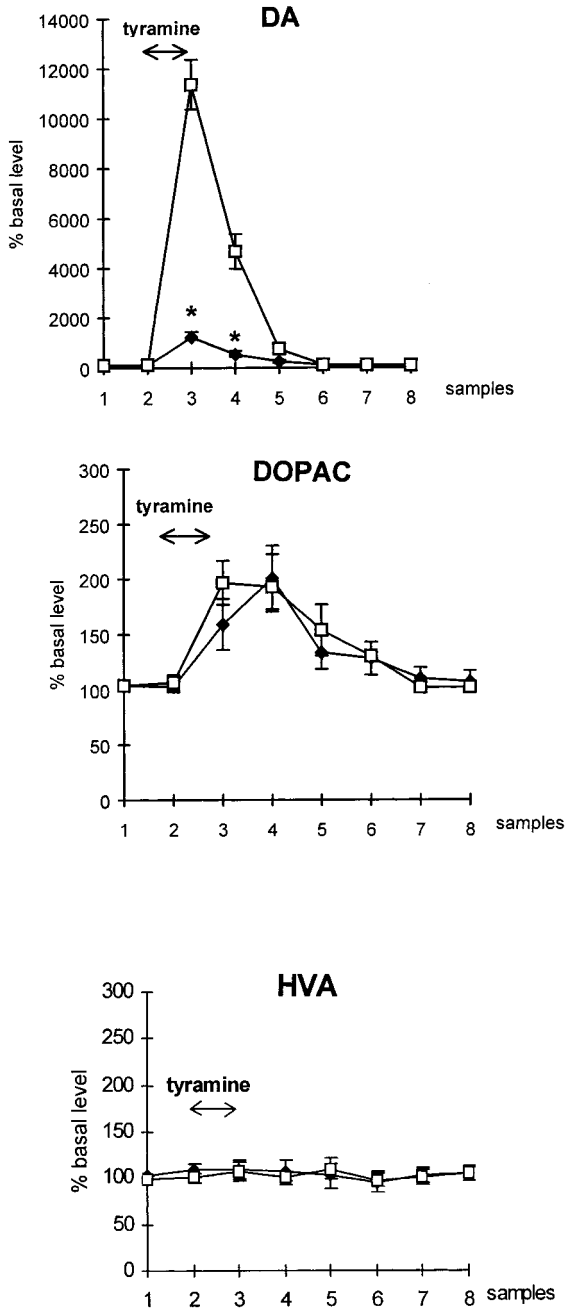

Fig. 4. Effect of in situ tyramine infusion (200 μmol/L) on DA and metabolite levels in dialysates collected from the nAcc shell of anesthetized control (□) and deficient rats (◆). Double arrows indicate the tyramine infusion period. Data are the mean percentages of basal levels ± SEM (n = 7 for each dietary group). * Increase in DA after stimulation was significantly different between control and deficient rats ($P < 0.05$, Student's *t*-test for unpaired values).

Table 3 shows the D_1 receptor, D_2 receptor, DAT, and $VMAT_2$ binding sites measured in the shell region of nAcc. The density of dopamine D_1 receptor sites, evaluated by the specific binding of [^{3}H]SCH-23390, was high in the nAcc (about 150 pmol/g of wet tissue) and was similar in both the deficient and control groups.

The specific binding of [^{3}H]-YM-09151-2 to D_2 receptors was consistently and significantly higher in the n−3 PUFA-deficient rats than in controls (+36%, $P < 0.05$). Mean specific binding of [^{3}H]-YM-09151-2 was 87.0 ± 22.8

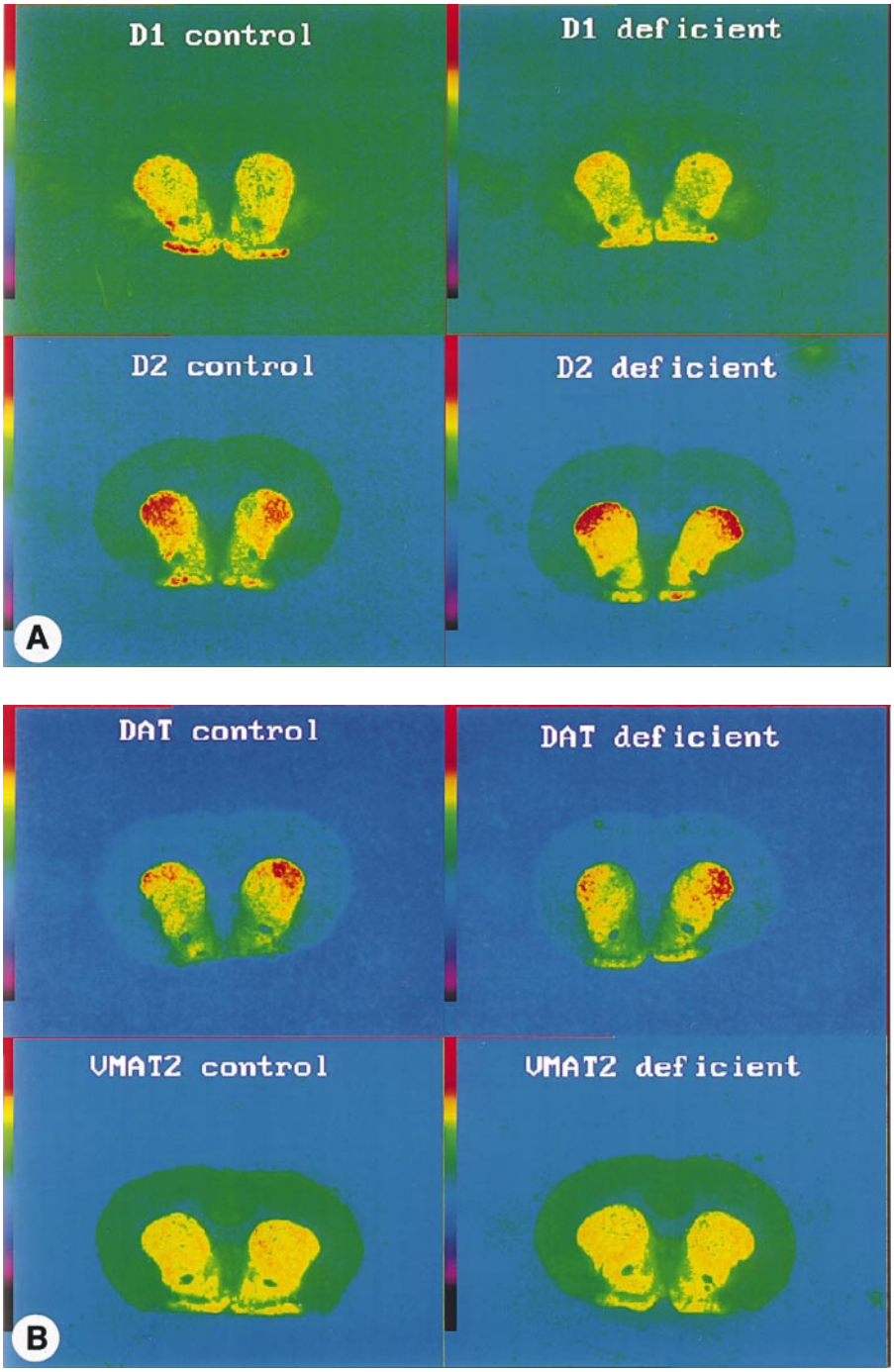

Fig. 5. Color-coded images of coronal sections of the anterior forebrain of control and n–3 PUFA-deficient rats showing (A) the distribution of dopaminergic D_1 and D_2 receptors and (B) the distribution of neuronal and vesicular transporters, DAT and $VMAT_2$. Color scale: from red color (high radioactivity) to blue color (low radioactivity).

pmol/g of wet tissue in the deficient rats compared to 64.0 ± 15.5 pmol/g of wet tissue in controls.

The DAT binding sites were revealed by [^{3}H]mazindol, and the mean specific binding was 90 pmol/g of wet tissue for both deficient and control rats.

The specific binding of [^{3}H]DTBZ to $VMAT_2$ was 60% lower in the nAcc of deficient rats compared to controls (11.3 ± 2.5 versus 28.1 ± 3.3 pmol/g of wet tissue, $P < 0.05$).

DISCUSSION

We have previously shown that an unbalanced supply (deficiency or excess) of n–3 PUFA changed several aspects of the dopaminergic neurotransmission in the frontal cortex of rats (6, 8, 9, 13). The results of the present study show that chronic deficiency of n–3 PUFA strongly acts on dopaminergic neurotransmission in the nAcc, af-

TABLE 3. Effect of dietary α-linolenic acid deficiency on dopaminergic and $VMAT_2$ binding sites in the shell nucleus accumbens in 2 month-old male rats

Binding Site	Control[a]	n−3-Deficient[b]
	pmol/g wet tissue	
D_1	153.0 ± 20.1	154.5 ± 14.2
D_2	64.0 ± 15.5	87.0 ± 22.8[a]
DAT	90.0 ± 14.1	93.5 ± 13.5
$VMAT_2$	28.1 ± 3.3	11.3 ± 2.5*

Values are given as means ± SD ; n = 8. The brain sections of each group of rats (one control, one deficient) were exposed on the same film (*, significantly different from control, $P < 0.05$, Student's *t*-test). D_1, dopamine D_1 receptors; D_2, dopamine D_2 receptors; DAT, dopamine transporters; $VMAT_2$, vesicular monoamine transporters.

[a] African peanut oil−rapeseed oil mixture (60.5%−39.5%).

[b] African peanut oil.

fecting both DA metabolism and several molecular targets of DA such as receptors and transporters. Chronic n−3 PUFA-deficient rats had higher basal levels of DA and lower basal levels of metabolites (DOPAC, HVA) in the shell part of the nAcc than animals receiving a dietary supply of n−3 PUFA corresponding to need (10). Moreover, the vesicular storage pool of DA was markedly decreased, whereas the cytoplasmic pool was unchanged. Examination of several DA binding sites in this cerebral region showed a 35% increase in D_2 receptors with no change in D_1 receptors, and a 60% decrease in the vesicular monoamine transporter ($VMAT_2$) with no change in the membrane dopamine transporter (DAT). These results therefore demonstrated that dopaminergic neurotransmission in the nAcc is modified by n−3 PUFA deficiency.

The nAcc belongs to the mesolimbic dopaminergic system and thus plays an important role in cognitive and affective functioning (7, 17). Various studies have indicated that the processes of positive reinforcement are mediated through DA release in the nAcc, and more particularly in the shell portion which is involved in emotional and cognitive responses (24). This cerebral region responds to novel stimuli or stimuli associated with rewards with a DA surge (25) which is decreased after environmental habituation (26). In addition, evidence has been accumulated that the mesocortical DA pathway indirectly inhibits the DA response of the mesolimbic pathway (27). This inhibition seems to be mediated through glutamate and GABA fibers, which globally inhibit release of DA from the nAcc nerve terminals (28). The basal output of DA terminals in the nAcc is therefore under tonic inhibitory control of the frontal cortex (29).

Our results revealed an enhanced basal DA level in the nAcc in n−3 PUFA-deficient rats. This could be related to previous findings that showed a lower endogenous DA concentration in the frontal cortex of these animals, with higher extracellular metabolite levels and reduced storage pool of DA (6, 8, 13). It can be hypothesized that the decreased levels of total and packaged DA in the frontal cortex of deficient rats would decrease inhibition of the nAcc and thus explain the enhanced DA release in the nAcc of these animals.

In addition to these changes in DA metabolism, we observed a 36% increase in [^{3}H]-YM-0915-2 binding in the shell of the nAcc in n−3-deficient rats compared to controls. [^{3}H]-YM-0915-2 is a D_2-like DA receptor antagonist (30) which binds both to D_2 and D_3 DA sites (31). In the nAcc, these DA binding sites are largely localized in afferent fibers where they act as autoreceptors and regulate the release of DA in the synaptic cleft (32, 33). Although our autoradiographic studies did not discriminate between modification in the affinity or number of binding sites, it can be hypothesized that the density of DA D_2 and/or D_3 autoreceptors was increased in the shell of the nAcc in deficient animals. This could be a response mechanism to the rise in the basal DA levels measured in this cerebral region.

Moreover, we studied both pools of intraneuronal DA, the cytoplasmic pool and the storage pool, using the microdialysis technique and specific in situ stimuli. We chose pharmacological stimulation in anesthetized animals in order to avoid any effects of external stimuli which could influence dopaminergic release in the nAcc (7). It is known that KCl infusion induces release of newly synthesized DA, whereas tyramine acts on DA from vesicular stores (34, 35). The KCl-induced release of DA was not different between deficient and control animals. In contrast, the tyramine response was markedly reduced in deficient animals. These results therefore demonstrated that n−3 PUFA deficiency induced the same decrease in the vesicular pool of DA in the nAcc as previously observed in the frontal cortex (13). In order to evaluate the mechanism leading to a decrease in this dopaminergic storage pool, we labeled the vesicular monoamine transporters ($VMAT_2$) using [^{3}H]dihydrotetrabenazine. The density of [^{3}H]DTBZ, which reflects the number and/or affinity of functional vesicular transporters (36), was markedly decreased in deficient animals. This result, associated with reduced DA release under tyramine stimulation, could correspond to a reduction in the number of dopaminergic vesicles. n−3 PUFA deficiency might significantly alter the physical properties of vesicular membranes (fluidity, permeability), as already suggested (10, 37), and might thus decrease vesicle formation. In accordance with this hypothesis, it has already been shown that α-linolenic acid dietary deficiency can affect vesicular density in the rat hippocampus after learning sessions (38). These results could be related to the known modifications in the fatty acid composition of neuronal membrane phospholipids occurring with this dietary deficiency (10, 39). Moreover, we previously reported that the level of docosahexaenoic acid (22:6n−3), the predominant n−3 PUFA in membrane phospholipids, was 75% lower in the ventral striatum (including nucleus accumbens) in our model of deficient rats (6). This low level of 22:6(n−3) was compensated for by a significantly higher amount of n−6 PUFA, particularly 22:5(n−6), in the same area. It can be therefore hypothesized that in deficient rats the high DA level under environmental stimulation (awake animals) could be the cumulative consequence of both low storage of newly synthesized DA and deinhibition of the nAcc related to hypofunction of the frontal cortex. Although metabolite levels

may not always be reliable indicators of DA release (40), the decreased levels of DOPAC and HVA in deficient awake rats could be explained by the high level of DA release before intra-neuronal metabolism.

Finally, our results agree with several studies reporting behavioral effects of n–3 PUFA deficiency. In particular, studies of visual discrimination abilities in behavioral procedures using food as positive reinforcement (3–5) demonstrated that n–3 PUFA deficiency acted on motivation or response to reward rather than through direct effect on learning ability. According to this assumption, greater reactivity to external stimuli related to a deficit in attention could interfere with and in turn delay the acquisition of learning in these animals (2). In view of the involvement of mesolimbic DA release in the pleasurable effect of reinforcing stimuli, it can therefore be assumed that in n–3 PUFA-deficient rats all environmental stimuli have the value of reward and lead to greater responses to the external environment. This could also explain the slower extinction process previously described by Yamamoto et al. (5). All these behavioral findings would be in agreement with the rise in basal DA levels observed in the shell of the nAcc. Moreover, the DA storage pool is particularly mobilized during cognitive sessions (41), and our results of tyramine stimulation indicated that this pool could be reduced in n–3 PUFA-deficient rats. It can therefore be hypothesized that the positive reward is less effective in deficient rats and thus disrupts learning. An increased tendency to engage with stimuli in the environment and a disrupted reinforcement process could then together contribute to the poorer performance in cognitive tasks observed in n–3 PUFA-deficient rats.

In conclusion, this study demonstrates that n–3 PUFA deficiency induces modifications in DA neurotransmission in the nAcc. The enhanced physiological DA release can be partly explained by a reduction in the effectiveness of inhibitory effect of the frontal cortex on the DA nerve terminals in the nAcc in deficient rats. This could result in a DA surge in the nAcc which might then cause an increased tendency to engage with stimuli in the environment, as already proposed by Reisbick and Neuringer (21). Thus, the present findings provide new neurochemical evidence of the mechanisms explaining the effects of n–3 PUFA deficiency on behavior.

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REFERENCES

1. Wainwright, P. E. 1992. Do essential fatty acids play a role in brain and behavioral development? *Neurosci. Behav. Rev.* **16:** 193–205.
2. Wainwright, P. E., H. C. Xing, L. Mutsaers, D. McCutcheon, and D. Kyle. 1997. Arachidonic acid offsets the effects on mouse brain and behavior of a diet with a low (n–6)/(n–3) ratio and very high levels of docosahexaenoic acid. *J. Nutr.* **127:** 184–193.
3. Okaniwa, Y., S. Yuasa, N. Yamamoto, S. Watanabe, T. Kobayashi, H. Okuyama, M. Nomura, and Y. Nagata. 1996. A high linoleate and a high α-linolenate diet induced changes in learning behavior of rats. Effects of a shift in diets and reversal of training stimuli. *Biol. Pharm. Bull.* **19:** 536–540.
4. Yamamoto, N., M. Saitoh, A. Moriuchi, M. Nomura, and H. Okuyama. 1987. Effect of dietary α-linolenate/linoleate balance on brain lipid compositions and learning ability of rats. *J. Lipid Res.* **28:** 144–151.
5. Yamamoto, N., A. Hashimoto, Y. Takemoto, H. Okuyama, M. Nomura, R. Kitajima, T. Togashi, and Y. Tamai. 1988. Effect of the dietary α-linolenate/linoleate balance on lipid compositions and learning ability of rats. II. Discrimination process, extinction process, and glycolipid compositions. *J. Lipid Res.* **29:** 1013–1021.
6. Delion, S., S. Chalon, J. Hérault, D. Guilloteau, J. C. Besnard, and G. Durand. 1994. Chronic dietary α-linolenic acid deficiency alters dopaminergic and serotoninergic neurotransmission in rats. *J. Nutr.* **124:** 2466–2476.
7. Le Moal, M., and H. Simon. 1991. Mesocorticolimbic dopaminergic networks: functional and regulatory roles. *Physiol. Rev.* **71:** 155–234.
8. Delion, S., S. Chalon, D. Guilloteau, J. C. Besnard, and G. Durand. 1996. α-Linolenic acid dietary deficiency alters age-related changes of dopaminergic and serotoninergic neurotransmission in the rat frontal cortex. *J. Neurochem.* **66:** 1582–1591.
9. Chalon, S., S. Delion-Vancassel, C. Belzung, D. Guilloteau, A. M. Leguisquet, J. C. Besnard, and G. Durand. 1998. Dietary fish oil affects monoaminergic neurotransmission and behavior in rats. *J. Nutr.* **129:** 2512–2519.
10. Bourre, J. M., A. Youyou, O. Dumont, M. Piciotti, G. Pascal, and G. Durand. 1989. The effects of dietary α-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning tasks in rats. *J. Nutr.* **119:** 1880–1892.
11. Enslen, M., H. Milon, and A. Malnoë. 1991. Effect of low intake of (n–3) fatty acids during development on brain phospholipid fatty acid composition and exploratory behaviour in rats. *Lipids.* **26:** 203–207.
12. Wainwright, P. E., Y-S. Huang, B. Bulman-Fleming, S. Lévesque, and D. McCutcheon. 1994. The effects of dietary fatty acid composition combined with environmental enrichment on brain and behavior in mice. *Behav. Brain Res.* **60:** 125–136.
13. Zimmer, L., S. Hembert, G. Durand, P. Breton, D. Guilloteau, J. C. Besnard, and S. Chalon. 1998. Chronic n–3 polyunsaturated fatty acid diet-deficiency acts on dopamine metabolism in the rat frontal cortex : a microdialysis study. *Neurosci. Lett.* **240:** 177–181.
14. Salamone, J. D., M. S. Cousins, L. D. McCullough, D. L. Carriero, and R. J. Berkowitz. 1994. Nucleus accumbens dopamine release increases during instrumental lever pressing for food but not free food consumption. *Pharmacol. Biochem. Behav.* **49:** 25–31.
15. Beckstead, R. M., V. B. Domestick, and W. J. Nauta. 1979. Efferent connections of the substantia nigra and ventral tegmental area in the rat. *Brain Res.* **175:** 191–217.
16. Christie, M. J., L. B. James, and P. M. Beart. 1985. An excitant amino acid projection from the medial prefrontal cortex to the anterior part of nucleus accumbens in the rat. *J. Neurochem.* **45:** 477–482.
17. Le Moal, M. 1995. Mesocorticolimbic dopaminergic neurons: functional and regulatory roles. *In* Psychopharmacology: the Fourth Generation of Progress. F. E. Bloom and D. J. Kupfer, editors. Raven Press, New York, NY. 283–294.
18. King, D., and J. M. Finlay. 1995. Effects of selective dopamine depletion in medial prefrontal cortex on basal and evoked extracellular dopamine in neostriatum. *Brain Res.* **10:** 117–128.
19. King, D., M. J. Zigmond, and J. M. Finlay. 1997. Effects of dopamine depletion in the medial prefrontal cortex on the stress-induced increase in extracellular dopamine in the nucleus accumbens core and shell. *Neuroscience.* **77:** 141–153.
20. Neuringer, M., G. J. Anderson, and W. E. Connor. 1988. The essentiality of n–3 fatty acids for the development and function of the retina and brain. *Annu. Rev. Nutr.* **8:** 517–541.
21. Reisbick, S., and M. Neuringer. 1997. Omega-3 fatty acids deficiency and behavior: a critical review and directions for future research. *In* Handbook of Essential Fatty Acid Biology. S. Yehuda and D. I. Mostfsky, editors. Humana Press, Totowa, NJ. 397–426.
22. Paxinos, G., and C. Watson. 1986. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York, NY.

23. Wilson, J. M., and S. J. Kish. 1996. The vesicular monoamine transporter, in contrast to the dopamine transporter, is not altered by chronic cocaine self-administration in the rat. *J. Neurosci.* **16:** 3507–3510.
24. Deutsch, A. Y., and D. S. Cameron. 1992. Pharmacological characterization of dopamine systems in the nucleus accumbens core and shell. *Neuroscience.* **46:** 49–56.
25. Hooks, M. S., and P. W. Kalivas. 1995. The role of mesoaccumbens-pallidal circuitry in novelty-induced behavioral activation. *Neuroscience.* **64:** 587–597.
26. Broderick, P. A., F. T. Phelan, F. Eng, and R. T. Wechsler. 1994. Ibogaine modulates cocaine responses which are altered due to environmental habituation: in vivo microvoltammetric and behavioral studies. *Pharmacol. Biochem. Behav.* **49:** 711–28.
27. Banks, K. E., and A. Gratton. 1995. Possible involvement of medial prefrontal cortex in amphetamine-induced sensitization of mesolimbic dopamine function. *Eur. J. Pharmacol.* **282:** 157–167.
28. Mitchell, J. B., and A. Gratton. 1992. Partial dopamine depletion of the prefrontal cortex leads to enhanced mesolimbic dopamine release elicited by repeated exposure to naturally reinforcing stimuli. *J. Neurosci.* **12:** 3609–3618.
29. Karreman, M., and B. Moghaddam. 1996. The prefrontal cortex regulates the basal release of dopamine in the limbic striatum: an effect mediated by ventral tegmental area. *J. Neurochem.* **66:** 589–598.
30. Niznik, H. B., D. E. Grigoriadis, I. Pri-Bar, O. Buchman, and P. Seeman. 1985. Dopamine D_2 receptors selectively labeled by a benzamide neuroleptic: [^{3}H]-YM-09151-2. *Naunyn Schmiedebergs Arch. Pharmacol.* **329:** 333–343.
31. Yokoyama, C., H. Okamura, T. Nakajima, J. Taguchi, and Y. Ibata. 1994. Autoradiographic distribution of [^{3}H]YM-09151-2, a high-affinity and selective antagonist ligand for the dopamine D2 receptor group, in the rat brain and spinal cord. *J. Comp. Neurol.* **344:** 121–136.
32. Filloux, F., T. J. Richards, G. F. Huff, and J. K. Wamsley. 1991. Quinolinic acid lesion of nucleus accumbens reduces D_1 but not D_2 dopamine receptors: an autoradiographic study. *Life Sci.* **48:** 2535–2542.
33. Sokoloff, P., B. Giros, M. P. Martres, M. L. Bouthenet, and J. C. Schwartz. 1990. Molecular cloning and characterisation of a novel dopamine receptor (D_3) as a target for neuroleptics. *Nature.* **347:** 146–151.
34. Fairbrother, I. S., G. W. Arbuthnott, J. S. Kelly, and S. P. Butcher. 1990. In vivo mechanisms underlying dopamine release from rat nigrostriatal terminals : II. Studies using potassium and tyramine. *J. Neurochem.* **54:** 1844–1851.
35. Zetterstrom, T., T. Sharp, A. K. Collin, and U. Ungerstdt. 1988. In vivo measurement of extracellular dopamine and DOPAC in rat striatum after various dopamine-releasing drugs; implications for the origin of extracellular DOPAC. *Eur. J. Pharmacol.* **13:** 327–334.
36. Scherman, D., G. Boschi, R. Rips, and J. P. Henry. 1986. The regionalization of [^{3}H]-dihydrotetrabenazine binding sites in the mouse brain and its relationship to the distribution of monoamines and their metabolites. *Brain Res.* **370:** 176–181.
37. Murphy, M. G. 1990. Dietary fatty acids and membrane protein function. *J. Nutr. Biochem.* **1:** 68–79.
38. Yoshida, S., A. Yasuda, K. Kawazato, K. Sakai, T. Shimada, M. Takeshita, S. Yuasa, T. Kobayashi, S. Watanabe, and H. Okuyama. 1997. Synaptic vesicle ultrastructural changes in the rat hippocampus induced by a combination of alpha-linolenate deficiency and a learning task. *J. Neurochem.* **68:** 1261–1268.
39. Bourre, J. M., G. Pascal, G. Durand, M. Masson, O. Dumont, and M. Piciotti. 1984. Alterations in the fatty acid composition of rat brain cells (neurons, astrocytes, and oligodendrocytes) and of subcellular fractions (myelin and synaptosomes) induced by a diet devoid of n–3 fatty acids. *J. Neurochem.* **43:** 342–348.
40. Abercrombie, E. D., K. A. Keefe, D. S. DiFrischia, and M. J. Zigmond. 1989. Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. *J. Neurochem.* **52:** 1655–1658.
41. Garris, P. A., L. B. Collins, S. R. Jones, and R. M. Wightman. 1993. Evoked extracellular dopamine in vivo in the medial prefrontal cortex. *J. Neurochem.* **61:** 637–647.