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Age-related changes in phospholipid fatty acid composition and monoaminergic neurotransmission in the hippocampus of rats fed a balanced or an n–3 polyunsaturated fatty acid-deficient diet

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Abstract The influence of aging (2, 6, 12, and 24 months) on hippocampal lipid composition and neurochemical markers (endogenous noradrenaline, serotonin levels, monoamine oxidase (MOA) activities) was studied in rats fed a control or an n-3 polyunsaturated fatty acid (PUFA)-deficient diet. The n-3 PUFA deficiency reduced the 22:6(n-3) level, compensated by the increase in 22:5(n-6). However, the difference in 22:6(n-3) content between control and deficient rats was less between 2 and 12 months and then became stable. There was an overall age-induced decrease in the major phospholipid classes phosphatidylethanolamine (PE) and phosphatidylcholine (PC) whereas the minor classes, phosphatidylinositol (PI), phosphatidylserine (PS), and sphingomyelin (SM), were greatly increased, regardless of diet. The n-3 PUFA deficiency induced a reduction in the PS level, concomitant with a higher level in MAO-B activity as compared to control rats at the age of 24 months. The age-related evolution of the MAO-B activity was parallel with that of noradrenaline levels in both dietary groups. The noradrenaline and serotonin levels were modified according to age but without effect of the n-3 PUFA deficiency. Results showed that the hippocampus sustained specific age-induced modifications in lipid composition and neurotransmission factors, often with a transition period between 6 and 12 months.—**Delion, S., S. Chalon, D. Guilloteau, B. Lejeune, J-C. Besnard, and G. Durand.** Age-related changes in phospholipid fatty acid composition and monoaminergic neurotransmission in the hippocampus of rats fed a balanced or an n-3 polyunsaturated fatty acid-deficient diet. *J. Lipid Res.* 1997. **38:** 680–689.

Supplementary key words aging • dopamine • monoamine oxidase • (n-3)PUFA deficiency • phosphatidylserine • serotonin

The hippocampus is known to be a central component of the memory-related neural system (1, 2). Extensive research has demonstrated that damage to this cerebral area produced severe amnesia for specific events that occurred within a defined spatiotemporal context (3, 4), whereas memory for nonspecific information was relatively preserved (5). Moreover, these studies have shown that aging induces signs of both prefrontal and hippocampal dysfunction. Normal aging is accompanied by some loss of memory, whereas senile dementia is defined by dramatic or rapid memory loss. In the same way, dysfunctions of the hippocampus are involved in the memory deficits associated with Alzheimer's disease (6). As reviewed by Decker (7), aging diminishes the rate of acetylcholine synthesis and release but without changes in basal concentration. However, other neurotransmission systems are involved in the mediation of hippocampal function such as dopaminergic, noradrenergic, and serotoninergic systems. Selective depletion of monoamines has thus been shown to reduce long-term potentiation in the rat hippocampus (8). Particular alteration of the monoaminergic function is also associated with the progressive age-related cognitive deficit, although the findings have varied according to studies (9–11).

In addition to neurochemical changes, aging is associated with modifications of the biophysical properties of brain membranes in rats (12) and also in humans (13). A relationship between the level of cholesterol in

Abbreviations: 5-HT, serotonin; DA, dopamine; DHA, docosahexaenoic acid; FA, fatty acid; MAO, monoamine oxidase; MUFA, monounsaturated fatty acid; NA, noradrenaline; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, phospholipid; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid; TFA, total fatty acid; SFA, saturated fatty acid; SM, sphingomyelin.

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the hippocampal region, membrane lipid fluidity, and the learning performances in training tests was described in the rat (14), suggesting that the age-related biophysical changes in the hippocampal membranes could be implicated in the decrease in age-related mental activity.

Moreover, it seems that interactions exist between aging and lipid intake, as it has been shown that a dietary supply of n-3 and n-6 PUFA is able to act on aged-related behavioral disorders. In the aged rat, animals fed an α-linolenic acid-rich diet had a longer mean survival time and an increased learning ability (15). In the same way, it was demonstrated in an animal model of memory deficit occurring during age (senescence accelerated mouse) that intake of an α-linolenic acid-rich diet improved learning ability (16).

By contrast, a diet deficient in n-3 PUFA in rats has been shown to be associated with impaired performance in a variety of learning tasks (for review see 17). Thus, rats fed an n-3 PUFA-deficient diet since fetal development showed poorer performances in a visual discrimination task maze (18, 19), a reduced speed of habituation process (20), and displayed a lower exploratory activity in a novel environment, but without modification in locomotor activity (21, 22). Moreover, Yamamoto and coworkers have described a greater resistance to extinction of behavioral process in this type of deficiency (23, 24). In mice, data are sometimes unclear as an n-3 PUFA-deficient diet has been associated with longer latencies on the Morris water task (25, 26) although Wainwright et al. (27, 28) did not observe modification of spatial learning. These latter investigators rather suggest that changes in emotional reactivity occurred, as it is also suggested in n-3 PUFA diet-deficient mice exhibiting a lower motivation to escape in the low rota-rod testing (29).

We have recently shown (30, 31) that a diet devoid of n-3 PUFA led to modifications in the lipid composition of specific cerebral areas such as the striatum and the frontal cortex, as it has previously been described for the whole brain (18, 32). Moreover, we demonstrated that this deficiency specifically altered monoaminergic function in the frontal cortex of young and aged rats. These studies suggest that behavioral impairments could be linked to disturbances of monoaminergic function in a specific cerebral area, the frontal cortex.

However, because of the involvement of the hippocampus in the behavioral and memory processes, we studied and present here the double effect of age and of an α-linolenic acid-deficient diet on this cerebral region. Lipid composition and several aspects of the monoaminergic neurotransmission, i.e., the levels of monoamines (dopamine, noradrenaline, serotonin), and the catabolic enzyme monoamine oxidase activities were evaluated. We suggest that aging and an n-3 PUFA-deficient diet could influence each other to affect the properties of hippocampal membranes and rat learning abilities.

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
Peanut oil[c]	23.6	60
Rapeseed oil[c]	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; $CrK(SO_4)_2 \cdot 2H_2O$, 0.5; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.02; KI, 0.04; $CoCO_3$, 0.02; $Na_2SeO_3 \cdot 5H_2O$, 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*-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.

MATERIALS AND METHODS

Animals and diets

Female Wistar rats originating from our laboratory were given an α-linolenic acid-deficient diet (peanut oil). This deficient diet provided about 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 from the second generation of α-linolenic-deficient rats were divided into two groups. The first group received the deficient diet, while the second group received a diet in which peanut oil was replaced by a mixture of peanut oil and rapeseed oil. This diet (control) provided the same amount of linoleic acid as the deficient diet, and also about 200 mg of α-linolenic acid per 100 g of diet (n-6/n-3 = 6). The composition of diets and the composition in fatty acids are reported in **Table 1** and **Table 2.** Diets were given ad libitum for both groups. At weaning, the male progeny (third generation) of the two groups of female rats received the same diet as their respective dams and water ad libitum. Animals were

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TABLE 2. Fatty acid composition of dietary lipids

Fatty Acids	Diet	
	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
Σ SFA	13.1	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
Σ MUFA	60.2	61.9
18:2n-6	21.2	21.3
Σ n-6 PUFA	21.2	21.3
18:3n-3	3.6	<0.1
Σ 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

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.

housed 2 by 2 per cage under standard controlled conditions of temperature, humidity, and dark–light cycles (8 h AM–20 h PM). They were studied at 2, 6, 12, and 24 months of age. Each measurement was realized on 6 male rats. A total of 15 litters receiving each diet were used; each litter provided a mean of 5 male rats which were mixed at weaning. The experimental protocol was in compliance with applicable guidelines from the Ministère de l'Agriculture, France.

Lipid analyses

The fatty acid (FA) composition in the hippocampus was determined as follows. Tissue was homogenized using a Polytron Kinematica PT 1200 (Bioblock Scientific, Strasbourg, France) in 5 mL of a chloroform–methanol solution 2:1 (vol/vol) in the presence of butylhydroxytoluene (0.02 g/L). Total lipids were extracted according to Folch, Lees, and Sloane Stanley (33). The phospholipids (PL) were then separated from total lipids on a silica-gel column (Supelclean tube LC-SI, St. Germain-en-Laye, France). Part of the PL was used for FA composition analysis. FA methyl esters were separated using a gas chromatograph (Carlo Erba 4180, Fisons Instrument, Arcueil, France) equipped with an on-column injector and capillary column (length = 50 m; diameter = 0.4 mm) (CP wax 52 CB, Chrompack, Les Ulis, France). Components were identified by their equivalent chain lengths in comparison with standards, the peaks being integrated by Nelson Software (Stang, Pavillons-sous-Bois, France).

Another aliquot of PL was used for separation of PL classes by high performance liquid chromatography (HPLC) (Beckman liquid chromatographic gold system model 126, Beckman Instruments, Gagny, France) equipped with a light-scattering detector (Cunow DDL 11, Cunow Cergy Pontoise, France). A fraction of total PL was separated by HPLC and each class (phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylcholine (PC), sphingomyelin (SM)) was related to the total amount of PL. For each determinations, two assays were performed by sample.

Determination of endogenous monoamine concentrations

Rats were decapitated and the brains were rapidly dissected on ice. The hippocampus was removed, weighed, and homogenized with an Ultra Turax T25 (Bioblock Scientific) in 0.5 mL perchloric acid 0.2 mol/L, 1 g/L EDTA, and 1 g/L $Na_2S_2O_5$ and then centrifuged at 7310 *g* (Beckman J2-21 M/E centrifuge; JA 21 rotor, Beckman Instrument) for 10 min at 4°C. The supernatant was kept at −80°C until use. Serotonin (5-HT), dopamine (DA), and noradrenaline (NA) levels were determined (two assays per sample) by HPLC with electrochemical detection (electrodetector model 460; Waters Millipore, Milford, MA).

Monoamine oxidase assays

2,5-Diphenyl-oxazole (PPO) was purchased from Sigma (France). [^{14}C]-5-OH-tryptamine creatinine sulfate ([^{14}C]5-HT, specific activity 55 mCi/mmol) was obtained from Amersham (Les Ulis, France) and [^{14}C]phenylethylamine hydrochloride ([^{14}C]PEA, specific activity 43.7 mCi/mmol) was obtained from NEN (France). Rats were decapitated and the brains were quickly removed. The hippocampus was dissected on ice and homogenized with an Ultra Turax T25 (Bioblock Scientific, Strasbourg, France) in 10 volumes (wt/vol) of ice-cold 0.1 mol/L phosphate buffer, pH 7.4. The resulting homogenate was used as enzyme sources to assay monoamine oxidase activity; each assay was conducted in triplicate. MAO [EC 1.4.3.4., amine: oxygen oxidoreductase (deaminating) flavine containing] activity was radiochemically assayed by a conventional method (high substrate concentration method). Aliquots (0.1 mL) of tissue homogenates were incubated at 37°C with [^{14}C]PEA (final concentration 8 µmol/L) for MAO-B activity for 1 min, or with [^{14}C]5-HT (final concentration 125 µmol/L) for MAO-A activity for 5 min in a total volume of 0.5 ml. The reaction

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was stopped by cooling the tubes on ice and acidifying with 0.2 ml of 4 M HCl. The deamination products, [^{14}C]indole acetic acid, were extracted in 7 ml of toluene–ethylacetate 1:1 (vol/vol). The tubes were then kept at −20°C for 1 h to allow the aqueous layer to freeze. The layer was poured into a scintillation vial and 10 mL of toluene containing PPO (0.4%, wt/vol) was then added. The total radioactivity was measured by scintillation beta counting (RACKBETA, LKB; Saint-Quentin-en-Yvelines, Paris, France). Enzyme activity was expressed as nmoles/min per mg of protein. Protein concentrations of the homogenates were determined according to Bradford (34).

Statistical analyses

Results were analyzed using a two-way ANOVA with both diet and age as factors, to take into account eventual significant interactions between both factors. A Newman-Keuls test was then applied, and different letters were assigned when means differed significantly with $P < 0.05$. For comparison at a same age, the same test was used (*, $P < 0.05$; **, $P < 0.01$).

RESULTS

Lipid analyses

Content of total lipids. There was no variation in the content of total lipids (approximately 8.2 mg/100 mg fresh weight) in the hippocampus throughout aging or between the two dietary groups (data not shown).

Fatty acid composition of total phospholipids. The principal saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) contents, i.e., 16:0, 18:0, 18:1(n-9), and 18:1(n-7) are shown in **Fig. 1.** In control rats, 16:0 (Fig. 1A), 18:1(n-9) (Fig. 1B), and 18:1(n-7) (Fig. 1D) showed fluctuations in their respective levels between 2 and 6 months, with a maximum or a minimum at 6 months of age ($P < 0.05$). α-Linolenic acid dietary deficiency induced modifications in the levels of 16:0 and 18:1(n-9) at 2 and 24 months of age and at 6 months of age, respectively. By contrast, the deficiency did not modify the age-related evolution of the amount of 18:0 and 18:1(n-7).

In control rats, the levels of the principal n-6 FA, 20:4(n-6), decreased between 2 and 6 months of age (−13%, $P < 0.05$), and then remained stable between 6 and 24 months (**Fig. 2A**). In the deficient group, the two principal n-6 PUFA were 20:4(n-6) and 22:5(n-6). The levels of 20:4(n-6) decreased between 2 and 6 months and, as in control group, remained stable after 6 months. The level of 22:5(n-6) (Fig. 2B) was greatly increased at all ages compared with the control group, but decreased between 2 and 24 months of age. This led to a considerable increase in the total n-6 PUFA in the deficient rats, and a subsequent increase in the 22:5(n-6)/22:6(n-3) ratio (Fig. 2E) and then in the total (n-6)/(n-3) ratio (Fig. 2F).

The major n-3 PUFA series was represented by 22:6(n-3) (DHA) (Fig. 2C). The level in the control group decreased from 15% to 12% of TFA between 2 and 6 months of age. After that, it remained stable up to 24 months. The deficient diet induced a dramatic decrease in DHA level as compared to controls. However, DHA levels significantly increased in deficient rats between 2 and 12 months of age and also became stable. Thus, the reduction in DHA levels in deficient versus control rats was 73%, 56%, 44%, and 50% at 2, 6, 12, and 24 months of age, respectively ($P < 0.01$). After 12 months there was thus no further recuperation of DHA as compared to control rats.

Throughout the period studied, the (n-6) + (n-3) levels in both groups of rats were around 30–35% of total FA (Fig. 2D). Total (n-6) + (n-3) levels significantly decreased between 2 and 6 months of age and then remained stable up to 24 months of age.

Proportions of phospholipid classes. Analysis of the proportions of the different PL classes showed some specific diet- and age-related modifications (**Fig. 3**). In control rats, the PE level decreased between 6 and 12 months of age (Fig. 3A), whereas the PC level remained stable between 2 and 12 months and then decreased (Fig. 3B). The contrary was observed in the evolution of PE and PC levels in deficient rats. The levels of PC were lower at 12 months and higher at 24 months in deficient rats as compared to controls (9%, $P < 0.01$), whereas PE levels were identical in the two groups except at 12 months when PE was higher in the deficient group. PS levels were not modified between 2 and 6 months (Fig. 3C), and PI levels were slightly decreased during this period (Fig. 3D). Thereafter, both PS and PI levels were greatly increased from 6 to 12 months (about 10-fold increase for PS and 2- to 3-fold increase for PI). These levels then tended to stabilize between 12 and 24 months of age. However, levels in these two classes were significantly lower in deficient rats than in controls at 24 months of age (−22%). The SM level remained stable between 2 and 12 months of age and approximately doubled from 12 to 24 months (Fig. 3E). However, no diet-related variation was observed in this PL class.

Endogenous monoamine concentrations

Endogenous dopamine levels were almost undetectable and we considered their levels insignificant. Indeed, the hippocampus is a brain region with relatively

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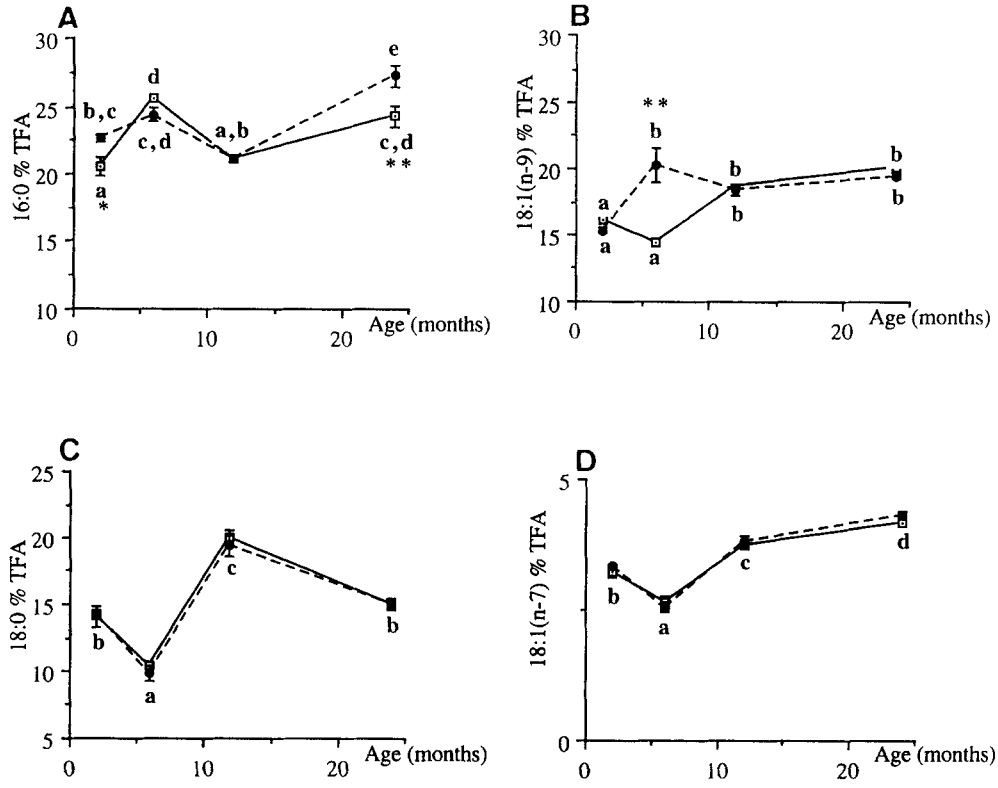


Fig. 1. Changes in saturated (16:0; 18:0) and monounsaturated (18:1(n-7); 18:1(n-9)) fatty acid levels in hippocampus of control (——) and α-linolenic acid-deficient rats (---) during aging. Values are means ± SEM (mg/100 mg of total fatty acids, TFA); n = 6 for each point. Results were analyzed using a two-way ANOVA with both diet and age as factors, and a post-hoc Newman-Keuls test. Different letters were assigned when means differed significantly with $P < 0.05$. For comparison at a same age, the same test was used (*, $P < 0.05$; **, $P < 0.01$).

little dopaminergic innervation. The results showed some specific age-related changes in NA and 5-HT levels, but independent of the diet (**Fig. 4**). Thus, the serotonin level significantly decreased by about 40% between 2 and 6 months (Fig. 4A) and then increased by 70% up to 24 months. The noradrenalin level was constant between 2 and 12 months of age and then decreased by 35% (Fig. 4B) between 12 and 24 months.

Monoamine oxidase activities

There was a predominance of MAO-A activity (60–70%) over MAO-B activity. The results showed a specific evolution of MAO-A activity according to age, whereas diet had no effect on this factor (**Fig. 5A**). In both dietary groups, MAO-A activity reached a maximum at 6 months of age, decreased slightly between 6 and 12 months, and then plateaued between 12 and 24 months. By contrast to MAO-A activity, evolution of MAO-B activity during aging was different according to the diet (Fig. 5B). The level of MAO-B in the control group increased from 40% between 2 and 12 months and then decreased significantly up to 24 months of age (−35%). The level in the deficient group reached the same maximum activity as early as 6 months and then remained stable up to the last age studied. This led to a significantly higher level of MAO-B activity in deficient versus control rats at 24 months of age (+40%).

DISCUSSION

This study examined the effects of age on the hippocampal membranes in rats fed a PUFA balanced diet or a chronically α-linolenic acid-deficient diet. The first contribution of this work concerns the evolution of lipid membrane composition during aging. It appeared that the variations in SFA and MUFA levels occurred in three phases and the period between 6 and 12 months was a transition period for these FA levels in hippocampal PL. Moreover, the major n-6 and n-3 PUFA, respectively 20:4(n-6) and 22:6(n-3), decreased between 2 and 6 months of age, but then remained stable until 24 months of age. It can therefore be assumed

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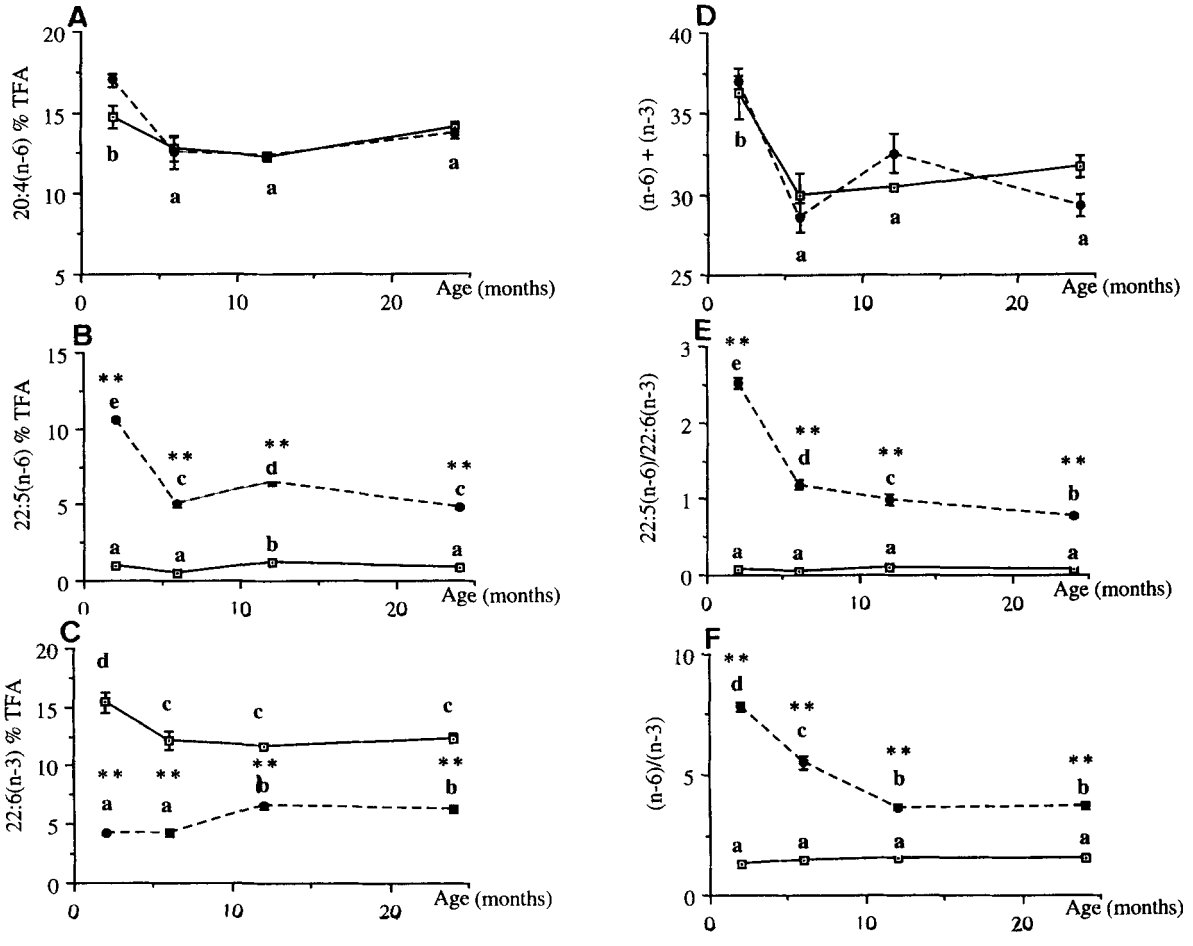


Fig. 2. Changes in levels of 20:4(n-6), A, 22:5(n-6), B, 22:6(n-3), C, (n-6) + (n-3), D, 22:6(n-3)/22:5(n-3), E, and (n-6)/(n-3), F, in hippocampus of control (——) and α-linolenic acid-deficient rats (---) during aging. Values are means ± SEM (mg/100 mg of total fatty acids, TFA); n = 6 for each point. Results were analyzed using a two-way ANOVA with both diet and age as factors, and a post-hoc Newman-Keuls test. Different letters were assigned when means differed significantly with $P < 0.05$. For comparison at a same age, the same test was used (*, $P < 0.05$; **, $P < 0.01$).

that described perturbations of behavioral and memory processes dependent on the hippocampal function are not related to the whole membrane PL composition. In addition, our data showed that the repartition of PL classes was little influenced by the diet, but, in contrast, was modified during aging. Between 6 and 12 months, the PE level decreased and PI and PS levels greatly increased in compensation. It has been well established that PI and PS are involved in the signal transduction pathways, involving phosphoinositide metabolism for the first and protein kinase C activity for the second (35). Borghese, Gomez, and Ramirez (36) recently showed that hippocampal slices perfused with PS liposomes exhibited increased synaptic transmission efficiency. Data have suggested that the beneficial effects of PS were linked to a stimulation role on brain catecholaminergic secretory tonus (37) and acetylcholine output from the cerebral cortex (38, 39). Moreover, Nunzi et al. (40) have shown that administration of PS to the brain reversed age-induced dendritic spine loss in the rat hippocampus. On this basis, we could speculate that the old brain tries to regulate the programmed monoaminergic, cholinergic, and cognitive decline occurring during aging by increasing the PS level, thus stimulating their turnover. However, our results showed that this rise did not continue after 12 months of age.

In α-linolenic acid-deficient rats, we observed that the levels for the n-3 PUFA family, especially 22:6(n-3), were dramatically decreased in the hippocampus, compensated by the increase in those of the n-6 family, especially 22:5(n-6) as already described for the whole brain (18, 32). However, we observed that the difference in 22:6(n-3) content between control and deficient groups tended to lessen from 2 to 12 months of age, due to a concomitant rise in DHA levels in deficient rats and a decrease in control rats. At the same time, a

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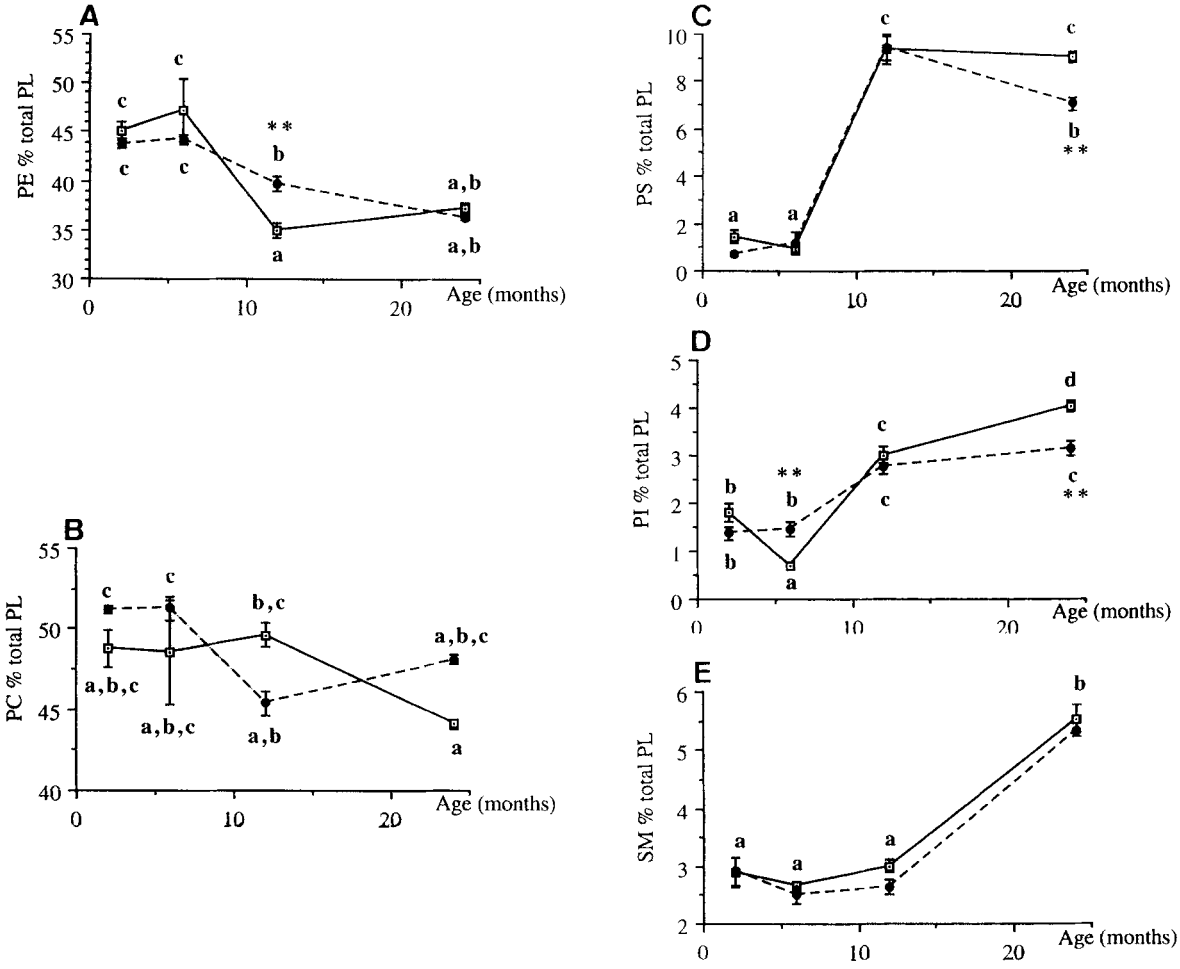


Fig. 3. Changes in phospholipid class levels in hippocampus of control (——) and α-linolenic acid-deficient rats (---) during aging: phosphatidylethanolamine, A, phosphatidylcholine, B, phosphatidylserine, C, phosphatidylinositol, D and sphingomyelin, E. Values are means ± SEM (mg/100 mg of total phospholipids); n = 6 for each point. Results were analyzed using a two-way ANOVA with both diet and age as factors, and a post-hoc Newman-Keuls test. Different letters were assigned when means differed significantly with $P < 0.05$. For comparison at a same age, the same test was used (*, $P < 0.05$; **, $P < 0.01$).

reduction in 22:5(n-6) levels occurred in the deficient rats. However, no recuperation of DHA levels occurred after 12 months of age. We have already observed this phenomenon in the striatum, the frontal cortex, and the cerebellum (31). The origin of this recuperation remains to be elucidated. However, it should be noted that there is not a complete balance between the age-related increase in DHA levels and the concomitant decrease in 22:5(n-6) levels in the deficient group at the oldest ages. This suggests that this partial recuperation of DHA with age could be due to the capacity of the central nervous system to take up and store n-3 PUFA present in very small proportions in the diet, and thus tends to compensate for the deficiency. Moreover, almost all 22:6(n-3) is recycled from PL breakdown, and this recycling assures a well-regulated level in brain membranes (41). Nevertheless, this does not explain why the capacity for DHA recuperation ceased after 12 months of age. It could be due to a lower age-related permeability of the blood-brain barrier for the PUFA or a loss of the ability of astrocytes to supply DHA to the neurons at this age, according to the process previously described by Moore, Yoder, and Spector (42) and Moore et al. (43).

The measured endogenous monoamine levels (NA and 5-HT) were not modified by the diet but they showed some specific age-related fluctuations. Our results showed a decrease in 5-HT level between 2 and 6 months of age and then a regular increase up to 24 months, although there was a reduction in the level of NA after 12 months of age. By contrast, Gozlan et al. (44) and Luine, Bowling, and Hearns (10) have shown an age-related decrease in 5-HT levels in the hippocampus but their experiments only concerned rats aged 3

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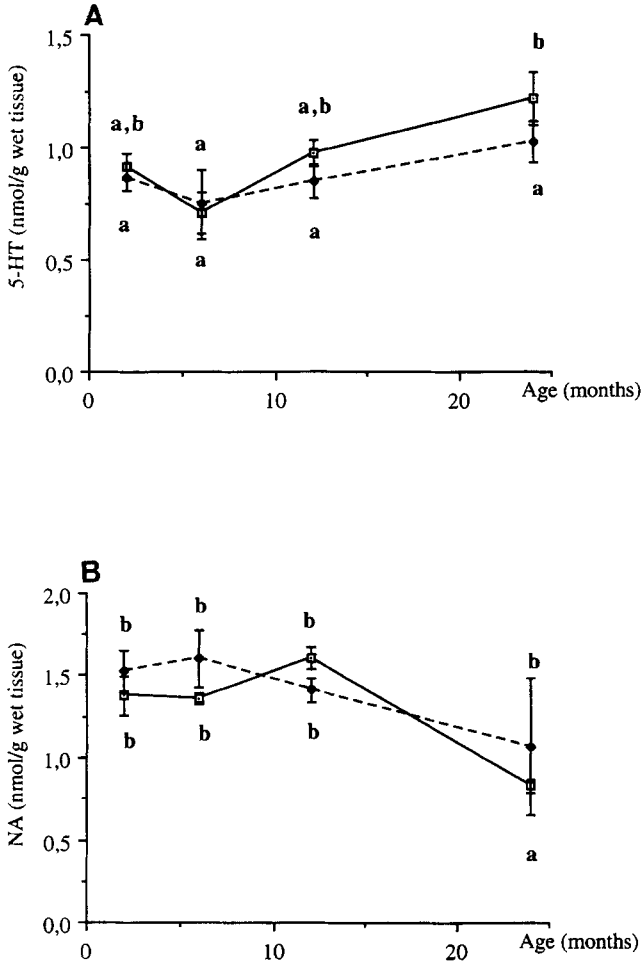


Fig. 4. Changes in serotonin, A, and noradrenaline, B, levels in hippocampus of control (——) and α-linolenic acid-deficient rats (---) during aging. Values are means ± SEM (nmol/g of wet tissue); n = 6 for each point. Results were analyzed using a two-way ANOVA with both diet and age as factors, and a post-hoc Newman-Keuls test. Different letters were assigned when means differed significantly with $P < 0.05$. For comparison at a same age, the same test was used (*, $P < 0.05$; **, $P < 0.01$).

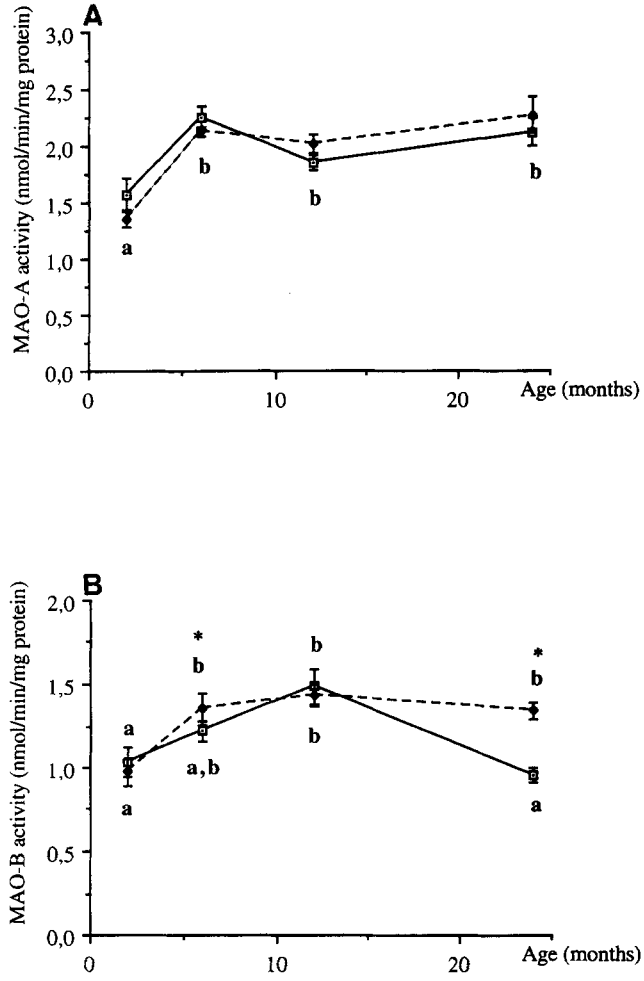


Fig. 5. Monoamine oxidase A and B activities in hippocampus of control (——) and α-linolenic acid-deficient rats (---) during aging. Values are means ± SEM (nmol/min/mg of proteins); n = 6 for each point. Results were analyzed using a two-way ANOVA with both diet and age as factors, and a post-hoc Newman-Keuls test. Different letters were assigned when means differed significantly with $P < 0.05$. For comparison at a same age, the same test was used (*, $P < 0.05$; **, $P < 0.01$).

and 26 months. Others (11, 45) observed no age-related variation in NA and 5-HT levels. It seems, therefore, that the evolution of monoamine levels during aging are debated in rats; environmental conditions or strain of animals might influence the results as already proposed (11). Nevertheless, our study showed that many age-induced fluctuations occurred between 2 and 12 months of age and the results obtained depended on the age considered. Therefore, the age selected as "young" is critical in this period. According to the choice, it could lead to erroneous conclusions because any intermediate fluctuations could be missed. However, the results reported here are in agreement with a previous study (46) that showed that the hippocampal 5-HT concentration increased during aging. We have demonstrated that this increase occurs only after 6 months of age.

The age-related reduction in monoamine content reported in the literature has moreover been described as accompanied by an increase in catabolic enzyme activity which intensifies the process of decline (47, 48). In this study, we observed specific evolutions in MAO activities according to age. The results showed that the age of 6 months seems to be a critical stage for this neurochemical parameter in the rat lifespan. However, we did not observe the age-induced rise in MAO activities previously described in the literature but a stabilization after 6 months of age for MAO-A. The diet deficiency induced a higher MAO-B activity compared to the control group at the age of 24 months. Orologas, Bukman, and

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Eiduson (49) showed that PS is a highly specific inhibitor of platelet MAO-B activity. If an identical PS-MAO interaction exists in brain cells, removal of the inhibition exerted by PS could explain the maintenance of the high level of MAO-B activity in deficient rats compared to control rats observed at 24 months of age.

The concomitant examination of both endogenous monoamine levels and MAO assays showed that NA levels and MAO-B activity varied in a comparable manner during the period studied. This phenomenon was observed but the reason for its occurrence remains to be elucidated. Otherwise, 5-HT levels and MAO-A activity varied in a counterbalanced way, which might be related to the fact that 5-HT is a major substrate for MAO-A activity.

In conclusion, this work shows that during aging the hippocampus sustains changes in both neurochemical and structural markers such as the distribution of PL classes and FA composition of total PL. There is thus a reduction in the major PL classes and an increase in the minor classes. Moreover, the age-related evolution of PS and PI levels, as well as other factors studied here, appears to occur in three phases, i.e., a "young period" from 2 to 6 months, an "old period" between 12 and 24 months, and a "transition period" between 6 and 12 months of age. Moreover, the α-linolenic acid deficiency induced a decrease in PS level, concomitant with an increase in MAO-B activity as compared to control group at the age of 24 months. The age-related evolution of MAO-B activity occurred in parallel with that of NA level in both dietary groups. However, our results showed that the diet deficient in α-linolenic acid has little effect on the monoaminergic neurotransmission parameters studied in the hippocampus, in contrast to the frontal cortex (30, 31). This could correspond to a different sensitivity of the cognitive processes mediated respectively by these two structures. Moreover, many other neurotransmission factors such as cholinergic or GABAergic systems are highly involved in the hippocampal function and remain to be studied.

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