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EFFECTS OF DIETARY ALPHA-LINOLENIC ACID DEFICIENCY ON NEUROMUSCULAR AND COGNITIVE FUNCTIONS IN MICE

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Summary

Mice were fed a diet deficient in α-linolenic acid [18 : 3 (n-3)] or a control diet and the effect of this deficiency was assessed by behavioral and pharmacological measurements. Three weeks before mating female mice were fed a diet containing either peanut oil poor in alpha-linolenic acid $(n\text{-}3)^-$ or peanut + rapeseed oil rich in alpha-linolenic acid $(n\text{-}3)^+$ = controls. Pups, aged 47 to 61 days, fed the same diet as their dams, were used for behavioral experiments. Muscular function and neuromuscular coordination assessed by the traction test, the elevated rotarod test and swimming endurance were unchanged by the $(n\text{-}3)^-$ deficiency. The level of anxiety assesed by the elevated plus-maze (anxiety protocol), the light-dark transition and the neophobia tests did not differ between $(n\text{-}3)^-$ and control $(n\text{-}3)^+$ mice. Defensive behavior was not changed by the diet. The pentobarbital-induced loss of the righting reflex had the same duration in males, females, and controls as in (n-3) deficient mice; the latency to pentobarbital-induced loss of the righting reflex was significantly shorter in females than in males but did not differ according to the diet. Mice fed the $(n\text{-}3)^-$ deficient diet showed less efficient learning in the elevated plus-maze (learning protocol) and poorer understanding of the situation (or less motivation to escape) in the low rotarod test than mice fed the control $(n\text{-}3)^+$ diet.

Key Words: α-linolenic acid, diet, muscular function, anxiety, cognitive function

Polyunsatured fatty acids, linoleic acid [18:2(n-6)] and alpha-linolenic acid [18:3(n-3)] cannot be synthesized by animals. These precursors must be obtained from the diet; they are then converted to longer chain polyunsatured derivatives via a series of steps involving desaturation and subsequent chain elongation. The main polyunsatured fatty acids synthesized from linoleic and alpha-linolenic acid are arachidonic acid [20:4(n-6)] and the docosahexaenoic acid [22:6(n-3)]. These control the composition of membranes and hence their fluidity, and, as a result, their enzymatic activity, the binding between molecules and their receptors, cellular interactions, and the transport of nutrients. Rats fed a diet restricted in n-3 fatty acids during development show a significant decrease in the levels of [22:6(n-3)] in phospholipids of synaptic membranes, myelin, retina, brain, sciatic nerve, liver, heart, kidney, and muscle compared to rats fed a diet with normal n-3 fatty acid levels (1, 2). Thus it can be expected that the biochemical modifications induced by

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dietary restriction in n-3 fatty acids would induce physiological and behavioral modifications and, in fact, behavioral alterations, learning impairment and altered responses to drugs have been described (1, 4).

The aim of the present study was to examine the effect of dietary restriction in alpha-linolenic acid on the anxiety level and cognitive function of mice, on their defensive behavior, emotionality, and also on their muscular function and motor coordination since, in mice, the phospholipid content (mg/kg) of striated muscles is about 3 times higher than in humans, rats, guinea-pigs, pigeons, frogs, and cattle (5). In addition, the effect of pentobarbital was also investigated.

Methods

Animals and diet : Female OF1 mice originating from IFFA-CREDO (L'Arbresle, France) and bred in our laboratory were divided into two groups three weeks before mating. The two groups were fed purified diets that were similar except for lipids (Table 1). The total amount of lipids was 6% in each diet. In the group fed a diet deficient in α-linolenic acid [$(n\text{-}3)^-$], the lipids were peanut oil containing 1200 mg of linoleic acid [18:2(n-6)] / 100 g diet ; and traces of α-linolenic acid/100g diet. In the group fed a control diet [$(n\text{-}3)^+$], the lipids were a mixture of peanut oil and rapeseed oil containing 1200 mg of linoleic acid / 100g diet, and 200 mg of α-linolenic acid / 100 g diet. Diets were given *ad libitum*.The quality of the oils used was carefully examined with regard to fatty acids and antioxidants. The toxicological analysis (performed by the Institut National de la Recherche Agronomique - Dijon - France) showed the lack of any contaminants and a non-detectable level of oxidized fatty acids or of "trans" structures. The diets were prepared in the Institut National de la Recherche Agronomique (INRA-CNRS), 78350 Jouy-en-Josas, France.

Pups from 16 dams in the $(n\text{-}3)^-$ group and from 12 dams in the $(n\text{-}3)^+$ group were fed the same diet as the dams and were used for behavioral tests from day 45. Breeding and experimental room were thermostatically maintained at 21 ± 1° C. At weaning all pups were housed in four big cages : $(n\text{-}3)^+$ males, $(n\text{-}3)^+$ females, $(n\text{-}3)^-$ males, $(n\text{-}3)^-$ females. Then in each group 10 pups, taken at random, were placed in the same home cage. Experiments were performed between 10 am and 4 pm by an observer blind to the diets.

Allotment of animals : Mice were allotted to different groups, each mouse being used in several tests. Three groups of male mice from each diet group were established. The first one was used in the low rotarod test, the traction test, the elevated plus-maze (learning protocol) and, lastly, in the forced swimming test. The second group was used in the elevated rotarod, the elevated plus-maze (anxiety protocol), the pentobarbital-induced loss of the righting reflex and, finally, in the defensive behavior test. The third group was used in the light-dark transition and neophobia tests. Two groups of female mice from each diet group were established. The first group was used in the elevated plus-maze (learning protocol), then in the light-dark transition test and the pentobarbital-induced loss of the righting reflex. The second group was used for measuring anxiety in the elevated plus-maze and neophobia tests.

Lipid analysis: Four mice of each diet group were anesthetized with diethyl ether and killed by decapitation. Forebrains were quickly removed, lyophilized and stored at -70°C until the lipid analyses were performed. Heptadecanoic acid was added as internal standard and the forebrain total fatty acids were transesterified according to the method of Lepage and Roy (6). The methyl esters were analyzed on a Delsi gas chromatograph equipped with a flame ionization detector and a silica capillary column (length 25m, internal diameter 0.22 mm, stationary phase BPX 70,SGE France). Helium was used as the carrier gas. The oven temperature was programmed to maintain 165°C for 12 min, then to increase by 1.5°C/min up to 215°C; the injector and detector temperatures were maintained respectively at 230°C, and 250°C. Peaks were identified by comparison with authentic commercial standards and with mixtures of known fatty acid composition. Areas were calculated with a Merck-Hitachi 2 500 integrator, and fatty acid concentrations are presented as a percentage of total fatty acid content.

TABLE I

Diet Composition

	Diet	
	control	(n-3) deficient
	g/kg diet	
Casein	220	220
DL-Methionine	1.6	1.6
Cornstarch	432.4	432.4
Saccharose	216	216
Cellulose	20	20
Mineral mixture [1]	40	40
Vitamin mixture[2]	10	10
Peanut oil[3]	23.6	60
Rapeseed oil[3]	36.4	--

[1] Composition of mineral mixture (g/kg diet): $CaHPO_4.2H_2O$, 15.2 ; K_2HPO_4, 9.6; $CaCO_3$, 7.2; NaCl, 2.8; MgO, 0.8; $MgSO_4.7H_2O$, 3.6; $FeSO_4.7H_2O$, 0.34; $ZnSO_4.H_2O$, 0.2; $MnSO_4.H_2O$, 0.2; $CuSO_4.H_2O$, 0.04; NaF, 0.03; $CrK(SO_4)_2.H_2O$, 0.02; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.8 x 10^{-3}; KI, 1.6 x 10^{-3}; $CoCO_3$, 0.8 x 10^{-3}; $Na_2SeO_3.5H_2O$, 0.8 x 10^{-3}.

[2] Composition of vitamin supplement, triturated in dextrose (mg/kg diet): retinol acetate, 10; cholecalciferol, 0.0625; acetate all-*rac*-α-tocopherol, 50; menadione, 1; thiamine HCl, 10; riboflavine, 10; nicotinic acid, 45; D-calcium pantothenate, 30; pyridoxine HCl, 10; inositol, 50; D-biotin, 0.2; folic acid, 2; cyanocobalamin, 0.0135; L-ascorbic acid, 100; *p*-aminobenzoic acid, 50; choline chlorhydrate, 750.

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

Traction test : The apparatus consisted of a horizontal fine metallic rod (1.5 mm in diameter) set 24 cm above the floor. The mouse was held in such a way that the two forepaws were very near the rod, then it was dropped. Normal mice grasp the rod.

Swimming endurance: Swimming to the point of exhaustion was evaluated according to the method of Jacob and Michaud (7). A tank (55x45x34cm) contained 18 cm of water at 20 ± 0.5°C. Artificial waves were produced by injected compressed air. Four mice were tested together in the same bath. A mouse was considered exhausted when its head plunged under the water for 7 seconds. The mouse was then removed.

Low rotarod test: A rotarod for mice (Apelex 92220 Bagneux, France) was used. The rod had a diameter of 30 mm and was 15 cm above the floor. The motor-driven rod revolved once in 4.25 seconds. Each mouse received 5 minutes of adaptation : after every fall, it was replaced on the rotating rod. After these 5 min of adaptation the ability of the mice to remain on the rod was evaluated; the cut-off time was 30 min.

Elevated rotarod test :Another rotarod for mice was used. The diameter of the rod was 2.5 cm, the rotation speed was one revolution in 5 seconds and the height from the floor was 80 cm. Each mouse received 5 minutes of adaptation : after every fall (on to soft rags) it was replaced on the rotating rod. After these 5 min of adaptation the ability of mice to remain on the rod was evaluated; the cut-off time was 30 min.

Elevated plus-maze test : A grey elevated plus-maze apparatus was used. Two open arms (25x5cm) and two (25x5cm) closed (15cm high) arms were attached at right angles to a central platform (5x5cm). The open arms and the central platform were covered with white plastic-coated paper. The apparatus was 40 cm above the floor. In one test to evaluate memory, a mouse was placed at the end of an open arm with its back to the central platform. The time for the mouse to cross a line half way along one of the closed arms was measured (transfer latency) on day 1 and day 2. The mouse had to have its body and four paws on the other side of the line.

In the other test to evaluate anxiety, a mouse placed on the central platform with its head towards an open arm. The frequency of entries onto the open and closed arms was noted and time spent on the open arms was measured over 5 minutes. In this test, entry onto either arm was counted when the mouse had its body and four paws on the arm.

Light-dark transitions : A box with two compartments was used. One of them (30x15x28cm) had a black floor and walls, the other (30x30x28cm) a white floor and walls. An opening (8x8cm) in the middle of the dividing wall allowed access from one compartment to the other. During the experiments a lid covered the black compartment, the other was brightly lit by a reading lamp. A mouse was placed in the middle of the white compartment. The time spent in the black and white compartments was noted as well as the number of crossings from one compartment to the other during the 5 minute test.

Neophobia : Male and female mice deprived of food for sixteen hours were placed in individual cages. After being weighed, a cup containing nearly 20 g of an unusual food (standard lab chow) was introduced in each cage. Three hours later, the cups were withdrawn and weighed. The amount of food consumed was obtained by subtraction.

Defensive behavior : The mice used in this test were fasted 18 h before the test . Two male mice from the same diet group but taken from two different home cages were confined under a reversed beaker (1 liter) placed on a grid floor through which they received electric shocks to the hind paws. The mice received five one millisecond 6 mA shocks per second for 3 minutes. Animals exhibited upright posture, boxing and squealing. A fight was considered finished when the two mice were no longer face to face (8).

Sensitivity to pentobarbital : Pentobarbital (40 mg/kg) was administered i.p. to mice placed in individual cages on soft paper. The time between pentobarbital administration and the loss of the righting reflex (latency to loss of the righting reflex) was measured as well as the time between the loss of the righting reflex and recovery of the righting reflex (duration of the loss of the righting reflex).

Drugs: Sodium pentobarbital as injectable solution (6g of sodium pentobarbital qsp 100 ml) was obtained from SANOFI (La Ballastière - 33501 Libourne - France).

Statistical analysis: For pentobarbital experiments, two-way ANOVA was used with sex and diet as between-group factors. For the elevated plus-maze experiment (learning protocol) a repeated measures analysis was performed with diet and sex as between-group factors and day as within-group factor. For the low rotarod test a t test with separated variances was used. Student's t test was used for analysing data for behavior in the elevated rotarod, swimming endurance, light-dark transition, and neophobia test, and the elevated plus-maze (anxiety protocol test).

Results

Lipid analysis: Lipid analysis (Table 2) had been performed in a previous series of experiments in the same strain of mice fed the same diets according to the same protocol. Results are given again for convenience. The major alteration in forebrain total fatty acid composition was a marked reduction in the content of 22:6(n-3) compensated by an increase in the (n-6) fatty acids : 22:5(n-6) and to a lesser extent 22:4(n-6) and 20:4(n-6).

Traction test:The thirty male mice used in the low rotarod test were used in the traction test. All mice in both diet groups were able to grasp the rod (Table 3).

Swimming endurance (Table 3) The $(n\text{-}3)^-$ deficient mice and the control $(n\text{-}3)^+$ mice did not differ significantlly for swimming endurance.

Rotarod tests : with the rod 15 cm above the floor:Thirty naïve male mice from the $(n\text{-}3)^+$ diet and 30 naïve male mice from the $(n\text{-}3)^-$ diet were used. Results (Table 3) show that the $(n\text{-}3)^+$diet mice did not remain on the rod as long as the $(n\text{-}3)^-$ diet mice. The difference was highly significant.

With the rod 80 cm above the floor:Twenty naïve male mice from each of the two diet groups were used. Results (Table 3) show that there was no significant difference between the time that the mice remain on the rotating rod.The data of these two experiments have been analysed separately since the two experiments were performed using two different apparatus and the second experiment involved a new parameter: fear of falling from a height.

Elevated-plus maze (anxiety protocol) : When a mouse was placed on the central platform, its head towards an open arm, the total number of entries onto both arms did not differ between the two diet groups either in males or in females (Fig.1). The percentage of entries onto open arms tended to be higher in the $(n\text{-}3)^-$ diet groups for both males and females, but the difference was not significant. The percentage of time spent on open arms was higher in the $(n\text{-}3)^-$ diet groups ; this effect was marginally significant for males ($p = 0.09$) but not significant in females.

TABLE II
Forebrain Fatty Acid Profile

Fatty acid	(n-3)$^+$ diet	(n-3)$^-$ diet
14:0	0.10 ± 0.01	0.11 ± 0.02
16:0	20.41 ± 0.42	20.70 ± 0.33
18:0	23.54 ± 0.42	23.21 ± 0.18
22:0	0.38 ± 0.02	0.40 ± 0.03
24:0	0.57 ± 0.16	0.54 ± 0.04
Σ Saturated	45.68 ± 0.36	45.74 ± 0.36
16:1n-9	0.25 ± 0.03	0.26 ± 0.02
16:1n-7	0.46 ± 0.06	0.44 ± 0.04
18:1n-9	13.57 ± 0.26	13.31 ± 0.29
18:1n-7	3.42 ± 0.16	3.64 ± 0.09
20:1n-9	1.11 ± 0.13	1.10 ± 0.06
20:1n-7	0.31 ± 0.04	0.34 ± 0.02
22:1n-9	0.16 ± 0.02	0.15 ± 0.01
22:1n-7	0.10 ± 0.01	0.11 ± 0.01
Σ MUFA	19.38 ± 0.42	19.35 ± 0.43
18:2n-6	0.34 ± 0.01	0.30 ± 0.01 **
20:3n-6	0.43 ± 0.09	0.29 ± 0.04 *
20:4n-6	11.11 ± 0.22	11.54 ± 0.23 *
22.4n-6	3.02 ± 0.18	3.89 ± 0.14 ***
22:5n-6	0.87 ± 0.05	11.25 ± 0.83 ***
Σ n-6	15.78 ± 0.19	27.27 ± 0.78 ***
22:5n-3	1.62 ± 0.19	1.63 ± 0.10
22:6n-3	17.54 ± 0.26	6.01 ± 0.17 ***
Σ n-3	19.16 ± 0.20	7.64 ± 0.18 ***
n-6+ n-3	34.94 ± 0.33	34.91 ± 0.69
n-6/n-3	0.82 ± 0.01	3.57 ± 0.16 ***

Effect of diet deficient in α-linolenic acid on the profile of fatty acids in the forebrain of 60-day-old mice. Means (± S.D.) of 4 determinations (4 mice) are given for each diet. Results are expressed as percentages of total fatty acids.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Memory protocol (Table IV): The results show that a memory of the situation (transfer latency TL2 less than TL1) occurred in both diet groups and in both sex. However the difference was significant only in the control (n-3)$^+$ diet group. In addition, the TL1 score was significantly smaller in the α-linolenate deficient group.

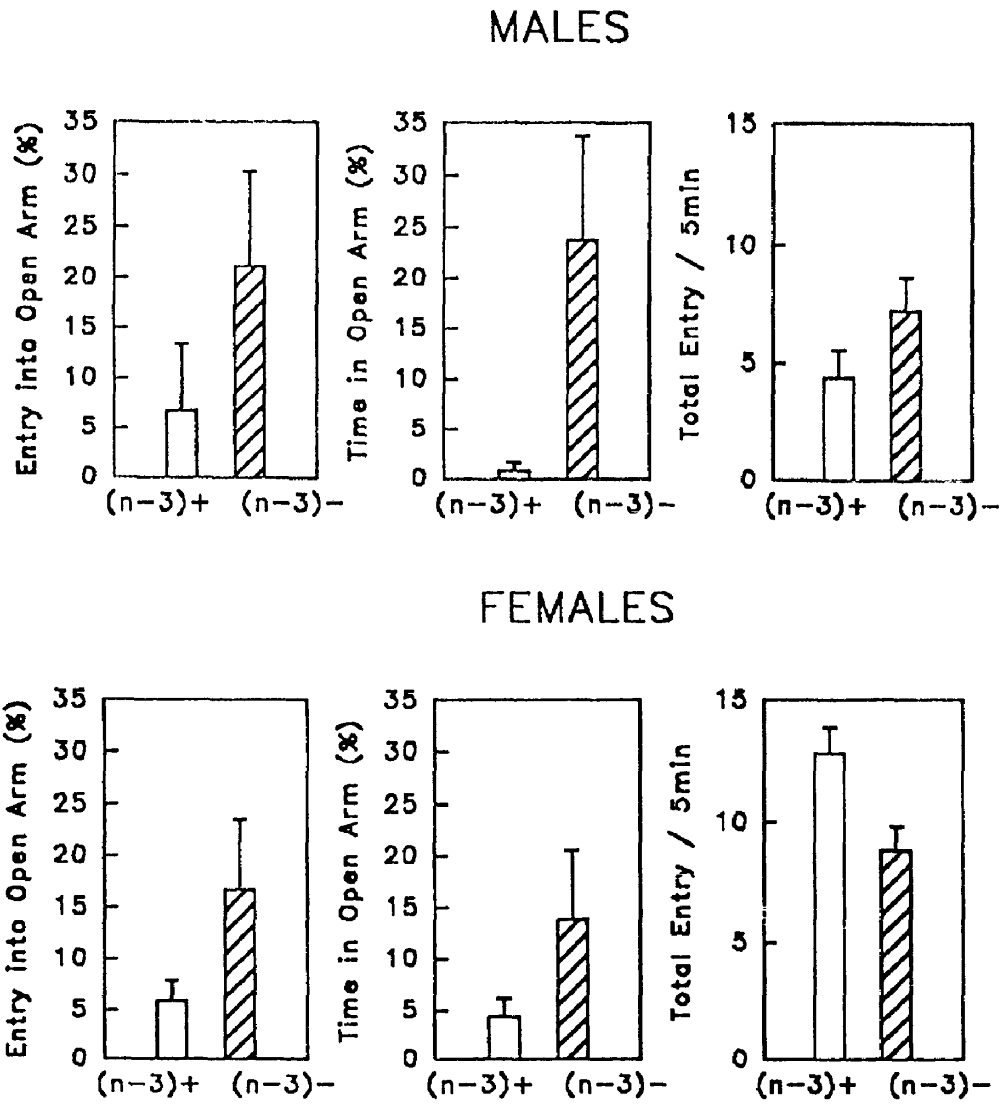

Fig. 1

Assessment of anxiety in the elevated plus maze; males, n = 15 mice in each diet ; females, n= 20 mice in each diet. The percentage of entries (m ± S.E.M) into the open arms was calculated with 100% = number of entries into open + closed arms during the 5 minutes of test. The percentage of time (seconds, m ± S.E.M.) spent in open arms was calculated with 100 % = total time spent in either an open or a closed arm (which is smaller than the 5 minutes of test because the mice spent some time on the central platform).

TABLE III

Assessment of Neuromuscular Function and Motor Coordination

	$(n\text{-}3)^+$	$(n\text{-}3)^-$	p
Time on the low rotarod sec (mean±S.E.M)	74.6 ± 21.4	669.2±134.3	<0.0002
Time on the high rotarod sec (mean±S.E.M)	1187.5±169.8	1294.3±162.6	N.S.
Traction test % of mice with successful grasping	100 %	100 %	
Swimming endurance sec (mean±S.E.M)	859±114 (n=20)	646±88 (n=18)	N.S.

n=number of mice. Student's t test. For the low rotarod test a t test with separated variances was used.

TABLE IV

Elevated Plus Maze : Learning Protocol

	males		females	
	$(n\text{-}3)^+$	$(n\text{-}3)^-$	$(n\text{-}3)^+$	$(n\text{-}3)^-$
TL1	46.8±2.1	34.1±2.6	43.7±2.9	32.5±3.4
	*	NS	**	NS
TL2	34.9±6.3	24.5±4.4	22.6±4.6	24.3±4.9
n	10	13	14	10

Transfer latency (sec, mean±S.E.M.) on day 1 (TL1) and on day 2 (TL2).Transfer latency was the interval before a mouse crossed the middle of one of the closed arms. n= number of mice. The repeated measure analysis indicated an effect of day ($F_{1,43}$= 16.62, p =0.00018), no effect of sex, and an effect of diet ($F_{1,43}$=9.35, p =0.004). The Bonferroni test was used for post-hoc comparisons: * p<0.05, ** p<0.01.

Light-dark transitions (Table V): The time spent in the black compartment was higher in both diet groups and in both sexes ; which means that the situation was anxiogenic for all mice. The number of crossings from one to the other compartment did not differ significantly between the diet groups in either sex. When the results from males and females were pooled, the percentage of time spent in the white compartment was 34% in the $(n\text{-}3)^-$ group and 32.5 % in the $(n\text{-}3)^+$ group.

Neophobia (Table VI):The amount of food consumed did not differ between the two diet groups in either males or females.

TABLE V

Number of Crossings between and Time Spent in the Black and White Compartments.

	males		females	
	$(n-3)^+$	$(n-3)^-$	$(n-3)^+$	$(n-3)^-$
Crossings (mean± S.E.M.)	11.2 ± 2.2	14.8 ± 2.1 NS	9.5 ± 1.9	15.0 ± 2.8 NS
Time (sec) (mean± S.E.M.)				
black	185.5 ± 18.0	221.3 ± 8.4	219.0 ± 18.4	172.6 ± 22.9
white	114.5 ± 18.1 (a)	78.7 ± 8.4 (b)	81.0 ± 18.4 (b)	127.4 ± 22.9 NS

n = 13 in each of the four groups.

Student's t-test : (a) $p < 0.05$, (b) $p<0.001$. The significance is expressed vs. the time spent in the black compartment.

TABLE VI

Neophobia

		$(n-3)^+$	$(n-3)^-$	p
Amount of food consumed (g) mean ± S.E.M	males	0.63 ± 0.06 (n=13)	0.69 ± 0.07 (n=13)	N.S.
	females	0.64 ± 0.05 (n=13)	0.70 ± 0.05 (n=13)	N.S.

Mice deprived of food for 16 hours were placed in an unknown location with an unusual food. The amount of food was weighed before and after the 3 hour test. Student's t-test

TABLE VII

Pentobarbital-induced Loss of the Righting Reflex

	$(n-3)^+$	$(n-3)^-$
Latency min (mean±S.E.M)		
males	11.05±3.04 (n=18)	12.7±4.7 (n=18)
females	8.0±0.5 (n=20)	6.6±0.7 (n=17)
Duration min(mean±S.E.M)		
males	44.6±2.4 (n=18)	58.7±3.1 (n=18)
females	46.8±4.8 (n=20)	46.8±4.4 (n=17)

For the loss of the righting reflex an effect of sex was observed: ($F_{1,69}$ =34.56, $p<0.00001$), but there was no effect of diet. For the duration of the loss of the righting reflex there were neither an effect of sex nor of diet nor a diet x sex interaction.

Defensive behaviour: During the 3 minutes of the test, the number of fights did not differ between the two diet groups : $(n-3)^+$: 4.92 ± 1.24 (n=13) ; $(n-3)^-$: 4.69 ± 1.61 (n=13).

Sensitivity to pentobarbital: Intraperitoneal injection of 40 mg/kg of pentobarbital induced a loss of the righting reflex. The latency to this loss was shorter in females than in males.

(Table 7).There was no effect of the diet. The duration of the loss of the righting reflex did not differ significantly according to the sex or the diet.

Discussion

The effects of the diets on the fatty acid composition of the brain are in agreement with biochemical measures obtained in rats fed a (n-3)$^-$ deficient diet in this (1) and an other laboratory (3) ; they suggest that the behavioral modifications may be linked to biochemical alterations in the forebrain.

For suspension from a horizontal rod and swimming endurance, there were no significant differences between the control (n-3)$^+$ and deficient (n-3)$^-$ diets. These tests are used to assess neuromuscular function (7,9,10). It may thus be concluded that the diet deficient in alpha-linolenic acid used in this protocol does not alter the neuromuscular function. The rotarod testing techniques were introduced in the 1950s (11) and are still widely used for assessing motor coordination in pharmacological and toxicological experiments (12,13). Using the low rotarod apparatus a highly significant difference was observed between the two diet groups: the time on the rotating rod was considerably greater in the diet group deficient in α-linolenic acid. The simple interpretation of these data leads to a surprising conclusion : the motor coordination of the deficient mice is better than that of the control mice ! However, several authors using the rotarod testing techniques have noted a number of problems (14) ; the main problem is encountered with long-term studies involving repeated testing : control animals become increasingly adept at escaping from the apparatus. To avoid these problems some authors used shock or height to motivate stable performance and avoid escape responses. In our procedure, the mice had 5 minutes habituation before measurement of the time on the rotating rod. During this period all mice that fell were replaced on the rod, so that they could learn that the fall was not very hard and this may have motivated an escape response. To test this hypothesis, the elevated rotating rod was used and, indeed, the deficient mice no longer differed from the control mice. Therefore, the two dietary groups did not differ regarding motor coordination. In addition, both control and deficient mice remained on the elevated rod longer than on the low rod ; this means that mice fed either diet are more motivated to remain on the elevated than on the low rod ; that is to say : they are conscious that the height is great. The reason for the greater escape response of the control mice on the low rotating rod is not evident : deficient mice may not clearly see the height of the rod (since it is known that the (n-3)$^-$ diet alters retina function), however, this is unlikely since they stay a longer time on the elevated rod ; alternatively, the (n-3)$^-$ deficient mice may habituate (understand or learn the situation) slower than the control mice as has been previously suggested (15) ; another possibility is that (n-3)$^-$ mice may have less motivation to escape.

Pain-induced aggression is typically a defensive behavior (16). In this test, there was no significant difference between the control (n-3)$^+$ and deficient (n-3)$^-$ mice. Since this model is based on the sensitivity of mice to foot-shock induced pain, it may be suggested that the two diets do not differ regarding the sensitivity. Bearing in mind that ventral frontal lesions in rats increase fighting in response to mild shocks (17,18) and that medial frontal operated male rats engage in more fights (19), it may be tentatively suggested that the (n-3)$^-$ deficient diet did not alter dramatically the function of the ventral and medial frontal brain structures.

In the light-dark transition test, one of the chambers is white, large, and brightly lit and the other is black, smaller, and poorly lit : the light serves as an anxiogenic stimulus, and there is an apparent conflict between the desire to explore and the desire to avoid the brightly lit part of the apparatus (20). The above results show that the brightly lit compartment is anxiogenic for both diets and for both sexes since the time spent in it is shorter than the time spent in the smaller black compartment. However, the situation appears to be equally anxiogenic for both diet groups.

An animal presented with food or drink to which it is not accustomed consumes only a small part of it ; the word "neophobia" has been introduced by Barnett (21) to characterize this behavior in rodents. A marked reduction in food consumption may be obtained in mice (22) when the food and the cage are both new to the animal. This behavioral inhibition has been linked to the stress induced by the novelty of the situation.The two diet groups did not differ significantly regarding the amount of new food consumed in the unfamiliar cages ; this was true for both males and females. So the nutritional deficiency in α-linolenic acid does not appear to modify the neophobia, that is to say the situation is no more stressing for the deficient than for the control mice.

The elevated plus-maze is a test developed from the work of Montgomery (23). It has been extensively studied by Pellow and coworkers (24) in rats and validated in mice by Lister (25). The test is based on the natural aversion of rodents for open spaces and for the height of the maze. When using the elevated plus-maze to measure anxiety, two indices of anxiety are used : the proportion of entries that are made onto the open arms of the maze, and the time spent on the open arms of the maze expressed as a percentage of the total time spent on both open and closed arms. This test has been found to be sensitive to the effects of both anxiolytic and anxiogenic agents, anxiolytic agents increasing and anxiogenic agents decreasing the two measures.

The percentage of entries onto the open arms and the percentage of time spent on the open arms were higher in the $(n-3)^-$ group than in the control $(n-3)^+$ group for both males and females. These results are in accordance with those obtained in female mice by Nakashima et al. (4), however, as reported by these authors, the difference was not significant. The total number of entries onto both open and closed arms did not differ significantly between the two diet groups for either males or females. This result differs from that of Nakashima et al. (4) in females ; however the lack of significance is not surprising since the total number of entries onto both open and closed arms reflects global motor activity (25) and motor activity is not affected by a diet deficient in α-linolenic acid (1,26).

The elevated plus-maze may be used for other purposes than measuring anxiety : Itoh (27) first described the use of this model to measure learning and memory in mice ; their results were validated by Sharma and Kulkarni (28). The latency to crossing the middle of the closed arm was shorter on day 1 for the $(n-3)^-$ diet group for both males and females. On day2, the latency was shorter than on day1 in every group, and the difference was significant in the control diet group for males and females, but not significant in the $(n-3)^-$ diet group. This means that learning was more efficient in the control diet group. Nakashima et al. did not observe such an effect perhaps because they used a different criterion. They defined latency as the time required for the body and four paws of the mouse to cross onto the closed arm, whereas we used the time required for the body and four paws of the mouse to cross the line drawn in the middle of the closed arm.

The latency to pentobarbital-induced loss of the righting reflex did not differ according to the diet but was shorter in females than in males whatever the diet. The duration of the loss of the righting reflex did not differ in females or in males fed either diet.These results differ from those of Nakashima et al. (4). These authors found a decrease in the latency to pentobarbital anesthesia in the $(n-3)^-$diet group and an increase in the duration of anesthesia. The present experiments also showed a trend to a longer duration of anesthesia in deficient male mice; however, this effect was not significant. It is suggested that the sensitivity to pentobarbital may be higher in the strain of mice used by Nakashima et al. than in ours since, for the same dose of 40 mg/kg, the latency to loss of the righting reflex was nearly 200 sec in their experiments and between 396 and 480 in female mice in our experiments.It cannot be excluded that the minor differences between Nakashima's results and ours are due to the presence of an unidentified component in the rapeseed oil we used. However, until the presence of such a component is proved, the more likely explanation rests on differences in the strains of mice or in the experimental protocols.

In general, these results show that mice fed a diet deficient in alpha-linolenic acid did not differ from control mice for level of anxiety as measured in the neophobia, light-dark transition or elevated plus-maze tests. Similarly, they did not differ from control mice for defensive behavior, muscle strength or motor coordination. Mice fed a (n-3) deficient diet showed less efficient learning in the elevated plus-maze test and a poorer understanding of the situation (or less motivation to escape) in the low rotarod test. These behavioral alterations may be linked to biochemical modifications induced by the diet. Indeed, a decrease in the dopamine level as well as a decrease in the density of dopamine D2 receptors and an increase in the density of serotonin 5-HT2 receptors have been measured in the frontal cortex of rats fed a diet chronically deficient in α - linolenic acid (29). Several studies have been conducted in rodents which tend to show an alteration in learning behavior in animals fed a diet deficient in n-3 fatty acids (1, 3, 30). In a review of these studies, P. Wainwright (31) stated that: " ...further studies are needed to separate effects on learning performance from those on related functions before effects on cognitive ability can be supported ". The present experiments constitute an additional argument in favour of selectively altered cognitive functions following dietary n-3 deficiency.

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