Diets containing long-chain *n*-3 polyunsaturated fatty acids affect behaviour differently during development than ageing in mice

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The effect of a standard diet providing essential fatty acids enriched in fish oil or palm oil was studied in young, mature and old mice. Two groups of pregnant and lactating OF1 mice were fed on diets with or without high levels of long-chain *n*-3 polyunsaturated fatty acids. Offspring were maintained on these diets after weaning. The litter size did not differ. The weight increased more quickly in fish-oil-fed mice than palm-oil-fed mice. The fish-oil diet induced a significant increase in exploratory activity in young mice which was not found in mature and old mice. The level of locomotor activity was significantly higher in young, no different in mature, and lower in old fish-oil-fed mice than in controls. Habituation, the simpler form of learning, occurred to the same extent in the two diet groups. For the place learning protocol of the Morris water maze there was no difference between the two diet groups; however, in the probe trial, the mature fish-oil-fed mice remembered the situation well compared with the control mice. In the active avoidance test, on the first day of acquisition the young fish-oil-fed mice made more avoidances than control mice, whereas in contrast, mature and old-fish-fed mice made less avoidances than control mice. These results suggest a positive effect on arousal and learning ability of a diet enriched in long chain *n*-3 polyunsaturated fatty acids in young mice and a detrimental effect in old mice.

***n*-3 Fatty acids**: **Learning**: **Ageing**

One of the reasons for studying the effect of *n*-3 polyunsaturated fatty acid (PUFA) deficiency is the need to determine whether and to what extent these fatty acids should be added to infant artificial formulas (Foreman-Van Drongelen *et al*. 1996; Lucas, 1997). These PUFA, mainly docosahexaenoic acid (22:6*n*-3), play an essential role in brain and retinal physiology. Numerous studies indicate an alteration in fatty acid composition of brain membranes, smaller amplitudes in the electroretinogram, and decreased cognitive performances in animals fed on a diet deficient in *n*-3 PUFA (Lamptey & Walker, 1976; Yamamoto *et al*. 1988; Bourre *et al*. 1989; Connor *et al*. 1992; Yehuda & Carosso, 1993). Our own studies showed a reduction in learning abilities on several tests in *n*-3 PUFA-deficient young mice (Francès *et al*. 1995, 1996). However, Wainwright *et al*. (1994), using mice of the third generation in a study including appropriate behavioural controls were unable to demonstrate effects of *n*-3 PUFA deficiency on diverse measures of learning and memory.

The effects of dietary *n*-3 PUFA deficiency on nervous system function are well documented, but those of a diet high in *n*-3 PUFA are not well known. Rats fed on a diet enriched in perilla- or soyabean oil (rich in α-linolenic acid) have shown higher performance in a brightness discrimination learning task (Yamamoto *et al*. 1987) and in the Morris water maze (Yonekubo *et al*. 1993) than in rats fed on a deficient diet containing safflower oil. However, in these two reports, a diet enriched in *n*-3 PUFA was compared with a diet impoverished in *n*-3 PUFA. Consequently, it is not obvious whether *n*-3 PUFA enrichment improves the learning performances: true control diets are required. Several authors have therefore performed experiments comparing the effects of diets enriched in *n*-3 PUFA with true control diets. Yamamoto *et al*. (1988) observed better learning performances in a brightness discrimination task in rats fed on a diet rich in α-linolenic acid than in rats fed on a control diet. Similarly, Yonekubo *et al*. (1994) using rats fed on a diet enriched in sardine oil (rich in long-chain *n*-3 PUFA) obtained higher learning performances on the Morris water maze than in rats receiving a control diet. Rats fed on a diet enriched in soyabean oil for only 3 weeks performed better than control rats in the Morris water maze (Coscina *et al*. 1986); however, the same authors could not reproduce this result (Coscina, 1997). In spite of some discrepancies, it seems that a diet enriched in *n*-3 PUFA may have beneficial effects.

Abbreviation: PUFA, polyunsaturated fatty acid.

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These data have been obtained with young animals. If *n*-3 PUFA can improve learning and/or memory performance, they would be of great interest for elderly people since cognitive deficiency is a major problem of ageing, especially since life expectancy is increasing. Moreover, an epidemiological study performed in elderly men showed high fish consumption tended to be inversely associated with cognitive impairment (Kalmijn *et al*. 1997). Thus, studies were performed to test the effect of a diet rich in *n*-3 PUFA in old rodents. Two reports show that old rats (Yamamoto *et al*. 1991) or senescence-accelerated mice (Umezawa *et al*. 1995) exhibit improved learning ability when fed on a perilla-oil-enriched diet compared with animals fed on a safflower-oil-enriched diet. Learning ability in mice fed on a fish-oil diet for 12 months was higher than in those fed on a palm-oil diet (Suzuki *et al*. 1998). In neither case was a control equilibrated diet used. We thus decided to compare a diet enriched in long-chain *n*-3 PUFA, obtained by fish oil supplementation, with a control equilibrated diet, on learning and/or memory abilities. These experiments were conducted in young, mature and old mice.

Materials and methods

Animals

Female (Swiss OF1) mice originating from IFFA-CREDO (L'Arbresle, France) and bred in our laboratory were divided into two groups 3 weeks before mating: palm-oil group (palm-oil diet) and fish-oil group (sardine-oil diet). The pups were fed on the same diet as their dams. At weaning, pups were separated according to sex and were housed in such a way that each home-cage contained six pups from six different dams. As a result, the behavioural experiment did not include two mice with the same dam in the same group.

Mice were housed in an air-conditioned animal room illuminated from 08.00 hours to 20.00 hours and maintained at $21 \pm 1°$. They were given free access to their respective diets and water. Experiments were performed between 09.00 hours and 17.00 hours in rooms maintained at $21 \pm 1°$.

A sample of twenty female mice in each diet group was weighed monthly. Since some deaths occurred, additional mice taken at random were included in order to keep twenty mice in each group.

The tests were performed in different groups of young adults (7–11 weeks), mature (9–10 months), and old (17–19 months) female mice. For young mice, separate groups were used. Each behavioural test was performed with a different group of mice. For mature and old mice, the same animals were used but on different tests. For example, the order was open-field (mature) and active avoidance (old) in order to avoid a memory effect. Each test was performed simultaneously in both diet groups and at the same age. The reason for the use of the female is their lack of aggression. A mouse was considered old from 17 months onwards because at this age the mortality is 50 % according to the survival curve. To minimize the influence of age-related physical incapacitation on performance, old mice were preselected: animals with visible defects such as tumours or major incapacitation were discarded.

Table 1. Diet composition (g/kg diet)

Ingredient	g/kg
Casein	220
Maize starch	379·1
Sucrose	189·3
Cellulose	20
DL-Methionine	1·6
Mineral mixture*	40
Vitamin mixture†	10
DL-α-Tocopherol acetate	0·1
Oil‡	140

* Composition of the mineral mixture (g/kg of diet): $CaHPO_4.2H_2O$ 15·2; K_2HPO_4 9·6; $CaCO_3$ 7·2; NaCl 2·76; MgO 0·8; $MgSO_4.7H_2O$ 3·6; $FeSO_4.7H_2O$ 0·344; $ZnSO_4.7H_2O$ 0·2; $MnSO_4.H_2O$ 0·2; $CuSO_4.5H_2O$ 0·04; NaF 0·032; KI 0·0016; $CoCO_3$ 0·0008; $Na_2SeO_3.5H_2O$ 0·0008; $(NH_4)_6Mo_7O_{24}.4H_2O$ 0·0008; $CrK(SO_4)_2.12H_2O$ 0·02.

† Composition of vitamin supplements triturated in dextrose (United States Biochemicals Corp., Cleveland, OH, USA) (mg/kg of diet): L-ascorbic acid 100, choline chlorhydrate 750; D-calcium pantothenate 30; inositol 50; menadione 1; nicotinic acid 45; *p*-aminobenzoic acid 50; pyridoxine-HCl 10; riboflavin 10; thiamine-HCl 10; retinyl acetate 10; cholecalciferol 0·0625; D-biotin 0·2; folic acid 2; cyanocobalamin 0·0135; DL-α-tocopherol acetate 50.

‡ See Table 2.

Diets

The diets were prepared by the Institut National de la Recherche Agronomique (Jouy en Josas, France). Two groups received the same basal purified diet (see Table 1) enriched in fish oil or palm oil. The two diets were of equal energy density and provided the essential fatty acids linoleic acid (18:2*n*-6) and α-linolenic acid (18:3*n*-3). The composition of dietary oils is shown in Table 2.

Table 2. Oil content and fatty acid composition of dietary lipids (g/kg diet)

	Palm-oil diet	Fish-oil diet
Oils		
Peanut	15·0	15·0
Rapeseed	25·0	25·0
Palm	100	–
Sardine	–	100
Total	140	140
Fatty acid		
14:0	1·2	9·6
16:0	47·1	24·6
18:0	5·4	5·4
20:0	0·7	0·8
22:0	–	0·3
Σ SFA	54·3	40·7
16:1 *n*-7	–	6·4
18:1 *n*-9	56·6	26·5
18:1 *n*-7	2·9	3·1
20:1 *n*-9	0·7	1
22:1 *n*-9	–	0·4
Σ MUFA	60·2	49·9
18:2 *n*-6	17·4	10·7
20:4 *n*-6	–	1·1
Σ *n*-6	17·4	11·8
18:3 *n*-3	2·0	2·7
18:4 *n*-3	–	1·7
20:5 *n*-3	–	15·9
22:5 *n*-3	–	1·3
22:6 *n*-3	–	9·4
Σ *n*-3	2·0	31·1
Total	133·5	133·9

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid.

The influence of temperature and light on the conservation of the diet enriched in *n*-3 PUFA was studied. Five samples were placed in one of each of the following places: in front of the home-cage (ambient temperature, light of the animal room, 3 d), in the home-cage (ambient temperature, reduced light, 3 d), in a drawer (ambient temperature, darkness, 3 months), in a refrigerator (+4°, darkness, 3 months), in a freezer (−18°, darkness, 3 months). The fatty acid composition of each sample was analysed by GC. The results showed a decrease of 45 % in the level of *n*-3 PUFA in the samples exposed to light. In order to minimize any changes in sample composition due to fatty acid oxidation, the following procedure was adopted: on arrival, diets were put in the cold room (+4°) in darkness. In the following 24 h, they were separated into plastic bags containing nearly 500 g each and put in a freezer (−18°) in darkness. Every day, the required number of bags were taken out, the diet placed in mangers and put in the home-cages. This method was used to protect against bacteriological contamination and oxidation.

Open-field

A wooden white painted open-field (500 × 500 × 250 mm) in a light- and sound-attenuated chamber was used. The floor was divided by black lines into twenty-five squares (100 × 100 mm). A mouse placed in the middle of the open-field was observed for 5 min. The number of squares crossed and rearings against the walls were counted.

Locomotor activity and habituation

A photocell actimeter (APELEX, 91300 Massy, France) was used. Mice were individually placed in plexiglas cages (250 × 200 × 100 mm) equipped with two i.r. photoelectric cells 20 mm above the floor (in the middle of the longer and shorter sides). Cages were located in an aerated cupboard without illumination. The locomotor activity of mice was recorded daily for 20 min for 5 consecutive days.

Morris water maze

A white circular platform (60 mm diameter) was placed on a pedestal 190 mm above the floor of a grey plastic tank, 800 mm in diameter and 300 mm high. The tank was filled with water (21 ± 1°) to a level of 200 mm. The submerged platform was made invisible by adding a white opacifier (Lytron 631, Norton International, distributed by Brenntag France, Sartrouville, France).

Place learning. Each mouse underwent four trials per day for four consecutive days. For each trial, the mouse was placed in the water facing the pool wall at one of eight possible starting locations, which were regularly distributed around the tank. There were visual cues in the room including posters on the walls, a light and the experimenter, who always stood in the same position. Latency to finding the hidden platform (escape latency) was recorded. If a mouse did not find the platform after 120 s swimming, it was gently put on it. Once the mouse located the platform (or was put on it) it was permitted to remain there for 30 s. At the end of the four trials the mouse was dried with paper towels and returned to a holding cage positioned 400 mm under a lamp.

Probe trial. On the fifth day of the learning test, the platform was withdrawn and the time the mouse swam in each of the four quadrants of the tank was recorded for 100 s. Learning was defined as a mouse spending a time significantly longer than 25 s in the quadrant where the platform was located (training quadrant).

Active avoidance

A shuttle box consisting of two compartments (260 × 200 × 150 mm) separated by a guillotine-type door (90 × 115 mm) was used (Gemini Avoidance System, San Diego Instruments, San Diego, CA, USA). The system is designed to administer a series of trials in which the animal may receive several stimuli (light, sound signal, electric footshock). The user determines the number of trials, the inter-trial intervals, the adaptation period and the shock intensity.

The test comprised fifty trials per d and lasted 5 d. A mouse was placed in the apparatus, the guillotine-type door open, for 2 min adaptation and exploration. After 2 min, the mouse was placed in the right compartment and the test began. Each trial lasted 30 s with two stimuli: first, a sound signal of 78 dB (conditioned stimulus) was emitted for 5 s, followed by an electric footshock of 0·3 mA (unconditioned stimulus) for 25 s. The inter-trial interval ranged from 22 to 38 s, with a median of 30 s. During the trial, the guillotine-type door was open. It closed when the four paws of the mouse entered the opposite compartment or at the end of the trial. The successive trials started in the side where the mouse was located. There were three possible types of response: (1) avoidance: the mouse crossed during the sound signal, (2) escape: the mouse crossed during the electric footshock, (3) no response: the mouse did not cross into the opposite compartment. The results were registered each day. Learning was defined as an increase in the number of avoidances and a reduction in the number of escapes or of 'no responses'.

Statistical analysis

Data are reported as means with their standard errors. The number of mice used in each diet group was twelve for behavioural tests.

Body weight (growth), locomotor habituation, Morris water maze (place learning) and active avoidance were examined by multivariate analysis (Systat software; SPSS Inc., Chicago, IL, USA): two-way ANOVA for repeated measures, two factors (diet, age or time). The Bonferroni test was used as post test. Logrank test was used to compare survival curves. Student's *t* tests were performed for unpaired series of values to analyse the results of the open-field test, the probe trial of the Morris water maze and the first trial session of active avoidance.

Results

Growth and development

The litter size did not differ between mice fed on the control diet (14·04 (SE 0·6), *n* 23) and mice fed on the *n*-3 PUFA-enriched diet (14·72 (SE 0·5), *n* 25).

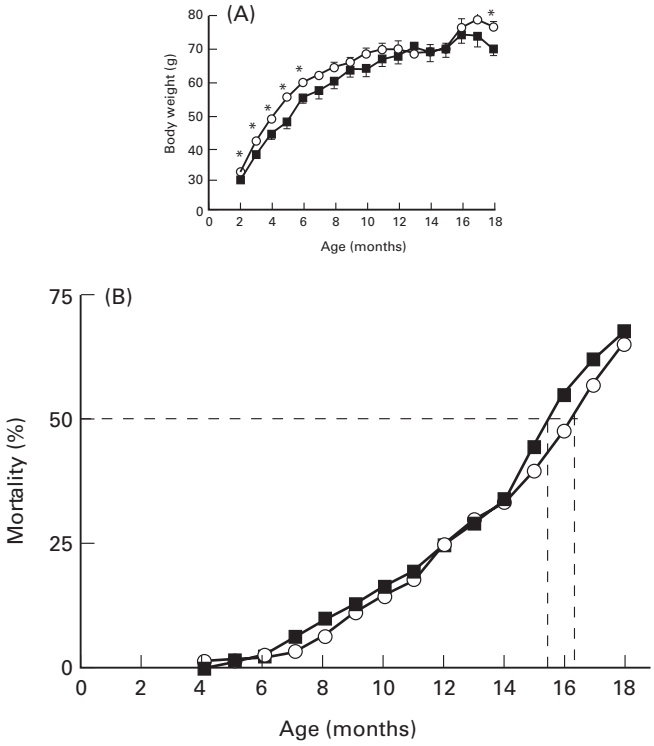

Fig. 1. (A) Effect of age on body-weight of mice fed on a palm-oil (■) or fish-oil (○) diet; mice (*n* 20) from each dietary group were weighed once per month up to 17 months of age. At 18 months, all available mice were weighed (palm-oil diet *n* 66, fish-oil diet *n* 75). Values are means and standard errors shown by vertical bars. Mean values were significantly different from that of the palm-oil-fed mice up to 6-months-old and at 18-months-old, * $P < 0{\cdot}05$. (B) Cumulated deaths (%) as a function of age (survival curve) in mice fed on a palm-oil (■) or fish-oil diet (○). (- - -), age at 50 % mortality. For details of composition of diets see Tables 1 and 2.

Multivariate analysis showed an effect of diet on growth ($P = 0{\cdot}007$), the fish-oil fed mice being heavier (Fig. 1). The post test indicated that fish-oil group body weight was significantly higher up to 6-months-old compared with the palm-oil group. There was no significant difference from 7 to 17 months. When all the available mice were weighed at 18 months of age, the *n*-3 PUFA-enriched mice were heavier: 76·1 (SE 1·5) g (*n* 75) than the mice fed on the control diet: 69·4 (SE 1·8) g (*n* 66), $P = 0{\cdot}005$.

In both diet groups the body weight was higher than in a group of mice of the same age fed on a standard laboratory chow diet. The body weight was 46·11 (SE 1·2) g in mice fed on a chow diet at 7 months and 49·5 (SE 1·5) g at 18 months.

Mortality was registered each month in the two diet groups (Fig. 1). The results indicate no effect of diet. When mice were 16-months-old, the mortality reached 50 %.

Open-field

In young mice, dietary enrichment in *n*-3 PUFA significantly increased the number of squares crossed (Fig. 2(A)) and the number of rearings (Fig. 2(B)) in the open-field ($P < 0{\cdot}05$). These effects were not apparent in mature and old mice.

Habituation

In young mice, there was a significant habituation in both control and fish-oil-fed mice, the locomotor activity decreased with time ($P < 0{\cdot}001$, Fig. 3). The level of locomotor activity was higher in fish-oil-fed mice indicated by a significant effect of diet ($P = 0{\cdot}03$); however, the diet did not influence the habituation.

In mature mice, the two curves are superimposable: there was no effect of diet on the level of locomotor activity; habituation was significant in both control and fish-oil-fed mice ($P < 0{\cdot}001$). There was no interaction between the diet and habituation.

In old mice, a significant habituation occurred in both control and *n*-3 PUFA-enriched mice ($P < 0{\cdot}001$); however, the level of locomotor activity was significantly lower in fish-oil-fed mice than in control mice ($P = 0{\cdot}008$). These results are the inverse of those obtained in young mice. There was no diet × habituation interaction.

Morris water maze

In the Morris water maze (Fig. 4) a significant decrease in latency to reach the hidden platform was evident in young control and fish-oil-fed mice ($P < 0{\cdot}001$). There was no

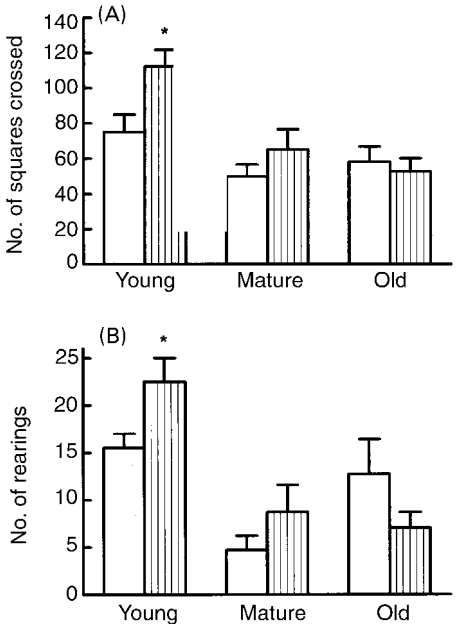

Fig. 2. (A) Number of squares crossed (motor activity) and (B) number of rearings (exploratory activity) in 5 min (open-field test; for details see p. 441) by young (7–11-weeks-old), mature (9–11-months-old) and old (17–19-months-old) mice fed on a palm-oil (□) or fish-oil (▥) diet. Values are means and standard errors represented by vertical bars (*n* 12 for each diet group). For details of the composition of diets see Tables 1 and 2. Mean values were significantly different from that of young palm-oil-fed mice. * $P < 0{\cdot}05$.

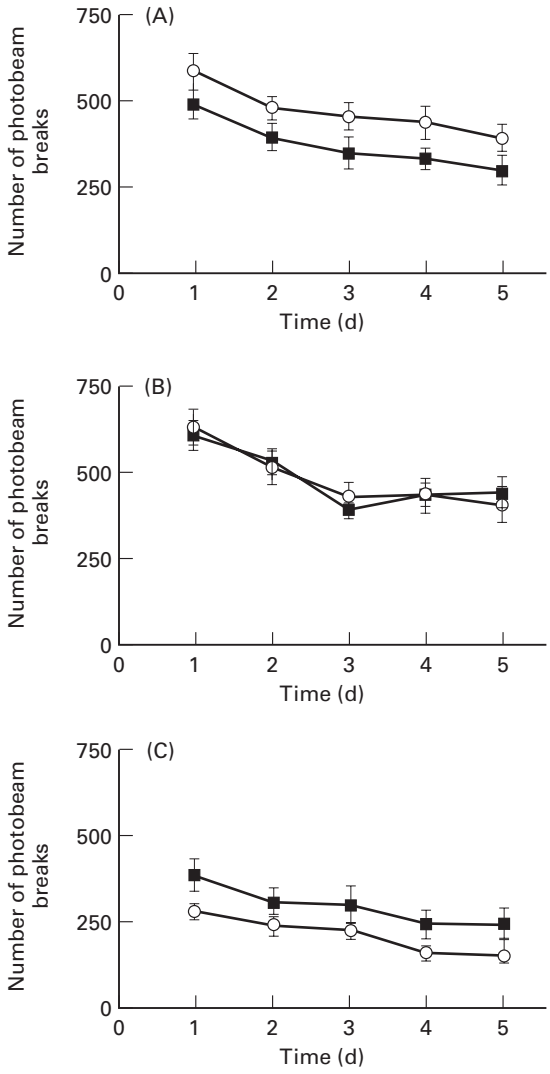

Fig. 3. Locomotor activity (habituation; for details of test see p. 441) measured for 5 d in (A) young (7–11-weeks-old), (B) mature (9–11-months-old) and (C) old (17–19-months-old) mice fed on a palm-oil (■) or fish-oil (○) diet. Values are means and standard errors represented by vertical bars (*n* 12 for each diet group). For details of the composition diets see Tables 1 and 2. The locomotor activity level of fish-oil-fed mice was significantly higher and lower respectively, in young ($P = 0{\cdot}022$) and old ($P = 0{\cdot}008$) mice than in the palm-oil-fed mice.

effect of diet and no interaction between diet and learning. Thus, there was no effect of the enriched diet on learning in this model after sixteen training trials performed over 4 d. In the probe trial (Table 3), the performance of young palm-oil-fed mice did not differ from that of mice fed on a diet enriched in *n*-3 PUFA, retention occurring in both diet groups.

In mature mice, significant learning was observed in the Morris water maze ($P < 0{\cdot}001$). Learning did not differ according to the diet. No interaction occurred between diet and learning. In the probe trial, the time spent swimming in the training quadrant was significantly longer than 25 s for the fish-oil group ($P = 0{\cdot}015$). So, mice fed on a diet enriched in *n*-3 PUFA remembered the platform location in contrast to control mice.

In old mice, the decrease in latency to finding the hidden platform (learning) was significant ($P < 0{\cdot}05$). This learning did not differ between control and fish-oil-fed mice. No interaction occurred between diet and learning. This learning, however, was rather poor since the decrease in mean latency between the first and the fourth day was 20·75 % in old mice, whereas it was 59·18 % in mature mice and 75·34 % in young mice. In these old mice, the probe trial did not show any retention in either control or fish-oil-fed mice.

Active avoidance

The age-induced decline in performance in the active avoidance test was evident because 50 % avoidance was achieved from the second training session in young mice, the fourth session in mature mice and was not reached during the last session in old mice (Fig. 5). In young fish-oil-fed mice, the percentage of avoidance on the first day of training was significantly higher than in control mice ($P = 0{\cdot}021$). The higher avoidance of electric shocks observed in young mice during the first training session was not found in the following sessions and the learning performance with the two diets regarded as a whole for the 5 d of training did not differ. There was significant learning ($P < 0{\cdot}001$) and a lack of effect of diet. There was no diet × learning interaction.

In mature mice, the learning curve of fish-oil-fed mice was below that of control mice; however, the two curves did

not differ significantly. There was significant learning ($P < 0{\cdot}001$) and no difference between the diets. There was no diet $\times$ learning interaction. When looking at the scores obtained for the first learning session, the two diets differ significantly, avoidance was less good in fish-oil-fed mice ($P = 0{\cdot}012$). This effect is the inverse to that seen in young mice.

In old mice, the learning curve of fish-oil-fed mice was also below that of control mice, but the difference between the two curves was only borderline significant ($P = 0{\cdot}062$). Effective learning took place in both groups ($P < 0{\cdot}001$). There was no diet $\times$ learning interaction. Thus, old fish-oil-fed mice tended to be slow to acquire avoidance behaviour compared with palm-oil-fed mice.

Table 3. Time (s) spent by mice fed on a palm-oil or a fish-oil diet in the training quadrant from which the platform was withdrawn (probe trial of Morris water maze)†

(Mean values with their standard errors)

	Palm-oil diet		Fish-oil diet	
Age of mice‡	Mean	SE	Mean	SE
Young§	32·9	2·4	33·2	1·7
Mature	25·3	2·0	32·0**	2·5
Old§	14·2	2·5	20·6	2·5

Mean value was significantly different from that of palm-oil-fed mice, ** $P < 0{\cdot}01$.
† For details of the composition of the diets see Tables 1 and 2; for details of the probe trial see p. 441.
‡ Young mice, 7–11-weeks-old; mature mice, 9–11-months-old; old mice, 17–19-months-old.
§ Both groups of young mice remembered the platform location, whereas there was no significant retention of the location by the old mice.

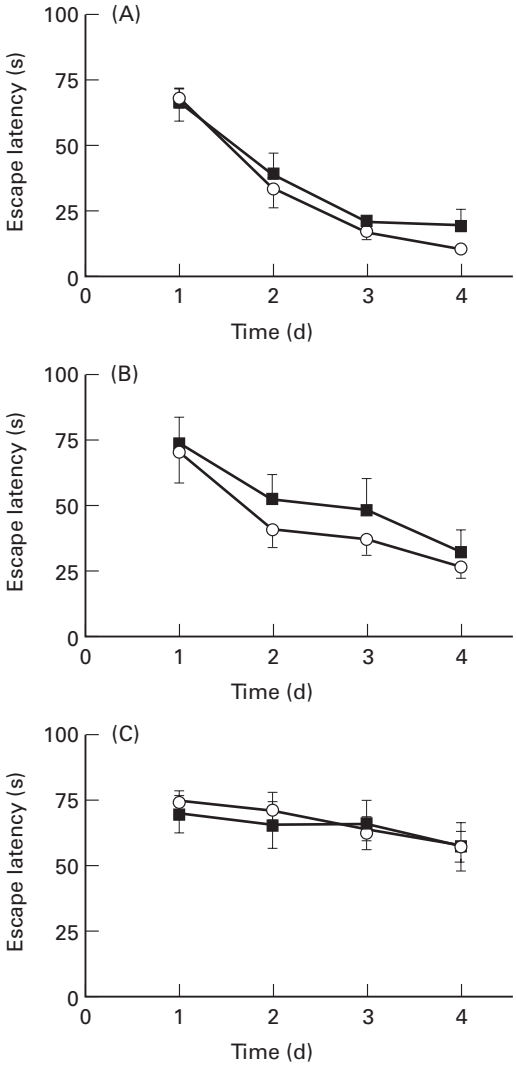

Fig. 4. Performance in the Morris water maze as measured by the time taken for mice (n 12 per diet group) to find the hidden platform (escape latency; for details of test see p. 441) in (A) young (7–11-weeks-old), (B) mature (9–11-months-old) and (C) old (17–19-months-old) mice. The mice were fed on a palm-oil (■) or fish-oil (○) diet. For details of the composition of the diets see Tables 1 and 2. Each point represents the mean group performance in four trials from day 1 to 4, and standard errors represented by vertical bars. There was a significant spacial learning for the two diet groups in each age group ($P < 0{\cdot}01$).

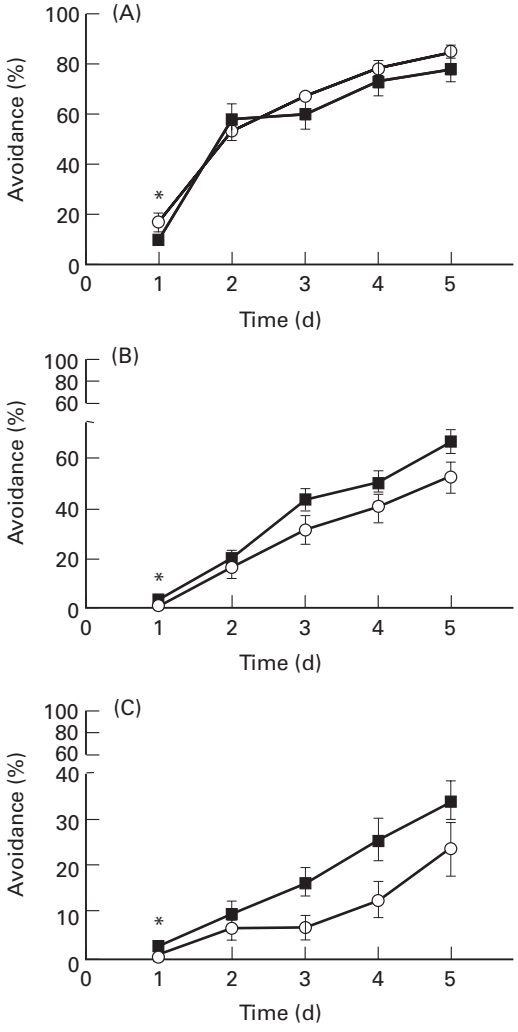

Fig. 5. Active avoidance (for details of test see p. 441) in (A) young (7–11-weeks-old), (B) mature (9–11-months-old) and (C) old (17–19-months-old) mice, fed on a palm-oil (■) or fish-oil (○) diet. For details of composition of diets see Tables 1 and 2. Each point represents the mean group performance of fifty trials each day (n 12 per diet group) and standard errors represented by vertical bars. The maximum percentage of avoidances on the fifth day decreased with increasing age. Mean values were significantly different from that of palm-oil-fed mice, * $P < 0{\cdot}05$.

Discussion

These results show that the fish-oil-diet transiently promoted body-weight gain, induced a positive effect in young mice since an increase in exploratory and locomotor activity was observed as well as a greater number of avoidances in the first trial of active avoidance (which indicates increased arousal). These advantages were not found in mature fish-oil-fed mice, who showed improved performance only on the probe trial of the Morris water maze. In old mice, the fish-oil diet was detrimental for locomotor activity and the first trial of active avoidance.

In young mice, dietary enrichment in *n*-3 PUFA significantly increased the number of squares crossed and the number of rearings in the open-field. The number of squares crossed is a measure of locomotor activity which reflects both the desire to explore an unknown place and the fear of being confronted by novelty. Rearings are more representative of exploratory activity. An increase in exploratory activity in the open-field was not observed by Coscina *et al.* (1986) in rats fed on a diet enriched in soyabean oil but the diet was only given for 1 month. Similarly, Wainwright *et al.* (1997) did not observe, in the open-field, a modification in the performance of mice fed on a diet enriched in *n*-3 PUFA. Contradicting these results, Mills *et al.* (1988) reported a reduction in the total number of squares crossed and in the number of rearings in rats of 26 d old whose mothers had been implanted with osmotic pumps delivering α-linolenic acid for 8 weeks. These results, however, are hardly comparable because of differences in the species, the duration and means of supplementation.

Interestingly, Enslen *et al.* (1991) have described the same results with a reciprocal study: exploratory activity in rats was reduced by a diet depleted in *n*-3 fatty acids. Our results are also consistent with the observation by Wilcock & Broadhurst (1967) that there is a positive correlation between locomotion measured in the open-field and avoidance latency.

The increase in exploratory activity of young *n*-3 PUFA-enriched mice was not found in mature and old mice: one explanation is that the greater weight of fish-oil-fed mice may be troublesome. Alternatively, and more likely, it may be suggested either that the effect of the fish-oil-diet may be an advantage after a short duration and a drawback after a long duration of consumption, or that the diet is only beneficial in young animals.

Habituation occurred in every age group and for both diets; so the fish-oil diet did not improve this very elementary form of learning. The level of locomotor activity was higher in young, equal in mature and lower in old *n*-3 PUFA-enriched mice than in control mice. This result for young mice is in agreement with the results of the open-field test where the score of experimental mice was higher than that of control mice. The reduced activity of old mice may be related to their heavier weight.

In the Morris water maze test, the decrease in mean latency to finding the hidden platform between the first and the fourth day of training progressively declined as age increased. This decrease in learning ability with ageing has been described in this test (Gallagher & Pelleymouter, 1988; Brandeis *et al.* 1989; Lamberty & Gower, 1991), and in our studies with old OF1 mice (Carrié *et al.* 1999). We observed no improvement in learning ability with fish-oil-diet in any of the three age groups.

Regarding the effect of the *n*-3 PUFA-enriched diet, our results do not agree with those of other authors. Nakashima *et al.* (1993) observed an improvement in learning in mice fed on a diet enriched in *n*-3 PUFA in the Morris water maze; however, they compared mice fed on a perilla-oil diet with mice fed on a safflower-oil diet, that is to say an enriched diet with a diet poor in *n*-3 PUFA without equilibrated controls. Coscina *et al.* (1986) found an improvement in learning in this model but their rats, fed on a diet enriched in *n*-3 PUFA using soyabean oil, differed from the controls after the twenty-fifth training trial only; in addition, they could not reproduce their own results (Coscina, 1997). For Jensen *et al.* (1996) diets enriched in *n*-3 PUFA improved learning in the Morris water maze, but this effect was significant only from the sixth day of training for diets enriched in seal oil or vegetable oil, but not fish oil. So, it may be suggested that the duration of training in our mice (sixteen trials on 4 d) was perhaps not sufficient to see an effect of diet. Alternatively, the positive results having been obtained with vegetable or seal oils but not with fish oils suggests a possible role of short-chain PUFA.

In the probe trial of the Morris water maze, the effect of age was also evident since significant retention of the platform location occurred in young and not in old mice. In mature mice, significant retention of the platform location occurred in mice fed the *n*-3 enriched diet but not in control mice, indicating a possible favourable effect of the diet.

In the active avoidance test, the decline in performance with age is in accordance with the observations of Stavnes & Sprott (1975), Miyamoto *et al.* (1986), and the results obtained in OF1 mice fed on a standard laboratory diet (Carrié *et al.* 1999). The higher percentage of avoidance in young fish-oil-fed mice than in controls on the first day of training is in agreement with the results of Mills *et al.* (1988) in rats. In addition, it corresponds in the opposite direction to results obtained in animals fed on a diet poor in *n*-3 PUFA, since Bourre *et al.* (1989) in rats and Galli *et al.* (1975) in mice, showed that the first two or first one (respectively) training sessions in the active avoidance test resulted in a reduced percentage of avoidance in animals fed on a diet poor in *n*-3 PUFA compared with controls. All these experiments, therefore, led to the same conclusion: enrichment in *n*-3 PUFA increased, and depletion reduced, the scores in the first session in the active avoidance test. For young fish-oil-fed mice, the quicker association between the sound signal and the electric shock may result from an increase of arousal as observed in open-field. It may be noted that the duration of diet administration does not seem to be a determinant factor. Indeed, the rats used by Bourre *et al.* (1989) were obtained from the third generation that had received the diet whereas the mice used by Galli *et al.* (1975) were born from dams receiving supplementation only during the last week of pregnancy and were themselves fed on a standard diet. The rats used by Mills *et al.* (1988) were born from dams implanted with a minipump that provided the oil supplementation. These pumps were placed 8 weeks before weaning. From weaning, the rats in the experiment were fed on standard laboratory chow. Our

mice were fed on the same diet as their mothers who began receiving the diets 3 weeks before mating; so it seems that the duration of the diet does not need to be long in order to have a beneficial effect.

For mature and old mice, the percentage of avoidances for the first training sessions was smaller in mice fed on the *n*-3 PUFA-enriched diet than in control mice. This effect is the opposite of that seen in young mice and has not been reported previously. This is not surprising since the authors who have studied animals fed on a diet enriched or depleted in *n*-3 PUFA in the active avoidance test used young animals only (60–83 d for Mills *et al*. (1988); 60 d for Galli *et al*. (1975) and Bourre *et al*. (1989)).

In general, in young mice the fish-oil-enriched diet increased the exploratory and locomotor activity and also the number of avoidances in the first trial of the active avoidance. The increase in body weight may be accompanied by an accelerated behavioural development. These advantages were not found in mature mice who showed improved performance only on the probe trial of the Morris water maze. On the other hand, mature mice made a greater number of errors that the controls in the first active avoidance trial. Old fish-oil-fed mice were either comparable with the control mice or performed less well for locomotor activity and the first trial of active avoidance.

There was no difference between the two diet groups regarding litter size. Weight increase differed significantly: the fish-oil diet induced accelerated growth. The extent to which the greater weight of the fish-oil-fed mice may be a handicap for their behavioural performance is unknown but could have an effect. Alternatively, the PUFA are readily oxidizable and the *n*-3 PUFA seem to be more oxidizable than the *n*-6 PUFA, since Sen *et al*. (1997) reported that a diet supplemented in fish oil shows 33 % higher lipid peroxidation than a diet supplemented with soyabean oil. In addition, Ide *et al*. (1996) reported that, in liver, the rates of mitochondrial and peroxisomal oxidation were significantly higher in rats fed on perilla oil than in those fed on other fats (saturated fat or safflower oil). Thus, the decreased performance in old fish-oil-fed mice may result from the formation of more oxidation products than in the control mice, leading with time to an accumulation and decreased performance. Nevertheless, Garrido *et al*. (1998) showed that in old rats fed on a fish-oil diet (100 g/kg) oxidative damage in brain did not increase compared with the control diet. These results recall the experiments of Weisinger *et al*. (1996) who showed alterations in the electroretinogram in guinea-pigs fed on a fish-oil-enriched diet compared with those fed on a control diet. These authors suggested oxidative stress as a possible explanation.

These results indicate a positive effect of a supply of *n*-3 polyunsaturated fatty acids from fish oil in young mice and a negative effect in old mice. However, in our protocol, the duration of the diet was shorter in young than in old mice. Thus, the question of the effect of a fish-oil diet given to old mice for a restricted period remains to be answered: it will be the subject of our next study.

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