Effect of a high linoleate and a high α-linolenate diet on general behavior and drug sensitivity in mice

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Abstract Semi-purified diets supplemented with either a high linoleate (n-6) (safflower) oil or a high α-linolenate (n-3) (perilla) oil were fed to mouse mothers and their offspring through 6 weeks of age. The proportions of n-3 and n-6 highly unsaturated fatty acids in brain phospholipids reflected the n-3/n-6 balance of the diets while no difference was found in phospholipid compositions or cholesterol/phospholipid ratios. In the elevated plus maze task, the total number of entries into the open- and enclosed-arms was smaller and the time spent in the dark enclosed arms tended to be longer in the perilla group than the safflower group. The time required to reach a safe platform in Morris's water maze test was less in the perilla group, but no significant difference was observed in the entries into the arms darkened with a movable cover in Y-maze dark-preference task. The safflower group was more sensitive to pentobarbital; the anesthesia onset time was less and the anesthetic time was longer than in the perilla group. Increased locomotion induced by scopolamine injection was less in the safflower group as compared with the perilla group. These results indicate that in mice the dietary α-linolenate/linoleate balance affects the n-3/n-6 ratio of brain phospholipid acyl chains and that this is accompanied by general behavioral changes as well as changes in sensitivities to drugs known to affect behavior.—**Nakashima, Y., S. Yuasa, Y. Hukamizu, H. Okuyama, T. Ohhara, T. Kameyama, and T. Nabeshima.** Effect of a high linoleate and a high α-linolenate diet on general behavior and drug sensitivity in mice. *J. Lipid Res.* 1993. **34:** 239–247.

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There is a widely held perception that functions of the central nervous system are not easily affected by the choice of foods in mammals. This is based mainly on two facts. First, high molecular weight food constituents such as proteins and nucleic acids are hydrolyzed during digestion in the gastrointestinal tract, and second, the blood–brain barrier discriminates carefully among blood-borne nutrients to maintain homeostasis of the central nervous system. However, certain fatty acids do not fit this generalization. Different foods contain different proportions of n-3 (α-linolenate) and n-6 (linoleate) fatty acids, and the choice of foods affects the n-3/n-6 ratio of membrane phospholipid acyl chains in all mammalian tissues, including those of the central nervous system (1).

The effects of dietary fatty acids on brain functions were first studied by evaluating the effect of fat-free diets (n-3 and n-6 dual deficiency) on animal behavior (2, 3) as reviewed by Wainwright (4). These studies were then extended to estimate the effect on brain functions of an n-3 deficiency in the presence of n-6 fatty acids (5–7); this latter question was of particular interest because docosahexaenoate (22:6n-3) derived from α-linolenate (n-3) is enriched in such tissues as brain and retina. Earlier studies using simple maze tests led to apparently conflicting conclusions. Lamptey and Walker (7) reported inferior learning by rats fed an α-linolenate-deficient diet in a simple Y-maze test; however, this finding could not be reproduced using a more complex X-maze test (5) as reviewed by Bivins et al. (8). Harman et al. (6) have reported that the number of errors in an intelligence test (maze) was the highest in an n-3-enriched (fish oil) diet group as compared with n-3-deficient corn oil and safflower oil diet groups. Only recently, we (9–12) and others (13–16) have established, using more sophisticated experimental conditions, that long-term α-linolenate (n-3) deficiency affects retinal function and some aspects of behavior in mammals.

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In establishing the essentiality of α-linolenate for the maintenance of brain functions, we used an operant-type brightness-discrimination learning apparatus (10–12). In this test, discrimination between a bright light and a dim light was conditioned. However, other experiments by Benolken, Anderson, and Wheeler (13), Neuringer et al. (16), and Watanabe et al. (9) revealed that n-3 deficiency also causes a decrease in retinal function. This raises the question of whether decreased retinal function is the only defect induced by n-3 deficiency and whether the decreased discrimination performance in the brightness-discrimination learning test is secondary to decreased visual sensitivity (17). Circumstantial evidence supports the conclusion that n-3 deficiency induces decreases in both brain and retinal functions as discussed previously (11, 12), but more direct evidence is necessary to support this conclusion. The next step would be to find a clue to the biochemical bases for a link between essential fatty acids and behavior.

In this study, we have examined the effects of the dietary α-linolenate/linoleate balance on behavior and on the sensitivities to drugs known to affect behavior of mice.

MATERIALS AND METHODS

Animals and diets

Semi-purified diets (Nihon Clea Co., Ltd.) containing 10% perilla oil or safflower oil (10) were fed to ICR mice (Nihon SLC Inc., Shizuoka, Japan) from weaning. These mice were then mated at 11 weeks of age, and the offspring were fed the same diet as their dams. The major fatty acids of safflower oil were 16:0 (8.6%), 18:0 (2.5%), 18:1n-9 (12.5%), 18:2n-6 (73.5%), and 18:3n-3 (0.2%) while those of perilla oil were 16:0 (6.5%), 18:0 (1.9%), 18:1n-9 (19.6%), 18:2n-6 (13.6%), and 18:3n-3 (57.8%).

The offspring (both male and female from several mothers) at 6 weeks of age were used for behavioral tests; 1 week later some of the offspring were subjected to tests for either drug sensitivity or pain threshold and then they were killed. The combinations for the tests were: elevated plus maze test (memory)-diethyl ether treatment (10 males), Y-maze-scopolamine treatment (10 males), water maze (19 males)-pain threshold test (10 males), elevated plus maze test (anxiety)-diazepam treatment (10 females), Y-maze-pain threshold test (10 females), and pentobarbiturate treatment (15–18 females, 5 or 6 animals/dose). Animals were kept at 23 ± 2°C, 55 ± 5% humidity on a 12-h dark-light cycle (8:00–20:00). Diets and water were fed ad libitum. Behavioral tests were performed between 10:00 and 18:00.

Lipid analysis

After the behavioral tests described below, mice were killed and their brains were stored at −80°C until analysis. Lipids were extracted from the frozen brains with chloroform–methanol, separated by silica gel thin-layer chromatography, extracted from the silica gel, and fatty acids of individual phospholipids were analyzed by gas chromatography with a capillary column (Supelco Wax 10) essentially as described previously (10). Cholesterol was measured by gas–liquid chromatography as its trimethylsilyl ether with 5α-cholestane as an internal standard.

Elevated plus maze test

An elevated plus maze apparatus was made with two white open-arms (21 × 7.5 cm) and two black enclosed-arms (21 × 7.5 × 20.5 cm high) attached cross-wise to a white central platform (7.5 × 7.5 cm) (18, 19). The apparatus was placed 50 cm above the floor. In one test to evaluate anxiety and dark preference, a mouse was placed on the central platform with its head toward an open-arm. The frequency of entry into the open- and enclosed-arms and the time spent in the open-arms during 5 min were determined. Most mice prefer a dark, enclosed space. In another test to evaluate memory, a mouse was placed at the end of an open-arm with the head toward the outside of the arm. The time it took the mouse to enter its body and four limbs into the enclosed-arms was measured (transfer latency) on day 1 and on the next day (18).

Water maze test

Morris' water maze (20) was made of a vinyl pool 80 cm in diameter and 30 cm deep. A platform 5 cm in diameter was placed 1 cm below the surface of the water and at the center of the pool. Black India ink was added to the water so the mice could not see the platform. On the walls of the room, there were many black, white, or gray boards in order to give mice clues to recognize the environment. Mice were placed into the pool facing the wall randomly from five different starting points, and the time it took for the mouse to reach the platform and stay there for more than 30 sec was measured. This trial was performed daily for 10 days. The cut-off time was 100 sec.

Dark-preference in Y-maze

Dark-preference in a Y-maze was measured using an apparatus made of three arms each 5 × 10 × 17 cm and 12 cm in height, which was set in a dark room (21). An electric bulb (100 W) was placed 50 cm above the apparatus. To darken one side of the Y-shaped arm, a movable black cover was set. The inside of the apparatus was white while the outside was black. A mouse was placed in an open arm and its entry into the darkened or open arms in the first trial was recorded. The cover was moved randomly and five trials/mouse were performed.

Pain threshold test

Pain threshold was determined by the tail-flick method (22). The tip of tail was painted with a black marker, radi-

ant heat was applied, and the time elapsed before the animal moved its tail was measured.

Sensitivity to anesthetics

Sensitivity to pentobarbital in inducing anesthesia was determined after intraperitoneal injection of 30–50 mg/kg of sodium pentobarbital. The time of onset and duration of anesthesia were measured using the loss of righting-reflex. In measuring the anesthetic action of ether, 10 ml of diethyl ether was put into an observation chamber (10 × 12 × 8.5 cm), which was then covered. After 1 min, a mouse was placed in the chamber and the onset time for anesthesia was measured using the loss of righting-reflex. Immediately after the loss of righting-reflex, the mouse was taken out of the chamber and anesthesia duration time was measured.

Sensitivity to muscle relaxant

Muscle relaxation was induced by subcutaneous injection of 7.5 mg/kg of diazepam; control animals received a 5% gum arabic solution which was used as a vehicle. Muscle relaxation was measured with a retraction test at 15-min intervals for 90 min. In the retraction test, a steel wire (2 mm diameter) was anchored 80 cm above the floor, and the mouse that showed no retraction reaction within 5 sec after touching the forelimb to the wire was judged as "muscle relaxation positive."

Sensitivity to scopolamine

Scopolamine-induced hyperlocomotion was determined by measuring locomotor activity with an Automex (Columbus Instruments) at 15-min intervals for 1 h after intraperitoneal injection of 2 mg/kg of scopolamine or saline as a control.

Statistical analyses

The Student's *t*-test was used for analyzing data for the behavior in elevated plus maze test, for the tail-flick pain test, for the sensitivity test to diethyl ether, and for the fatty acid compositions. The fatty acid compositions were also analyzed by the Bonferroni's test. Mann-Whitney's U test was used in comparing data for the locomotor activity and the darkness preference Y-maze test. Fisher's probability test was used for muscle relaxation induced by di-

TABLE 1. Fatty acid compositions of total lipids, ethanolamine phospholipid, and choline phospholipid from brains of mice fed a perilla oil diet or a safflower oil diet

	Ethanolamine Phospholipid		Choline Phospholipid		Total Lipids	
Fatty Acid	Perilla	Safflower	Perilla	Safflower	Perilla	Safflower
			% of total fatty acid			
14:0	2.3 ± 2.7	1.0 ± 0.6	0.7 ± 0.7	0.6 ± 0.5	0.6 ± 0.1	0.7 ± 0.3
14:1	0.4 ± 0.3[b]	1.2 ± 0.3	0.7 ± 0.8	0.2 ± 0.2	0.2 ± 0.1	0.2 ± 0.1
16DMA	4.4 ± 0.8	2.9 ± 1.0	0.4 ± 0.5	tr	1.7 ± 0.2	1.7 ± 0.3
16:0	7.5 ± 1.6	6.1 ± 1.0	42.2 ± 5.2	44.4 ± 2.4	17.9 ± 1.2	18.4 ± 1.2
16:1	0.7 ± 0.5	1.4 ± 0.5	1.6 ± 1.6	0.9 ± 0.7	1.2 ± 0.2[c,A]	0.2 ± 0.1
18DMA	7.6 ± 0.9	7.5 ± 0.4	0.4 ± 0.7	tr	3.2 ± 0.2	3.1 ± 0.2
18:1DMA	4.8 ± 0.8	5.0 ± 0.6	tr	tr	tr	tr
18:0	18.6 ± 1.5	17.7 ± 1.4	12.8 ± 1.0	13.6 ± 1.1	21.3 ± 0.6	21.3 ± 1.1
18:1	11.1 ± 1.2	11.8 ± 5.4	27.3 ± 3.5	25.8 ± 2.8	20.5 ± 3.0	16.8 ± 1.9
18:2n-6	0.3 ± 0.2	1.6 ± 1.6	0.4 ± 0.1	0.7 ± 0.2	0.9 ± 0.6	1.2 ± 0.4
18:3n-3	0.2 ± 0.2	0.6 ± 0.5	tr	tr	0.3 ± 0.3	0.2 ± 0.2
20:1	2.6 ± 0.4	2.8 ± 1.1	0.6 ± 0.1	2.3 ± 2.7	2.4 ± 1.3	2.4 ± 1.6
20:3n-3	0.6 ± 0.2	0.5 ± 0.2	0.4 ± 0.1	0.2 ± 0.1	0.9 ± 0.5	0.9 ± 1.2
20:4n-6	8.4 ± 1.2	10.2 ± 1.0	4.1 ± 0.3[c]	5.6 ± 0.4	7.7 ± 0.7[a,A]	10.3 ± 1.9
20:5n-3	0.3 ± 0.2	0.1 ± 0.2	0.3 ± 0.2	0.1 ± 0.2	0.3 ± 0.1	0.4 ± 0.2
22:4n-6	2.9 ± 0.3[b]	4.8 ± 0.8	0.5 ± 0.2	0.6 ± 0.1	1.6 ± 0.1	2.1 ± 1.3
22:5n-6	tr	2.9 ± 1.5	1.0 ± 1.9	0.7 ± 0.4	0.1 ± 0.0[a]	2.0 ± 1.2
22:5n-3	2.5 ± 2.4	0.2 ± 0.2	0.3 ± 0.2	0.1 ± .1	0.7 ± 0.2[a]	0.2 ± 0.2
22:6n-3	24.1 ± 2.4[a]	17.5 ± 3.1	3.3 ± 1.0	3.1 ± 0.3	17.2 ± 1.1[a]	14.5 ± 1.7
Total n-3	27.1 ± 4.6[a]	18.4 ± 3.5	4.0 ± 1.2	3.2 ± 0.2	18.5 ± 0.8[a]	15.3 ± 1.7
Total n-6	12.2 ± 1.5[b]	20.4 ± 2.7	6.4 ± 2.2	7.7 ± 0.8	11.4 ± 1.2[a]	17.8 ± 3.4
n-3/n-6	2.28 ± 0.71[a,B]	0.92 ± 0.26	0.64 ± 0.16[a]	0.42 ± 0.06	1.63 ± 0.20[b,B]	0.90 ± 0.27
			mg/g			
Total	1.19 ± 0.16	1.02 ± 0.20	0.89 ± 0.12	1.04 ± 0.08	3.52 ± 0.19	3.57 ± 0.25

Averages ± SD for four mice in each group are presented. Fatty acids are designated by the number of carbons: the number of double bonds, and the first double bond numbered from the methyl terminus is designated as n-3 or n-6. DMA denotes dimethylacetal derived from plasmalogen; tr, trace.

[a], $P < 0.05$; [b], $P < 0.01$; [c], $P < 0.001$: statistical significance in Student's *t* test.

[A], $P < 0.05$; [B], $P < 0.01$: significance in Bonferroni test.

azepam. Two-way ANOVAs were used for the Morris' water maze test, the scopolamine-induced hyperlocomotion test, and the sensitivity tests for pentobarbital.

RESULTS

Lipid compositions of the brain

The amounts of total lipids, ethanolamine phospholipids, and choline phospholipids were not different between the safflower and perilla groups (**Table 1**). The fatty acid compositions of the total lipids and phospholipids are shown in Table 1. The difference in the α-linolenate (18:3n-3)/linoleate (18:2n-6) ratios of the diets was reflected mainly in the differences in the proportions of 22-carbon highly unsaturated fatty acids (22:5n-3 + 22:6n-3)/(22:4n-6 + 22:5n-6) in the total lipids and ethanolamine phospholipids. The proportions of arachidonate (20:4n-6) were also affected, but to a lesser degree than the changes seen in 22-carbon highly unsaturated fatty acids. In choline phospholipids, essentially no difference was detected in the proportions of n-3 and n-6 highly unsaturated fatty acids with 22 carbons, although the proportions of arachidonate and the n-3/n-6 ratios were affected by the diets (Student's *t*-test). These changes in the fatty acid compositions seen in mice were quantitatively less than those seen in rats fed similar diets (10). When the fatty acid compositions of the two dietary groups were analyzed in Bonferroni test, the differences were significant for the limited number of parameters as shown in Table 1.

The cholesterol/phospholipid ratios (μg/nmol phospholipid) of brains were the same in the two dietary groups; 0.29 ± 0.01 and 0.28 ± 0.01 for the safflower group and the perilla group, respectively.

Behavior in elevated plus maze test

When a mouse was placed on the center platform with its head toward an open-arm, the total number of entries into the open- and enclosed-arms was significantly larger in the high linoleate (safflower oil) group than in the high α-linolenate (perilla oil) group (**Fig. 1**). The time spent in open-arms as well as the frequency of entry into the open-arms tended to be higher in the safflower oil group, but these differences were not statistically significant (only female mice were examined).

When a mouse was placed at the end of an open-arm with its head toward the outside of the arm, the time spent before entering the enclosed arm was not significantly different on day 1 between the two dietary groups of the male mice. On the 2nd day, the time spent before entering the enclosed arm was significantly shorter than that on the first day, but there was no significant difference in the times between the two dietary groups (data not shown).

Morris' water maze test

The time required to reach the platform (goal latency) decreased rapidly during the first few days, achieving plateau levels in both dietary groups. The goal latency was significantly longer ($F(1,360) = 31.56$, $P < 0.01$ in two-way ANOVA, diet vs. time) in the safflower group than in the perilla group, indicating that the performance in this task was poorer in the safflower group than in the perilla group (**Fig. 2**).

Dark-preference in Y-maze

The entries from the open arm, where the mouse was placed, to the arm darkened with a movable cover or to the other open arm not covered were measured. The frequency of entry into the darkened arm was ca. 60% and no significant difference was observed between the two dietary groups (male and female) (data not shown).

Pain threshold

When a radiant heat was applied to the black-painted tail, the elapsed time before the animal moved the tail (pain threshold) was 3.8–4.5 sec under the conditions used, and no statistically significant difference was observed between the two dietary groups or between male and female mice (data not shown).

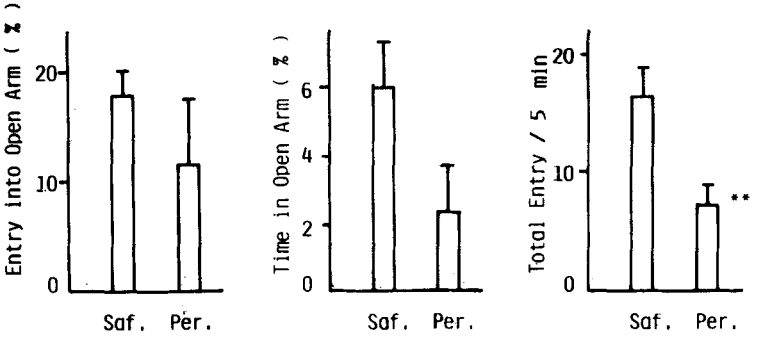

Fig. 1. Effect of dietary oils on behavior in an elevated plus-maze test. A mouse was placed on the central platform with its head toward an open-arm, and the entries into the open- and enclosed-arms as well as the time spent in the open-arms were determined. Averages of 10 female mice (± SEM) were presented. **, $P < 0.01$ vs. safflower group in Student's *t*-test.

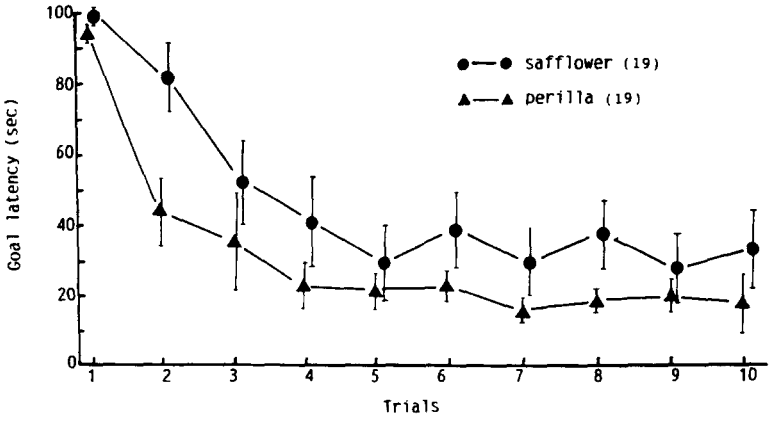

Fig. 2. Effect of dietary oils on acquisition in the water maze test. Mice were placed into the pool facing the wall randomly from five different starting points, and the time it took for the mouse to reach the platform was measured. Each point represents the average of 19 male mice (± SEM). The difference between the two dietary groups was statistically significant in a two-way ANOVA (dietary groups vs. time). $F(1,360) = 31.56$, $P < 0.01$.

Sensitivity to pentobarbital and diethylether

Intraperitoneal injection of 30–50 mg/kg of pentobarbital induced anesthesia. The time to enter anesthesia (anesthesia onset time) was significantly shorter in the safflower group than in the perilla group as analyzed by a two-way ANOVA (dietary group vs. dose of drug), $F(1,27) = 13.00$, $P < 0.01$. The duration of anesthesia was also significantly longer ($F(1,27) = 4.68$, $P < 0.05$ in two-way ANOVA) for the safflower group (**Fig. 3**). When diethyl ether was used, however, no significant difference was found in anesthesia onset or duration of the two dietary groups (data not shown).

Diazepam-induced muscle relaxation

Muscle relaxation was apparent at 15 min after diazepam injection (**Fig. 4**). No relaxation was seen in the control groups receiving vehicle. The action of diazepam tended to be weaker in the safflower group than in the perilla group, but the difference was not statistically significant in Fisher's probability test ($P > 0.05$) (Fig. 4).

Scopolamine-induced hyperlocomotion

Locomotor activities in the saline-injected control groups were similar between the safflower and perilla groups (**Fig. 5**). The locomotor activities measured after scopolamine injection increased significantly in both the safflower oil group ($F(1,216) = 500.79$, $P < 0.01$) and the perilla group ($F(1,216 = 617.97$, $P < 0.01$) in two-way ANOVA (drug vs. time). The scopolamine-induced locomotor activities were significantly lower in the safflower group than in the perilla group as examined by two-way ANOVA (dietary group vs. time of scopolamine), $F(1,216 = 28.67$, $P < 0.01$).

DISCUSSION

Many papers have reported that foods can affect the behavior of mammals. In addition to the protein/carbohydrate ratio of foods (23) and the food constituents (24), the types of fats and oils have been examined extensively

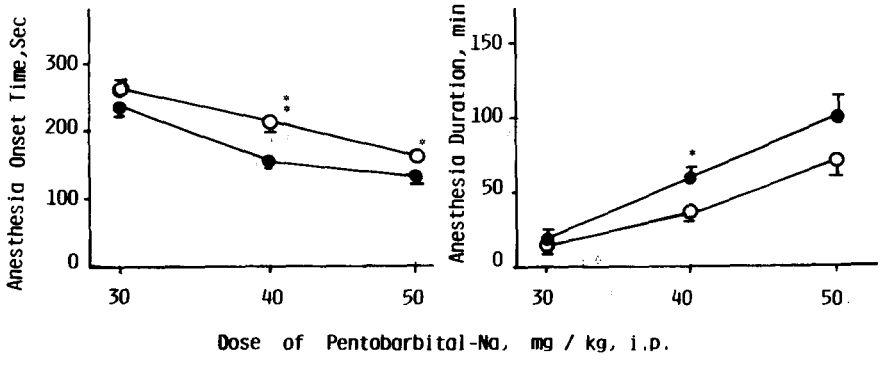

Fig. 3. Effect of dietary oils on pentobarbital-induced anesthesia. Sodium pentobarbital (30–50 mg/kg) was injected intraperitoneally and the time of onset of anesthesia and the duration of anesthesia were measured using the loss of righting reflex as an indication of anesthesia. Each point represents the average (± SEM) of six female mice (safflower group, ●) and five female mice (perilla group, ○). The difference in the onset times of the two groups was statistically significant in a two-way ANOVA (dietary groups vs. dose of drug, $F(1,27) = 13.00$, $P < 0.01$) and that of anesthesia duration was also significant in a two-way ANOVA ($F(1,27 = 4.68$, P 0.05. Significance in Student's t-test is shown as *, $P < 0.05$ or **, $P < 0.01$ vs. safflower group.

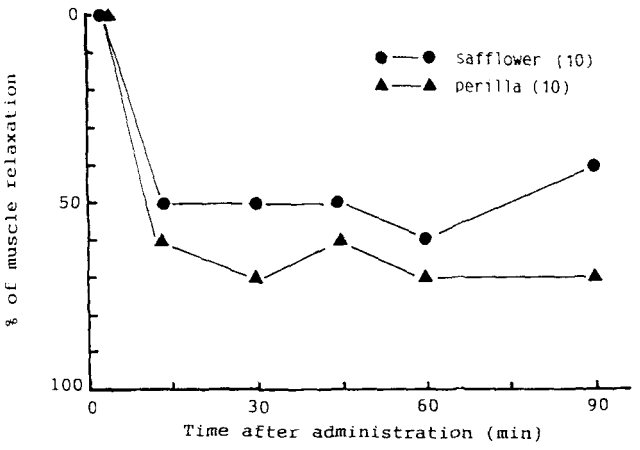

Fig. 4. Effect of dietary oils on diazepam-induced muscle relaxation. Muscle relaxation was induced by subcutaneous injection of 7.5 mg/kg diazepam; control animals received a 5% gum arabic solution which was used as vehicle. Muscle relaxation was measured by traction test. Each point represents the average of 10 female mice. The difference between the two dietary groups was not significant in Fisher's probability test.

in relation to behavioral patterns and drug sensitivity. The behavior known to be affected by dietary fats and oils includes exploratory activity and T-maze learning (25), retention of memory (26), pain threshold and thermoregulation (27), macronutrient selection (protein or carbohydrate) (28), pentobarbital-induced anesthesia (29), and interleukin-induced anorexia (30). Most studies have focused on evaluations of the effects of linoleate (n-6) deficiency, saturated/unsaturated ratios, or fatty acid chain lengths. Comparisons of component fatty acids, particularly of the n-3 and n-6 fatty acids, are difficult to estimate for these experiments. Furthermore, feeding times have been relatively short and changes in the fatty acid compositions of the nervous system have not been characterized (31).

Safflower oil and perilla oil provide for good experimental comparisons because the proportions of saturated and monoenoic acids as well as the tocopherol compositions are similar, but there is a major difference in the proportions of linoleate (18:2n-6) and α-linolenate (18:3n-3). By using these two dietary oils, we have shown that feeding α-linolenate-deficient and -enriched diets to mothers and their offspring induces significant changes in the n-3/n-6 ratio of brain phospholipid acyl chains, and that these changes are accompanied by changes in performance on a brightness-discrimination learning task (food-reinforced) (10-12) and retinal functions in rats (9). Bourre et al. (14) have observed similar changes in retinal function and learning performance using a different combination of vegetable oils, although Leat et al. (32) did not observe such a change. In the present experiments, performance in a spatial learning task (Morris's water maze) was affected by n-3 deficiency (Fig. 2) as reported by Coscina et al. (31). Very recently, Wainwright et al. (33) reported that n-3 deficiency during gestation and lactation did not affect discrimination learning performance in a water maze test or visual acuity in mice. These results appear to be inconsistent with the present observations.

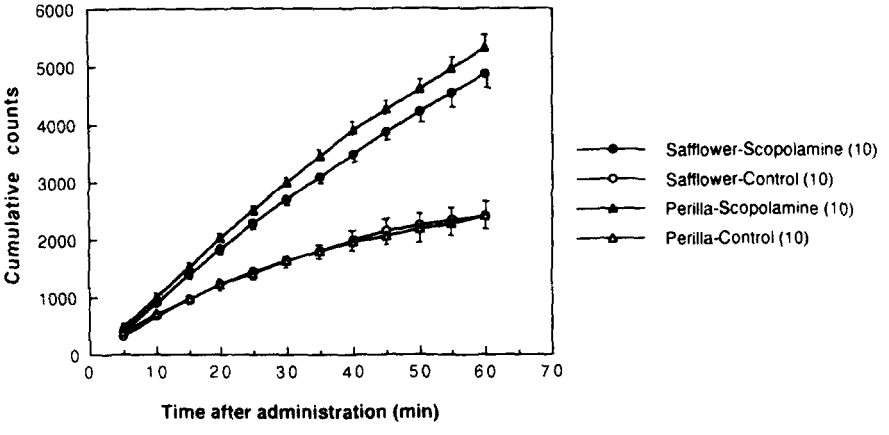

Fig. 5. Effect of dietary oils on scopolamine-induced hyperlocomotion in mice. Locomotor activity was measured with an Automex at 15-min intervals for 1 h after intraperitoneal injection of 2 mg/kg scopolamine or saline as a control. Each point represents the average of 10 male mice (± SEM). The difference between the two dietary groups treated with scopolamine was significant in a two-way ANOVA (dietary group vs. time). $F(1,216) = 28.67$, $P < 0.01$.

However, there is a critical difference in the dietary conditions used in these two studies; animals were weaned onto laboratory chow containing n-3 fatty acid in the experiments by Wainwright et al. (33), but in our experiments animals were tested while still undergoing dietary treatment. It should be noted that an n-3 deficiency-induced change in learning performance is a reversible process; supplementation with n-3 fatty acid after weaning reversed the inferior learning performance (Okuyama, H. 1992, Third International Congress on Essential Fatty Acids and Eicosanoids, Abstracts p. 146, Adelaide, Australia).

One of interesting questions related to these observations would be whether the inferior performance observed in the brightness-discrimination learning test, in the spatial learning test, or in a passive avoidance test is a secondary effect of decreased visual sensitivity or decreased pain threshold. One can define "learning per se" separately from other sensory and performance factors such as vision, sound, pain threshold, locomotor activity, emotionality, and so on. However, it is impractical experimentally to distinguish learning itself from sensory processes associated with learning. This is particularly true in the case of n-3 deficiency, because long term n-3 deficiency is known to induce changes in membrane lipid acyl constituents of all the cells of the body. The brain is an organ that integrates information from other sensory systems and makes decisions. Therefore, it may be impossible to define the effect of n-3 deficiency on learning per se when other performance factors are affected at the same time. In this context, it is noteworthy that a morphological difference has been found in a brain region of animals fed perilla oil and safflower oil (Yoshida, S., et al. 1992. Fifth Scientific Meeting of the Society for Research on Polyunsaturated Fatty Acids, Tokyo).

Animals having an n-3 deficiency were more sensitive to pentobarbital, but less sensitive to one dose of scopolamine. It is well known that pentobarbital and scopolamine produce their pharmacological effects through chloride channels in GABA receptor and through muscarinic cholinergic receptors, respectively (34). These results suggest that the function of the former may be potentiated, but that of the latter attenuated in the animals with an n-3 deficiency. Therefore, signal transduction systems involving these receptors may be affected by the dietary n-3/n-6 balance (35-38). Dysfunction of the cholinergic neuronal system produces decreased learning abilities in animals and humans (39, 40).

Aloia and Mlekusch (29) have reported that feeding animals hydrogenated coconut oil, which is characterized by a high proportion of medium chain fatty acids and a dual deficiency of n-3 and n-6 fatty acids (as compared with laboratory chow), caused a longer anesthesia duration induced by pentobarbital injection. Our results indicate that an n-3 deficiency in the presence of an excess of n-6 fatty acids is sufficient for the increased sensitivity to pentobarbital.

Some enzymes are reported to be affected by dietary n-3 and n-6 fatty acids (14, 41, 42), but we were unable to detect significant differences in 5'-nucleotidase, Na^+,K^+-ATPase, Ca^{2+}-ATPase, or cyclic nucleotide phosphodiesterase activities in the brains from the two dietary groups. Dietary oil-induced changes in cholesterol/phospholipid ratios and membrane fluidity have been reported (43, 44), but no significant differences in the cholesterol/phospholipid ratios of brains, in erythrocyte deformabilities (45), or in sensitivities to diethyl ether (which changes membrane fluidity (46)) were detected between the safflower and perilla groups. In contrast, the formation of certain cytokines (47) and lipid mediators (48, 49) has been shown to be affected significantly by dietary perilla and safflower oils. Thus, it seems likely that chemical mediator and neurotransmission systems are affected by dietary n-3 fatty acids. Changes in the rates of formation of these mediators in brain might affect behavior.

Although the precise mechanisms still remain to be clarified, it is now quite certain that the dietary n-3/n-6 balance affects behavioral patterns of mammals via changes in the fatty acid composition of the nervous system. This implies that the choice of foods affects behavioral patterns because different foods contain significantly different proportions of n-3 and n-6 fatty acids (1). Interestingly, the n-3/n-6 ratio of conventional diets (0.08 ~ 0.16) was not sufficient to elicit a maximum correct response ratio in a brightness-discrimination learning test (11), and a soybean oil-based diet containing 1.6 energy % as n-3 was not sufficient either for longevity (50) or for suppression of carcinogenesis and metastasis (51, 52) in rats. These findings raise the question as to whether diets in industrialized countries, which contain only about 1.5 energy % as n-3 fatty acid (53), meet the requirement for n-3 fatty acids or not. This issue is particularly important since symptoms of n-3 fatty acid deficiency have been reported in human beings (54, 55).

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