Effect of the dietary α-linolenate/linoleate balance on lipid compositions and learning ability of rats. II. Discrimination process, extinction process, and glycolipid compositions

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Abstract Donryu strain rats through two generations were fed semi-purified diets supplemented with safflower seed oil (rich in linoleic acid) or with perilla seed oil (rich in α-linolenic acid), or a conventional laboratory chow (normal control diet). Brightness-discrimination learning ability was determined to be the highest in the perilla oil-fed group, followed by the normal group, and then by the safflower group, extending our earlier observation in a different strain of rat that α-linolenic acid is a factor in maintaining high learning ability (Yamamoto, N., M. Saitoh, A. Moriuchi, M. Nomura, and H. Okuyama. 1987. *J. Lipid Res.* **28:** 144–151). After the brightness-discrimination learning test was administered, extinction of learning was measured. The time required for extinction was significantly longer in the safflower group than in either the perilla group or the normal diet group. Thus, the dietary α-linolenate/linoleate balance affected both the learning and the extinction of learning. The glycolipids of the cerebrum, cerebellum, and olfactory lobe were analyzed. Although the fatty acid compositions of the sulfatide and gangliosides were significantly different in the three parts of the brain, relatively little difference was observed in the fatty acids of glycolipids between the safflower group and the perilla group, suggesting that gross changes in brain glycolipids are not responsible for the differences in learning abilities between these dietary groups. — **Yamamoto, N., A. Hashimoto, Y. Takemoto, H. Okuyama, M. Nomura, R. Kitajima, T. Togashi, and Y. Tamai.** Effect of the dietary α-linolenate/linoleate balance on lipid compositions and learning ability of rats. II. Discrimination process, extinction process, and glycolipid compositions. *J. Lipid Res.* 1988. **29:** 1013–1021.

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Linoleic (18:2, n–6)[2] and α-linolenic (18:3, n–3) acids are synthesized in plants but not in animal tissues. When ingested by animals, linoleic acid and α-linolenic acid are converted to long-chain, highly unsaturated fatty acids. The essentiality of linoleic acid (n–6 series) was established by Burr and Burr (1), and can be ascribed to the fact that linoleate is converted to arachidonate which, in turn, is the major precursor of eicosanoids of the n–6 series. In contrast, rats can be raised, apparently normally, through three generations on diets containing linoleic acid but lacking α-linolenic acid (2). Prostaglandin synthesis from the n–3 series fatty acids has been recognized in some tissues (3–6), but the specific physiological activities of the n–3 derivatives tend to be lower than those of the n–6 derivatives. Therefore, according to the classical definition, α-linolenic acid is not an essential fatty acid (7, 8).

Docosahexaenoic acid (22:6, n–3) is known to be one of the major unsaturated fatty acids of phospholipids in brain, retina, and muscle. Several investigators have reported that α-linolenate, the precursor of docosahexaenoate, is essential for the functions of these tissues. Behavioral problems were described in monkeys fed diets lacking α-linolenate (9). In addition, defects in retinal function in rats and monkeys subjected to α-linolenate deficiency have been noted by several groups (10–14). As to the effects of n–3 fatty acid deficiencies on learning ability, Lamptey and Walker (15) were the first to describe an impaired learning ability of rats in a simple Y-maze test. However, Bowman and Davenport (16), in repeating and extending the work of Lamptey and Walker, found no

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[2] Fatty acids are abbreviated by the carbon chain length: the number of double bonds. The position of the first double bond numbered from the methyl terminus is designated as n–6 or n–3; h denotes hydroxy fatty acids.

difference between α-linolenate-fed and -deficient rats in the X-maze test as reviewed in ref. 8.

Previously, we used a brightness-discrimination learning test that appeared to allow more objective estimate of learning ability in rats (17, 18). The groups fed an α-linolenate-deficient diet through two generations were found to be inferior in learning ability to rats fed a diet enriched with α-linolenate (19).

In view of the potential implications of these studies on human nutrition, we have extended our earlier experiments to a different strain of rat (Donryu). A normal-diet control group was added in the evaluation of the learning process, and we also evaluated the process of extinction of learning. Furthermore, glycolipid compositions of brains from different dietary groups were analyzed since glycolipid is another important lipid component in brain and various glycolipids appeared to be involved in such physiologically important processes as nerve growth, differentiation, and cell recognition (20–22). Glycolipid compositions of rat whole brain have been analyzed (23–26), but only a few groups reported the glycolipid compositions of the different portions of the brain (27, 28). Here, we analyzed the acyl chain compositions of glycolipids from the cerebrum, cerebellum, and olfactory lobe by using an improved gas–liquid chromatographic method (29, 30).

MATERIALS AND METHODS

The compositions of conventional laboratory chow (normal diet) and semipurified diets supplemented with either safflower seed oil or perilla seed oil were described previously (19). The major fatty acids of the safflower diet were 11% saturated, 10% monoenoic, 78% linoleic, and 0.05% α-linolenic acids; those of the perilla diet were 11% saturated, 12% monoenoic, 13% linoleic, and 64% α-linolenic acids; and those of the normal diet were 19% saturated, 25% monoenoic, 49% linoleic, and 4% α-linolenic acids.

Donryu rats at 4 weeks of age (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were fed the test diets through two generations and the learning tests were started at 11 weeks of age. Weight gain, litter size, and gross physical appearance were not significantly different among the three dietary groups. In the perilla group, for which the supply of linoleic acid was relatively limited, no essential fatty acid deficiency symptoms as described by Burr and Burr (1) were observed.

Conditions for the brightness-discrimination learning test were described previously (19). Briefly, rats (12 rats in each group) were conditioned to depress a lever to obtain pellets. When a brighter light was given on the screen as a positive stimulus (S^+), the lever-pressing responses were reinforced. When a darker light (S^-) was present, no pellet was given in response to pressing the lever. Each stimulus was given for 20 sec with a Gellerman sequence (31). One session consisted of 20 times each of S^+ and S^-, and the session was performed every day. Correct response ratio, $R^+/(R^+ + R^-) \times 100$, was calculated from the number of correct responses (R^+) during S^+ presentation and that of incorrect responses (R^-) during S^- presentation in a session (32).

Evaluation of the extinction process

After 30 sessions of the brightness-discrimination learning test, new conditions were set; no pellet was given in response when the rat pressed the lever even when the brighter light was present on the screen. This extinction process was continued until the total number of responses decreased to below 20/session. Statistical analyses were performed using Student's *t*-test and a three-way analysis of variance (ANOVA).

Glycolipid compositions

Glycolipid compositions were analyzed essentially as described elsewhere (25). Briefly, lipids extracted with chloroform–methanol from the left hemispheres of brains (cerebrum, cerebellum, and olfactory lobe) were passed through a silicic acid column (Iatrobeads, 6RS 8060). The glycolipid fraction containing ceramide monohexoside (cerebroside) and cerebroside sulfate ester (sulfatide) was eluted with chloroform–methanol–28% NH_4OH 32:8:1, while the ganglioside fraction was eluted with chloroform–methanol–water 5:5:1. Cerebroside, sulfatide, and ganglioside were further purified by preparative thin-layer chromatography (TLC). In some cases crude glycolipid fractions were subjected to mild alkaline hydrolysis to remove contaminating glycerophospholipids. The purities of the isolated glycolipids were ascertained by TLC. Cerebroside and sulfatide were quantitated by densitometric scanning at 540/700 nm after first spraying the thin-layer plates with 2% orcinol in 2 N sulfuric acid and heating at 100°C (29). Gangliosides were quantitated with resorcinol reagent (33). To determine the amounts of tissue ganglioside from the estimated amounts of N-acetylneuraminic acid, a value of 1586 was used as the average molecular weight of gangliosides, and N-acetylneuraminic acid content was assumed to be present at an average level of 1.7 mol/mol ganglioside.

To determine the fatty acid compositions of glycolipids, the purified glycolipids were hydrolyzed with 5% hydrochloric acid in methanol for 3 hr at 100°C, and the total fatty acid methyl esters containing hydroxy and non-hydroxy components were analyzed on a gas chromatograph (Hewlett-Packard 5890A) equipped with a cool-on column mode of injection and fused-silica capillary column (0.32 mm × 30 m) coated with 5% phenylmethyl silicone. The column temperature was programmed from

50° to 200°C at a rate of 20°C and then raised to 300°C at a rate of 10°C. The detector temperature was 310°C. The chromatograms were recorded and the percentage composition of individual peaks was calculated with a Chromatopack C-R1A (Shimadzu).

RESULTS

The responses in the brightness-discrimination learning process are shown in **Fig. 1.** The total response ($R^+ + R^-$) tended to be lower in the perilla group than in the safflower group. The correct response (R^+) throughout the sessions increased similarly in all the dietary groups. In contrast, the R^- response increased similarly during the initial 10 sessions in all dietary groups, but thereafter the R^- response of the perilla group decreased rapidly whereas that of the safflower group remained relatively constant; the R^- of the normal diet group was intermediate between the perilla and safflower groups ($P < 0.05$ for the safflower group vs. the perilla group, $P < 0.05$ for the normal diet group vs. the perilla group in three-way ANOVA). Thus, the differences in the total responses of the three dietary groups are due mainly to the R^- responses.

The correct response ratios of the three dietary groups are shown in **Fig. 2.** In the first several days, the correct response ratios increased similarly in the three groups. In the perilla group, the ratio increased rapidly after about 10 sessions. The ratio of the normal diet group followed that of the perilla group and reached the same level at the end of the sessions. In contrast, the ratios of the safflower group were significantly lower than the others after about 10 sessions. The differences in the correct response ratios throughout the 30 sessions were statistically significant at $P < 0.05$ in three-way ANOVA only for the safflower group versus the perilla group; although in a two-way ANOVA, all three dietary groups were different from one another at $P < 0.01$. In the 11th to 20th sessions, the difference between the safflower group and the perilla group was significant at $P < 0.05$, while in the 21st to 30th sessions, the differences in the safflower group versus the perilla group and the safflower group versus the nor-

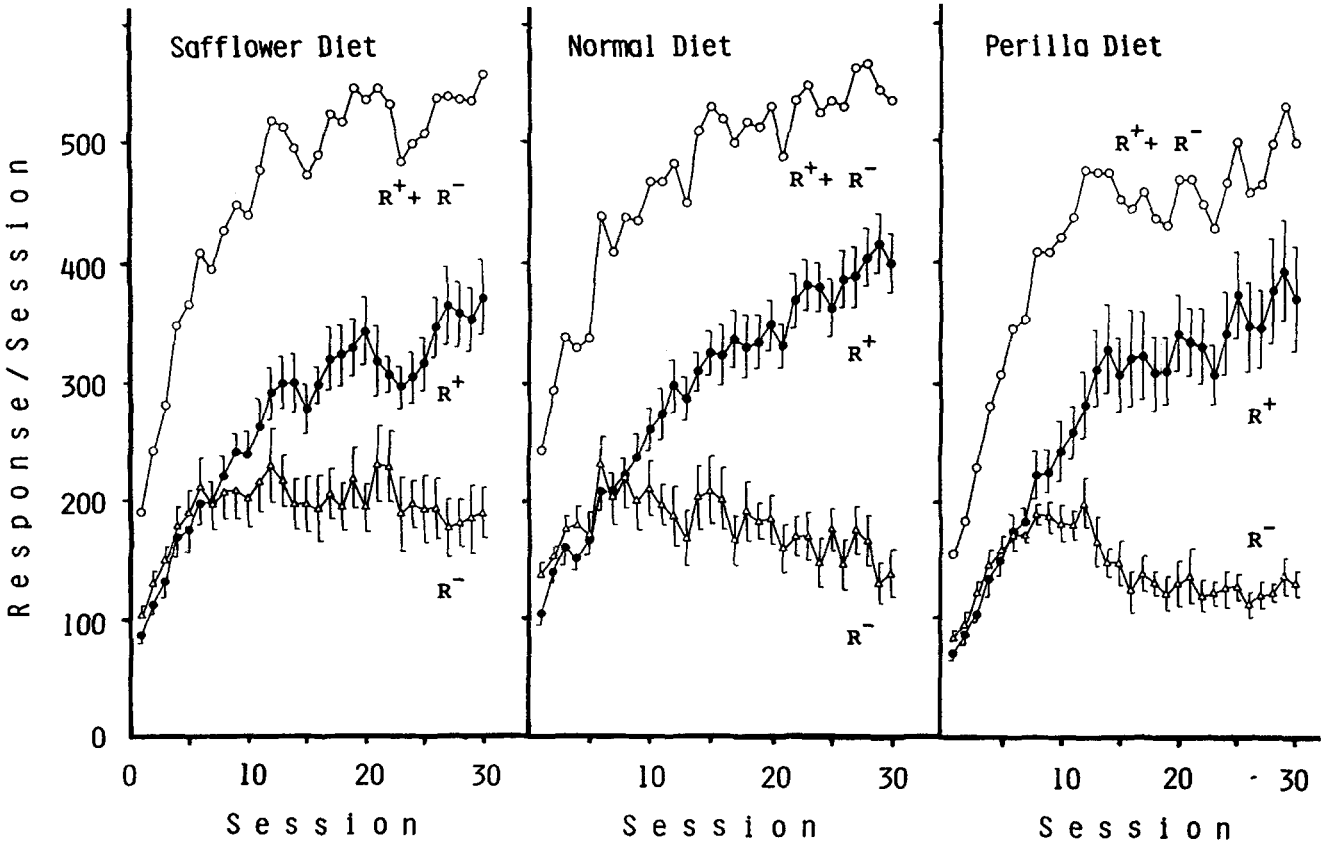

Fig. 1. Brightness-discrimination learning test in Donryu rats fed the safflower oil diet, the perilla oil diet, or the normal diet. Rats fed the test diets through two generations were first trained at 11 weeks of age to be reinforced to receive pellets upon pressing a lever. Then the brightness-discrimination learning test was begun. R^+ indicates the number of lever-press responses under the brighter light (S^+), and R^- represents the number of responses for the darker light (S^-). Values are the means of 12 rats (± SEM). Statistically significant differences at $P < 0.05$ in a three-way analysis of variance (ANOVA) were observed in the R^- responses of the safflower oil group versus the perilla oil group, and the safflower oil group versus the normal diet group; (●) R^+; (○) $R^+ + R^-$; (△) R^-.

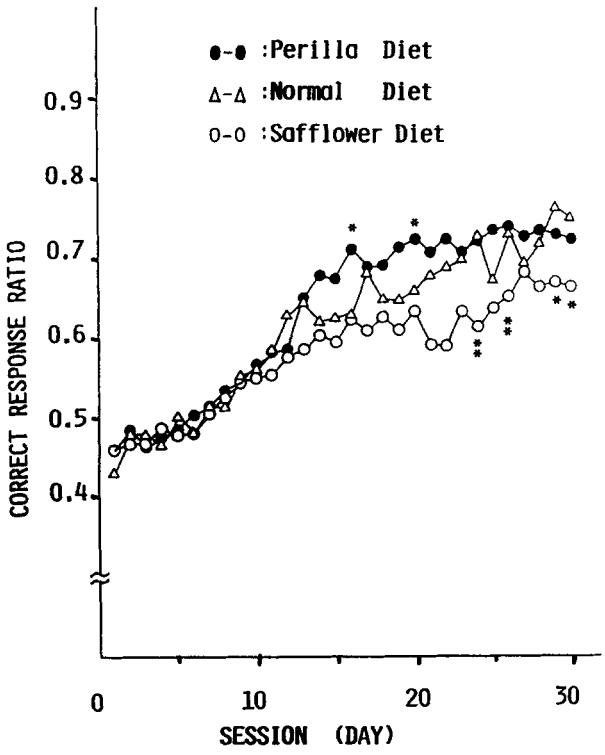

Fig. 2. The correct response ratio in the brightness-discrimination learning test. The correct response ratio, $R^+/(R^+ + R^-) \times 100$ was calculated from the data presented in Fig. 1. Statistical significance in Student's *t*-test is expressed as $P < 0.01$ (**) or $P < 0.05$ (*) only for the normal diet group versus the perilla group and the normal diet group versus the safflower group; statistical significance for the perilla group versus the safflower group is not shown, but was similar in extent to that reported previously (18). Statistical analyses between the perilla group and the normal diet group, as well as between the safflower group and the normal diet group, were carried out by two-way analysis of variance (ANOVA) or a three-way ANOVA. The differences among the three groups were statistically significant ($P < 0.01$) in two-way ANOVA. In three-way ANOVA, significant differences at $P < 0.05$ were observed for the safflower group versus the perilla group (1st session to 30th session), the safflower group versus the perilla group (11th to 20th session), the safflower group versus the perilla group (21st to 30th session), and the safflower group versus the normal diet group (21st to 30th session).

mal diet group were statistically significant at $P < 0.05$ in three-way ANOVA.

After 30 sessions of the brightness-discrimination learning test, the process of extinction of learning was followed (**Fig. 3**). Because no pellets were given when the lever was pressed, the total number of lever-pressing responses decreased rapidly. When the exponentials of the total responses were plotted versus time, straight lines were obtained (Fig. 3). The apparent differences in the slopes of three dietary groups were not statistically significant. When the total response per session decreased below 20, we judged that the rat had completed the extinction of learning process.

In **Fig. 4**, the number of rats remaining in the trials (i.e., >20 responses) is plotted versus the time from the beginning of the extinction of learning test. In the perilla group and the normal diet group, all rats completed the process by the 12th day of the test, while it took 20 days for all the rats in the safflower group to complete the extinction of learning process. The average number of days required for the completion of the extinction process was 7.7 ± 2.0 days in the perilla group, 8.2 ± 2.9 days in the normal diet group, and 10.8 ± 3.9 days in the saffllower group. The difference between the safflower group and the other two groups was statistically significant ($P < 0.05$).

The glycolipid compositions of the three major parts of the brain were analyzed (**Table 1**). Cerebroside was the major component, followed by sulfatide and gangliosides in the cerebrum, cerebellum, and olfactory lobe. Although the relative amounts of the three glycolipids were significantly different among the three parts of the brain, no differences were observed between the safflower group and the perilla group.

Fatty acid compositions of glycolipids from the cerebrum, the cerebellum, and the olfactory lobe are shown in **Table 2, Table 3,** and **Table 4,** respectively.

The component fatty acids were resolved completely by use of a capillary column. Fatty acid compositions of glycolipids from the safflower and perilla diet groups were relatively similar, in general, as compared with the phospholipid acyl chains (19), but some minor differences were observed: *a*) 18:1 was relatively less abundant and 22:1 was

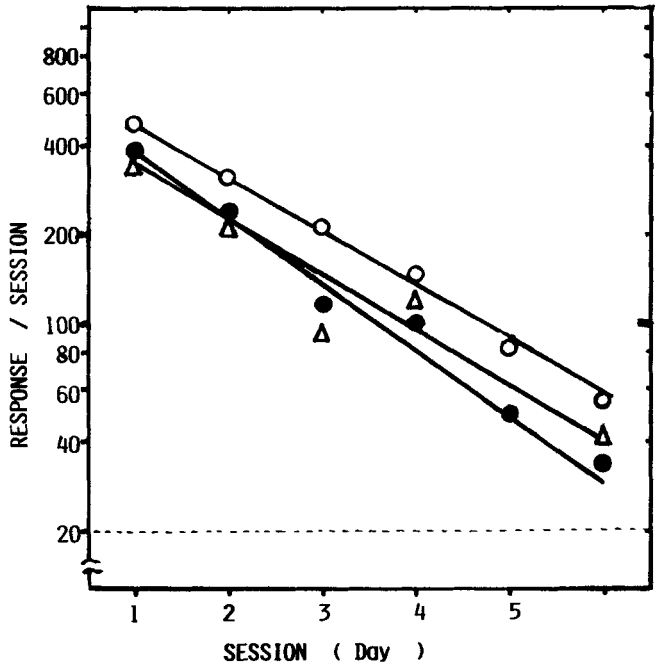

Fig. 3. Measurement of extinction of learning. After 30 sessions of the brightness-discrimination learning test, new conditions were set; no pellet was given in response to lever-pressing even with the brighter light on the screen. Exponentials of the total responses were plotted versus time. The differences in the slopes of three dietary groups were not statistically significant ($P > 0.05$). The safflower group (○), the perilla group (●), and the normal diet group (△) were examined.

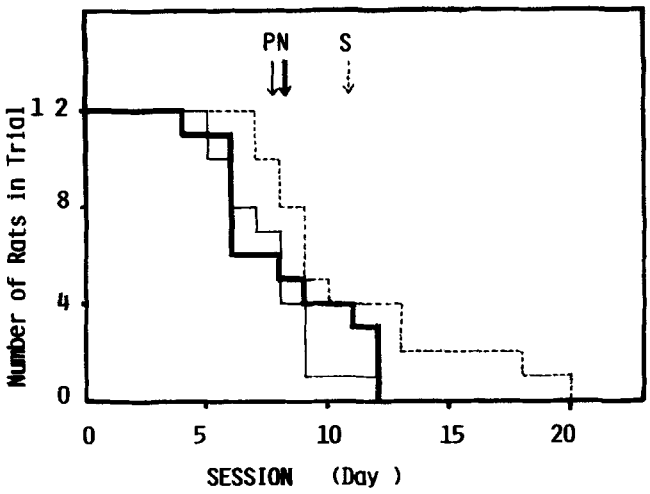

Fig. 4. The average time required for the completion of the extinction process. When the total number of responses decreased to below 20/session for any rat, we judged that the rat had completed the extinction process and it was thus excluded from the trials. Arrows indicate the average number of days required for the completion of the extinction of learning. The average time required for the completion was significantly ($P < 0.05$) longer in the safflower group (S) than in the perilla group (P) and the normal diet group (N).

relatively abundant in cerebral sulfatide from the safflower group; *b*) 16:0, 16:1, and 18:1 were less abundant but 18:0 was more abundant in cerebellar ganglioside from the safflower group; and *c*) more 24:1 was found in cerebroside and sulfatide of the olfactory lobe from the safflower group than in the perilla group.

The fatty acid compositions of glycolipids were significantly different among the three parts of brain. The cerebral sulfatides contained more 16:0, 18:1, and 22:1 but less 24:0 and 24:1 than cerebellar sulfatides, and the fatty acids of olfactory lobe were significantly different from those of the other two brain regions. In fact, 22:1 was essentially absent in cerebellar and olfactory sulfatides but was present in significant amounts in the cerebral sulfatides. The fatty acid compositions of gangliosides were also significantly different among the three parts of the brain; very little or no 16:1, 20:1, and 22:1 were found in the cerebral gangliosides and the total amounts of monoenoic fatty acids were much lower in the cerebrum (1.7–1.9%) than in either the cerebellum (7.7–16.9%) or olfactory lobe (10,5–15.6%) gangliosides. The average chain lengths of saturated fatty acids in the gangliosides were also significantly different; 18.04–18.25, 18.02–18.10, and 17.84–17.93 in the cerebral, cerebellar, and olfactory lobe gangliosides, respectively.

In summary, the glycolipid compositions and glycolipid acyl chain compositions were significantly different among the three parts of the brain, but the differences in these compositions between the two dietary groups (safflower and perilla) were relatively minor.

DISCUSSION

Behavioral tests are generally accompanied by a relatively high degree of uncertainty. However, the effects of dietary α-linolenate/linoleate balance on the brightness-discrimination learning test were quite reproducible in three strains of rats. The learning ability was superior in the perilla group followed by the normal diet group and then by the safflower group. Thus, we conclude that α-linolenic acid is essential for maintaining high learning ability in rats or perhaps high levels of n-6 fatty acids impair learning. It took a longer time to complete the extinc-

TABLE 1. Glycolipid compositions of brains of rats fed the safflower oil diet or the perilla oil diet

Composition	Glycolipid Content	
	Safflower Diet	Perilla Diet
	mg/g wet weight	
Cerebrum		
Cerebroside	13.1 ± 0.4 (n = 3 × 3)	13.5 ± 0.8 (n = 3 × 3)
Sulfatide	3.8 ± 0.4 (n = 3 × 3)	3.7 ± 0.4 (n = 3 × 3)
Ganglioside	1.28 ± 0.02 (n = 2 × 3)	1.30 ± 0.01 (n = 2 × 3)
Cerebellum		
Cerebroside	16.9 ± 0.5 (n = 3 × 3)	16.7 ± 1.2 (n = 3 × 3)
Sulfatide	4.3 ± 0.7 (n = 3 × 3)	4.3 ± 0.4 (n = 3 × 3)
Ganglioside	0.51 ± 0.02 (n = 2 × 2)	0.60 ± 0.01 (n = 2 × 2)
Olfactory lobe		
Cerebroside	4.9 ± 0.4 (n = 1 × 3)	4.5 ± 0.3 (n = 1 × 3)
Sulfatide	1.2 ± 0.1 (n = 1 × 3)	1.1 ± 0.1 (n = 1 × 3)
Ganglioside	0.58 (n = 1 × 1)	0.55 (n = 1 × 1)

The left hemispheres of the brains were obtained from Wistar/Kyoto rats fed the safflower diet or the perilla diet through two generations. Glycolipid compositions were determined as described in the text. The number of assays (n) in the cerebrum and cerebellum is expressed as the number of samples from different rats × the number of determinations; in the case of olfactory lobe, three rat brains were combined. Averages ± SD are presented.

TABLE 2. Fatty acid composition of cerebral glycolipids

Fatty Acid	Ceramide Monohexoside		Sulfatide		Ganglioside	
	Safflower (n = 2 × 3)[a]	Perilla (n = 2 × 3)	Safflower (n = 2 × 3)	Perilla (n = 2 × 3)	Safflower (n = 3 × 3)	Perilla (n = 3 × 3)
16:0	0.5 ± 0.0	0.7 ± 0.1	2.5 ± 0.1*	3.0 ± 0.1	1.8 ± 0.5*	1.5 ± 0.0
16:1	–	–	–	–	–*	–
18:0	1.4 ± 0.1	1.4 ± 0.0	4.0 ± 0.4	3.0 ± 0.2	86.7 ± 0.5	86.7 ± 0.9
18:1	0.5 ± 0.0	0.6 ± 0.0	2.4 ± 0.2↑*	5.4 ± 2.8	1.5 ± 0.8*	1.3 ± 0.6
20:0	1.7 ± 0.1	1.7 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	7.2 ± 0.5*	7.6 ± 0.4
20:1	–	–	1.1 ± 0.1	0.9 ± 0.2	–*	–
22:0	2.1 ± 0.1	2.1 ± 0.1	3.8 ± 0.2	3.2 ± 0.4	0.9 ± 0.1	0.9 ± 0.0
22:1	–	–	6.5 ± 0.2↑*	3.7 ± 2.9	–*	–
23:0	1.4 ± 0.0	1.4 ± 0.1	1.4 ± 0.0	1.4 ± 0.0	1.2 ± 0.1	1.1 ± 0.0
23:1	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	–	–
24:0	7.3 ± 0.9	6.8 ± 0.2	12.6 ± 0.5*	12.8 ± 0.1	0.5 ± 0.0	0.5 ± 0.0
24:1	10.7 ± 0.1	9.9 ± 0.4	15.4 ± 0.1*	15.1 ± 0.2	0.4 ± 0.2*	0.4 ± 0.1
25:0	tr.	0.3 ± 0.2	1.0 ± 0.1	1.2 ± 0.1	–	–
25:1	0.8 ± 0.0	0.8 ± 0.0	1.0 ± 0.0	1.1 ± 0.0	–	–
26:0	0.5 ± 0.0	0.4 ± 0.0	1.0 ± 0.1	1.3 ± 0.2	–	–
26:1	1.2 ± 0.0	1.1 ± 0.1	0.5 ± 0.2	2.0 ± 0.5	–	–
20h:0	1.0 ± 0.1	1.1 ± 0.1	0.5 ± 0.0	0.5 ± 0.1	–	–
20h:1	–	–	–	–	–	–
22h:0	11.1 ± 0.5	11.0 ± 0.1	5.7 ± 0.1	5.5 ± 0.2	–	–
22h:1	tr.	0.5 ± 0.1	0.3 ± 0.0	0.2 ± 0.1	–	–
23h:0	10.4 ± 0.2	10.4 ± 0.1	6.0 ± 0.4	5.9 ± 0.1	–	–
24h:0	31.1 ± 0.2	32.1 ± 0.8	21.4 ± 0.3	21.1 ± 0.5	–	–
24h:1	15.1 ± 0.3	14.7 ± 0.8	8.5 ± 0.2	8.0 ± 0.1	–	–
25h:1	2.4 ± 0.1	2.3 ± 0.0	1.8 ± 0.1	2.2 ± 0.1	–	–
26h:1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.4 ± 0.1	–	–
NOH/OH[b]	28.2/71.9	27.1/72.9	54.8/45.2	55.3/44.7	–	–

Glycolipids were extracted and purified as described in the text. Fatty acids were analyzed as methyl esters by fused-silica capillary gas chromatography. Fatty acids are abbreviated as the number of carbon chains:the number of double bonds, and h denotes hydroxy fatty acid. The data are presented as the percent of total fatty acids analyzed. When the safflower group and the perilla group were compared, arrows (↑) were used to indicate statistical significance ($P < 0.05$) while the asterisk (*) was used to indicate statistically significant difference ($P < 0.05$) between the cerebral glycolipids (this table) and cerebellar glycolipids (Table 3).

[a] The number of assays (n) was expressed as the number of samples from different rats × the number of determinations, and the SD values were calculated based on the number of rats.

[b] NOH/OH indicates the nonhydroxy fatty acid/hydroxy fatty acid ratio.

tion process in the safflower group than in the normal diet group and the perilla group (Fig. 4), but the total responses at day 0 of the extinction process were not the same among the three dietary groups, although these differences were not statistically significant. Therefore, it is possible that slight differences in the total responses at day 0 and slight differences in the slopes of the extinction process (Fig. 3) produced statistically significant differences in the times required to complete the extinction process (Fig. 4). Whether or not similar effects can be expected in humans remains to be examined. A case report of an α-linolenic acid-deficiency in a human subject (34) described symptoms suggestive of nerve dysfunctions. Behavioral problems were also noted in monkeys deficient in α-linolenic acid (9).

Since the brightness discrimination was used in evaluating the learning ability, and since we (14) and others (10–13) noted defective retinal functions in animals deficient in α-linolenic acid, it is possible that the apparently inferior learning ability is due to impaired vision in these animals. However, we suspect that differences in learning abilities observed in our studies are not simply due to differences in retinal functions among the dietary groups because brightness discrimination is not involved in the process of the extinction of learning, and even the darker light used in the learning test is enough to evoke a significant electroretinographic response as shown in **Fig. 5** (14). Statistically significant differences in electroretinograms of the two dietary groups were seen only at light intensities above the darker light used in the present experiments, which might be related to the nocturnal behavior of rats.

The cerebroside and sulfatide contents of the cerebrum as determined in this study (Table 1) were in good agreement with the published values (23–25, 35), but the ganglioside content was slightly lower than the value reported by Wells and Dittmer (23). The cerebroside and sulfatide contents in the olfactory lobe were only one-third the contents in the cerebrum and cerebellum while the ganglioside contents in the cerebellum and olfactory lobe were one-

TABLE 3. Fatty acid compositions of cerebellum glycolipids

Fatty Acid	Ceramide Monohexoside		Sulfatide		Ganglioside	
	Safflower (n = 2 × 3)	Perilla (n = 1 × 3)	Safflower (n = 2 × 3)	Perilla (n = 1 × 3)	Safflower (n = 3 × 3)	Perilla (n = 2 × 3)
16:0	0.8 ± 0.5	0.2	1.8 ± 1.2	1.2	4.6 ± 0.4↓	8.8 ± 1.0
16:1	–	–	–	–	0.8 ± 0.1↓	2.5 ± 1.1
18:0	1.8 ± 0.2	1.5	3.3 ± 1.3	2.3	81.6 ± 0.4↑	68.9 ± 1.3
18:1	0.6 ± 0.0	0.1	2.1 ± 1.9	0.8	4.1 ± 0.4↓	10.6 ± 2.7
20:0	2.3 ± 0.1	2.2	2.2 ± 0.2	1.8	4.3 ± 0.3	3.4 ± 0.5
20:1	–	–	0.7 ± 0.1	tr.	1.4 ± 0.3	2.0 ± 0.8
22:0	2.3 ± 0.2	2.3	3.8 ± 0.4	3.8	0.8 ± 0.0	0.8 ± 0.1
22:1	–	–	–	–	0.8 ± 0.0	0.8 ± 0.2
23:0	1.2 ± 0.1	1.3	1.7 ± 0.2	1.7	0.6 ± 0.1	0.6 ± 0.0
23:1	0.2 ± 0.0	0.2	0.4 ± 0.1	tr.	–	–
24:0	5.8 ± 0.3	6.1	15.2 ± 0.2	17.4	0.6 ± 0.0	1.1 ± 0.0
24:1	9.1 ± 0.6	10.7	20.4 ± 2.6	22.0	0.6 ± 0.1	1.0 ± 0.3
25:0	0.2 ± 0.1	tr.	1.1 ± 0.1	1.2	–	–
25:1	0.5 ± 0.0	0.5	1.2 ± 0.2	1.3	–	–
26:0	0.3 ± 0.0	0.4	1.1 ± 0.1	1.3	–	–
26:1	0.7 ± 0.1	0.8	2.0 ± 0.1	1.7	–	–
20h:0	1.6 ± 0.1	1.2	0.6 ± 0.2	0.5	–	–
20h:1	0.1 ± 0.0	0.1	–	–	–	–
22h:0	13.1 ± 0.6	12.5	5.8 ± 0.3	5.1	–	–
22h:1	0.5 ± 0.1	0.5	0.3 ± 0.0	0.3	–	–
23h:0	10.4 ± 0.1	10.4	5.3 ± 0.4	5.7	–	–
24h:0	32.6 ± 1.1	30.3	21.4 ± 0.1	20.3	–	–
24h:1	13.5 ± 1.4	15.2	7.6 ± 1.0	8.3	–	–
25h:1	2.3 ± 0.1	2.4	2.1 ± 0.5	2.2	–	–
26h:1	1.1 ± 0.0	1.1	1.1 ± 0.2	1.2	–	–
NOH/OH	25.1/74.9	26.3/73.7	56.1/43.9	56.4/43.6	–	–

See legend to Table 2.

TABLE 4. Fatty acid compositions of olfactory lobe glycolipids

Fatty Acid	Ceramide Monohexoside		Sulfatide		Ganglioside	
	Safflower (n = 1 × 3)	Perilla (n = 1 × 3)	Safflower (n = 1 × 3)	Perilla (n = 1 × 3)	Safflower (n = 1 × 3)	Perilla (n = 1 × 3)
16:0	0.5	0.7	1.2	1.4	12.8	14.8
16:1	–	–	–	–	2.8	1.7
18:0	2.3	2.3	3.3	4.7	65.2	67.9
18:1	0.1	0.3	0.5	0.7	10.3	7.3
20:0	2.1	1.5	2.2	2.6	4.2	5.8
20:1	–	–	–	–	1.5	1.5
22:0	2.7	2.2	4.1	4.6	0.8	0.7
22:1	–	–	–	–	0.5	tr.
23:0	1.4	1.1	1.9	2.0	0.7	tr.
23:1	0.2	tr.	0.4	tr.	–	–
24:0	6.2	5.4	12.3	13.2	0.8	0.2
24:1	10.3	7.0	17.6	12.9	0.5	tr.
25:0	–	–	0.7	1.3	–	–
25:1	0.8	1.1	1.2	1.1	–	–
26:0	0.6	0.8	1.0	1.0	–	–
26:1	1.5	2.1	1.5	1.8	–	–
20h:0	1.2	1.3	0.8	0.5	–	–
20h:1	–	–	–	–	–	–
22h:0	13.4	15.1	8.3	8.1	–	–
22h:1	0.8	0.7	0.2	0.1	–	–
23h:0	10.3	11.6	6.5	6.9	–	–
24h:0	26.6	29.1	23.7	25.3	–	–
24h:1	14.4	12.5	9.4	7.0	–	–
25h:1	3.2	3.5	2.3	3.3	–	–
26h:1	1.2	1.4	1.0	1.8	–	–
NOH/OH	28.9/71.1	24.7/75.3	47.8/52.2	47.1/52.9	–	–

See legend to Table 2.

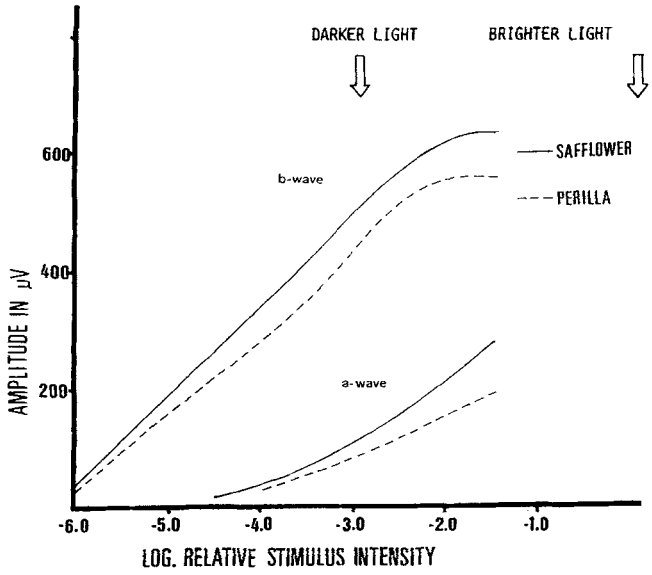

Fig. 5. Relationship between the light intensities used in the learning test and the electroretinographic responses. The intensities of brighter and darker lights used in the brightness-discrimination learning tests (shown by arrows) were compared with the amplitudes of a- and b-waves of electroretinograms determined earlier (14). Learning abilities of rats (SHR, WKY, and Donryu strains) were tested at 11–14 weeks of age and electroretinographic responses were measured at 13 weeks of age (WKY).

half the content in the cerebrum. The fatty acid compositions of the individual glycolipids in the cerebellum and the olfactory lobe of rat brain have not been reported so far. The contents and the compositions of glycolipids as well as the compositions of the acyl chains of the major glycolipids in brain were relatively similar in the perilla and safflower groups. This observation was not especially surprising since glycolipids contain almost no highly unsaturated fatty acids. A slight difference noted in the fatty acids of cerebellum glycolipids between the safflower group and the perilla group may be related to higher glycolipid synthesis in the cerebellum than in the whole brain (30). As shown previously (19), the contents and compositions of phospholipids as well as the ratios of saturated/unsaturated fatty acyl groups of the individual phospholipids were also very similar. So far, substantial biochemical differences were noted only in the proportions of highly unsaturated n-3 fatty acids and n-6 fatty acids as expected from the essential fatty acid balance of the diets. The biochemical mechanisms underlying the causal relationship between the essential fatty acid balance and learning ability remain to be elucidated.

Besides the essentiality of α-linolenic acid in learning ability and retinal functions, we noted that raising the ratio of α-linolenate (18:3 n-3)/linoleate (18:2 n-6) balance in the diet is beneficial in lowering systolic blood pressure of spontaneously hypertensive rats (36) and also decreasing the number of metastatic foci on pulmonary surfaces (37). Recently, a few research groups noted α-linolenate deficiency symptoms (38–40). Beneficial effects of raising the eicosapentaenoic acid (20:5 n-3)/arachidonic acid (20:4 n-6) ratios of diets for subjects with thrombotic diseases and inflammatory diseases have also been reported by many investigators as summarized by Lands (41). Thus, the n-3/n-6 ratio of the diets appears to have a number of significant effects. Different groups of human beings are reported to be eating foods having a wide range of average n-3/n-6 ratios (42–44). It is clearly important to begin determining the optimal essential fatty acid balance or the optimal n-3/n-6 ratio of human diets.

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