Pathophysiological effects of dietary essential fatty acid balance on neural systems

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1. Abstract

Dietary fatty acid balance has been revealed to affect neural functions as well as chronic diseases such as cancer, cerebro- and cardiovascular diseases, and allergic hyper-reactivity. In this review, we focused on the pathophysiological effects of n-6 and n-3 fatty acids on brain functions. Long-term n-3 fatty acid deficiency in the presence of n-6 fatty acids has been shown to affect learning behavior, drug sensitivity, and retinal functions. Some membrane enzymes and ion channel functions have been shown in experimental animals to be regulated by membrane fatty acid modifications. We also summarized the effects of these fatty acids in diets on human psychotic aspects and brain diseases. Although biochemical mechanisms remain to be elucidated, investigations on the effect of dietary fatty acids on neural networks may provide an important clue to clarify complex brain functions.

Running Title: Dietary Fatty acids and Neural Systems

Key words: arachidonic acid, docosahexaenoic acid, learning behavior, memory, psychoses.

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Abbreviations

Fatty acids were designated by the carbon chain length:the number of double bonds, and the position of the first double bond numbered from the methyl terminus as n-9, n-7, n-6 or n-3.

DHA, docosahexaenoic acid (22:6n-3); EPA, eicosapentaenoic acid (20:5n-3);

AA, arachidonic acid (20:4n-6); PKC, protein kinase C; CoA, Coenzyme A

I. Introduction

- Modification of membrane fatty acids and biochemical parameters-

Major dietary fatty acids can be classified into three series depending on their metabolism in mammals: the saturated and monounsaturated fatty acid series, the linoleic acid (18:2n-6) series and the α-linolenic acid (18:3n-3) series (Fig.1).

Fig.1

No interconversion occurs in mammals among these three series. Linoleic and α-linolenic acids are synthesized in plants but not in mammals. However, these fatty acids are desaturated and elongated in mammals to form very-long-chain polyunsaturated fatty acids as shown in Fig.1. In brain and retina, the major polyunsaturated fatty acids are arachidonic acid (20:4n-6, AA) and docosahexaenoic acid (22:6n-3, DHA). When the supply of n-3 fatty acids is limited, e.g., α-linolenic acid deficiency, the DHA content decreases but the decrease is compensated for by the increase in docosapentaenoic acid (22:5n-6) derived from arachidonic acid(n-6), and the total

amounts of 22 carbon polyunsaturated fatty acids are kept relatively constant in these tissues (Fig.2).

Fig.2

Dietary n-6 and n-3 fatty acid balance has been revealed to affect neural functions as well as chronic diseases such as cancer, cerebro- and cardiovascular diseases, and allergic hyper-reactivity. In this review, we focused on pathophysiological effects of dietary fatty acids, particularly n-6 and n-3 fatty acids, on brain functions. Before describing the details, we first summarize lines of evidence for the presence of a new biosynthetic pathway for very-long-chain polyunsaturated fatty acids and outlines of signal transduction in brain cells.

DHA is synthesized mainly in and transported from liver to brain (1, 2) , although human brain (brain vascular endothelial cells and astrocytes) has a capability to synthesize DHA from α-linolenic acid. In the synthesis of DHA from eicosapentaenoic acid (20:5n-3, EPA), the steps of elongation (20:5 -> 22:5 -> 24:5), desaturation (24:5 -> 24:6; delta-6) and subsequent β-oxidation (in peroxisomes) (24:6->22:6) are involved rather than the previously presumed "delta-4 desaturase pathway (22:5 -> 22:6)" (3-5). This newly revealed pathway of DHA biosynthesis is in accordance with the observation that Zellweger syndrome patients have significantly decreased DHA contents in the brain in which peroxisomes are defective (6, 7). Delta-6 desaturase is reported to have two distinct forms, one acting on 18:3(n-3) (to 18:4) and the other on 24:5 (to 24:6) (8).

The condensing enzyme, which is the first and rate-limiting enzyme in the elongation system, may have several distinct forms with different substrate specificities

(polyunsaturated, monounsaturated, and saturated fatty acyl-Coenzyme As(-CoAs)) (9-14). Moreover, in the elongation of AA, the subsequent elongation to 22:4 and 24:4, was observed through channeling without release of the presumed intermediate to outside of the enzyme or membranes, and several distinct condensing and reductase enzyme systems are involved (9-14). Consistently, Luthria and Sprecher (15) have shown in hepatic microsomal system that metabolite channeling also occurs in the elongation of 20:5(n-3) to 24:5 via the synthesis of 22:5. The competitive regulation by n-3 fatty acids of n-6 fatty acid metabolism or vice versa is an important aspect in understanding dietary fatty acid effects on complex behavioral patterns of animals. For example, increased DHA intake enhanced the accumulation of free AA (16), which was possibly caused by enhanced exchange of AA and DHA in phospholipids. Such a competitive aspect of various n-6 and n-3 fatty acids has also been reported at the elongation and desaturation steps (17 , 18, 19) .

Second, we summarize below the recent advances in research on the effect of fatty acids on signal transduction system in neural cells. In understanding the effect of n-3 fatty acids (deficiency) on neural functions, it is important to know the action mechanism of fatty acids on the signal transduction, especially protein kinase C (PKC) as well as Ca^{2+}- and K^{+}-channels (20). Among various fatty acids, AA has been reported, <1> to enhance PKC activity in cooperation with diacylglycerol or ACPD(1-aminocyclopentane-1,3-dicarboxylic acid) (21, 22), <2> to increase calcium current in cooperation with oxygen radical (23), <3> to enhance activity-dependent synaptic transmission (24), and <4> to affect presynaptic glutamate receptor (25, 26) and to increase glutamate release (27) , and <5> to inhibit glutamate uptake by glia cells (28). However, the activation of PKC by various acyl-CoAs in the presence of

diacylglycerol, phosphatidylserine and calcium ion was not specific to a certain chain length or extent of unsaturation; many acyl-CoAs such as stearoyl-(18:0), oleoyl-(18:1), and linoleoyl-(18:2) CoAs were also active. Acyl-CoAs were reported to affect Ca^{2+}-ATPase in pancreatic beta-cells (29) and Ca^{2+}-release channel as well (30). On the other hand, a PKC isotype in neutrophils was reported to be inhibited by acyl-CoA (31). Glutamate release in synaptosomes evoked by 4-aminopyridine was reported to be inhibited by AA in a PKC-independent manner (25). Another report showed that AA induced a synergistic facilitation of calcium-dependent glutamate release from hippocampal mossy fiber nerve endings depending on PKC (32). A lipoxygenase metabolite (12-hydroperoxy eicosatetraenoate, 12-HPETE) of AA has been shown to be important for the second messenger system in neural cells (33, 34). AA also activates K^+-channels (35). On the other hand, DHA acted differently from the other fatty acids including EPA and AA (36). DHA did not activate PKC but rather inhibited the phosphatidylserine/dioleoylglycerol-dependent stimulation of PKC activity (37). Fish oil is reported to protect the increase of cytosolic calcium ion through dihydropyridine-sensitive calcium-channel (38). These results indicate that arachidonic acid may act as an enhancer in signal transduction via PKC or K^+-channel, while DHA may act as a suppresser for PKC or calcium-channel. Besides PKC, the effect on phospholipases (39) may also be important to understand the mechanism of action of fatty acids in signal transduction.

In cultured PC12 cells, the addition of nerve growth factor (NGF) induce neurite outgrowth. We found that DHA (n-3) supplemented in the medium promoted and arachidonic acid (n-6) suppressed neurite outgrowth induced by NGF (40).

Phospholipid biosynthesis was elevated during differentiation and it was suppressed by arachidonic acid but not by DHA.

These effects of n-3 and n-6 fatty acids on signal transduction components and neurite outgrowth in neural cells may provide clues to understanding the effect of dietary fatty acids on neural function and learning behavior of laboratory animals. However, it may not be so simple to apply these in-vitro data directly to animal behavior, because it has not been shown clearly, for example, that such amounts of fatty acids (free or CoA-ester forms) as used in the in-vitro experiments are actually released to regulate synaptic functions in vivo. In this context, we need to examine carefully the results obtained at subcellular levels, culture cell levels, and animal behavior levels in order to find out consistent explanations (41).

Recently, Tonegawa's group (42) and Kandel's group (43) have developed sophisticated techniques to identify specific protein(s) in a specific site of brain to correlate them with learning performance. If the effect of a certain fatty acid on learning and memory is explained by some specific proteins involved, we might be able to apply these sophisticated techniques to study the detailed mechanisms. However, the effect of fatty acids on neural systems appears to be pleiotropic; changes in fatty acid composition would affect membrane structure and membrane enzymes as well as many other metabolic processes. This indicates that the interpretation of the results should be made with caution as a single factor may not always explain the whole phenomena of learning affected by dietary fatty acids, but rather multiple pathways with crosstalking could contribute to small but statistically significant changes in learning performance and general behavior.

II. Learning performance and dietary n-6 and n-3 fatty acids

II-1. Measurement of learning behavior

Learning behavior may be quantitatively measured in laboratory animals using several methods. Brightness-discrimination learning, water-maze, elevated plus-maze, exploratory performance, habituation task, and shock-avoidance task have been frequently used for the examination of dietary effect on learning performance (44-46). Early in 1976, the effect of n-3 deficiency in the presence of n-6 fatty acids was examined, but two types of simple maze tests led to opposite conclusions (47, 48). Later studies using more sophisticated apparatus revealed that long-term deficiency of α-linolenic acid (n-3) in the presence of n-6 fatty acid induce inferior learning performance and altered general physiology (49, 50). A typical example of brightness-discrimination learning test in rats is shown in Fig.3, in which the lever-pressing response under a bright light (R^+) was reinforced with diet pellets while the response under a darker light (R^-) was not. Statistically significant differences were observed mainly in the negative responses and in the correct respopnse ratios ($R^+/(R^+ + R^-)$) of the safflower oil (α-linolenate-limited) and perilla oil (α-linolenate-enriched) dietary groups.

Fig.3

As these learning tests were applied to laboratory animals (rats or mice) and are related to very complex neural systems in the animals, the interpretation of the results may not be simple. For example, the diet may also affect spontaneous locomotive activity which is not directly related to cognitive learning ability. However, it may affect the learning performance through modulation of motional activity related to the

test. In fact, locomotor activity of some animals or spontaneous motor activity of the senescence-accelerated mouse(SAM) was higher in the safflower oil (n-3 fatty acid deficient) dietary group (46, 51-53) than the perilla oil(n-3 rich) dietary group. On the other hand, habituation in the safflower oil group rats was slower than in the perilla oil group (51), which would also affect learning performance.

Similarly, brightness discrimination was used in evaluating the learning ability while retinal functions were also impaired in animals under n-3 fatty acid deficiency (54, 55). Then, it is possible that the apparently inferior learning ability is due to impaired vision in these animals. However, differences in learning ability were also observed in the process of extinction of learning in which brightness-discrimination was not involved, and even the darker light used was enough to evoke a significant electroretinographic responses (55). Altered learning performance was also noted in passive avoidance test in which vision was not the critical clue to test the learning performance (45, 56). One can define “learning per se” separately from other sensory and performance factors such as vision, pain threshold, sound, 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 has been shown to induce changes in membrane lipid acyl chains of all the cells of the body. The brain is an organ that integrates information from other sensory systems and make 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 (45).

II-2. Neural networks

Learning itself is supported definitely by a complex neural system. In the case of brightness-discrimination learning test, for example, this is more complex than the simple maze test. Because the brightness-discrimination task may involve declarative and associative memories as well as simple space memory, it may link functionally to many regions of brain, such as retina, visual cortex, basal ganglia, thalamus, limbic system (amygdala, hippocampus), cerebral cortex, motor neurons, and moreover neural excitation and suppression systems in those regions. In this system, neural connections may be modulated even during the learning task before completion of the learning. This modulation of neural connectivity itself may be equal to the process of learning, and the brain regions mentioned above are all possible targets to examine possible changes by the dietary fatty acids. Especially, the hippocampus has been reported to support episodic and declarative memory as two powerful benefits of the networking of cortical memories.(57) There are still many arguments for the role of specific brain regions on the learning and memory functions. In the case of brightness-discrimination learning performance, the difference of the performance between the two dietary groups (n-3 fatty acid-deficient and -sufficient groups) was observed particularly in the correction performance of "incorrect response" (49, 58) rather than in the performance of "correct response". However, it is not known how the two responses are modulated by neural networks in the brain, and which brain regions or neural systems are concerned.

II-3. Dopaminergic neuron

It was reported that dopamine neurons in basal ganglia of monkey were involved in the responses to reward and conditioned stimuli during successive steps of learning a delayed response task (59). Dopaminergic and serotoninergic

(monoaminergic) neurotransmission in the rat frontal cortex was affected by dietary α-linolenic acid-deficiency (60) and other fatty acids (61, 62). These results suggest that dopaminergic neurons in basal ganglia may play an important role in the conditioned learning task such as the discrimination learning. The alteration of the content of dopamine or other monoamines is still controversial; no change in n-3 deficiency (Tosaki et al, Masters Thesis; Nagoya City University), or decrease in n-3 deficiency (60).

II-4. Other neurotransmitters in n-3 deficiency

The content of neurotransmitters in brain regions was determined to find some changes in n-3 deficiency, but the changes were relatively small (±20 %) and we have reached no definite conclusion. Minami's group has shown that choline and acetylcholine contents in cortex of stroke-prone spontaneously hypertensive rats were 40 % high in DHA-supplemented group as compared with n-3 deficient group, although the difference in acetylcholine in hippocampus was less (68, 69). GABA (γ-aminobutyric acid) content was higher in the n-3 deficient group (Tosaki, T. et al., unpublished), which may be related to higher sensitivity to pentobarbital, because pentobarbital is known to enhance the binding of GABA to GABAa receptor and prolongs the chloride channel opening period potentiating the GABArgic inhibitory neurons. Glutamate content did not change by n-3 deficiency. These biochemical parameters (neurotransmitter content and membrane enzyme activities) of brain in the n-3 deficiency did not show marked changes in spite of relatively large differences in the n-6/n-3 fatty acid balance in the neural membranes. When the learning performance was measured by the brightness-discrimination learning task, the performance

difference between the n-3 deficient and sufficient rats in the second generation was at most 15-points (i.e., at the final stage 75-points in the correct response ratio (50) for n-3 sufficient group and 60-points for n-3 deficient group in 100-points full score). The DHA content of brain decreased by roughly 50 %, which was compensated for by the increase in docosapentaenoate (22:5n-6) even after prolonged n-3 deficiency (64), e.g., through two generations, without changes in phospholipid and cholesterol contents in brain. We might be able to expect greater changes in biochemical parameters if we could raise animals essentially devoid of DHA in neurons.

II-5. Membrane enzymes in n-3 deficiency

Synaptic transmission efficacy of neurons in the concerned brain regions may be a plausible factor in the alteration of brightness-discrimination learning performance in n-3 deficiency. This transmission efficacy may depend on the probability of transmission / quantal size of transmitters from presynapses and efficacy of postsynaptic signal transduction system. The excitatory and inhibitory neurons may function simultaneously and in a concerted manner in those brain regions, and the synaptic efficacy in those neurons may be heterogeneous. Those efficacy may be affected by some enzymes or channel proteins, such as Na^+, K^+-ATPase and protein kinases, and by some components concerned with membrane recycling system, although the role of Na^+, K^+-ATPase in the dietary fatty acid-dependent alteration of learning performance is controversial (63, 64). The activity of Na^+, K^+-ATPase at a suboptimal concentration of ATP in rat brain synaptosomes tended to be lower in the n-3 deficiency than in the n-3 rich dietary group (64). Gerbi et al. (63) reported that Na and ouabain-sensitivity of Na^+, K^+-ATPase isozymes was altered with more extensive n-3 deficiency. Cardiac Ca^{2+}-ATPase activity was reported to be decreased in the n-3

sufficient (menhaden oil) diet (65), although other report observed no significant change in Ca^{2+}-ATPase activity (66) nor in some other membrane enzymes in the n-3 deficient diet group. Na^{+}, K^{+}-ATPase was reported to be modulated by dopamine and hormones (67), and it is likely that dietary n-3 fatty acid deficiency lowers neural Na^{+}, K^{+}-ATPas activity indirectly through other hormonal pathways. In our hands, no significant change by n-3 fatty acid deficiency was observed in the activities of 5'-nucleotidase, cyclic nucleotide phosphodiesterase and acetylcholine esterase (64).

II-6. Morphological changes seen in brain under n-3 deficiency

It seemed important to examine possible morphological changes in synapses in relation to dietary fatty acids and learning task. As the biochemical changes in the bulk area of brain represent averages of heterogeneous cells measured, neurons or glias more specifically related to learning should preferably be investigated. We first counted the synaptic vesicles in hippocampus CA1 region (stratum radiatum). Electron micrographic analyses of synaptic vesicles revealed a significantly decreased vesicle density (vesicle number per unit area) in the release site in the n-3 deficient rat hippocampus CA1 region after the learning task as compared with n-3 sufficient rats. However, this difference was not observed without the learning task (58, 70), indicating the associated effect of both learning task itself (or certain extent of neural activity) and dietary fatty acid status. Fig. 4 shows the histogram patterns of synaptic vesicle density for the two dietary groups (safflower oil- and perilla oil-groups).

Fig.4

Those rats with inferior learning performance and decreased synaptic vesicle density in hippocampus have other biochemical changes in brain microsomes (70, 71); increased calcium-induced aggregation rate and increased reactivity against

phospholipase A2 and sialidase-treatment after the learning task. There was no significant difference in the inositol trisphosphate-induced calcium release from microsomes.

These morphological and biochemical studies of brain organella suggest that the altered neural membrane fatty acid composition may trigger further changes in membrane surface and its turnover dynamics. In this regard, it is noteworthy that circadian changes in the size and number of phagosomes in retinal pigment epithelium were also affected by n-3 deficiency (72).

III. Psychotic Aspects, Brain Diseases and Dietary Fatty Acids

A historical review on possible causal relationship between n-6 and n-3 fatty acid metabolism and human psychosis and neurosis was published elsewhere (41). More recent clinical studies will be reviewed below.

III-1. Schizophrenia

Dietary fatty acids may affect not only the learning performance in rodents and visual acuity in monkey but also psychotic aspects of human. Peet et al. (73, 74) reported that schizophrenic patients who eat more n-3 fatty acids in their normal diet have less severe symptoms, and that n-3 fatty acid supplementation significantly improved both schizophrenic symptoms and tardive dyskinesia over a 6 weeks period. Moreover, Mahadik et al. (75) have shown that docosahexaenoic acid as well as total n-3 essential fatty acid contents was significantly lower in cultured skin cell lines from schizophrenic patients than in cell lines from bipolar patients and normal subjects. Red blood cell membranes and plasma in schizophrenia showed a highly significant

decrease in the levels of polyunsaturated fatty acids, particularly 18:2(n-6), 20:4(n-6) and 22:6(n-3), suggesting non-enzymatic oxidative damage of the membranes (76-78).

III-2. Depression

It was also reported that arachidonic acid to eicosapentaenoic acid (n-6/n-3) ratio in blood correlates positively with clinical symptoms of depression (79). In major depression patients, the serum cholesteryl ester formation was decreased and its 18:3 n-3 content was reduced more than in the normal controls. They had significantly higher 20:4(n-6)/20:5(n-3) ratio in both cholesteryl esters and phospholipids (80). Administration of fish oil or 20:5n-3 supplements was reported to reduce depressive symptoms in post-viral fatigue syndrome (81), and Smith (82) has hypothesized that treatment with fish oil may offer a prophylaxis against depression. Hibbeln and Salem (83) have pointed out that long-chain polyunsaturated fatty acids, particularly DHA, play a role in mental functioning and depression. Depression is thought to be a common clinical symptom of hypercalcemia (84). One finding in depressed patients is an increase in platelet intracellular calcium (85). This process is considered to be dependent on the stimulation of protein kinase C and other membrane-dependent second messengers. An increase of neural DHA, mediated by diet, may lead to a more optimal environment for protein kinase C-mediated signal transduction system and thus DHA may dampen development of depressive cycles in response to stress as DHA may suppress PKC or calcium-channel (described in Introduction) . Needless to say, the mental disorders as described above are associated primarily with impaired protein functions, but it is likely that dietary essential fatty acid balance modifies the disorders. In this context, it is noteworthy that DHA supplementation suppressed aggressiveness

against others in young adult (86), and increasing the intake of linoleic acid (n-6) resulted in increased incidence of violent death (41).

III-3. Senile dementia and Alzheimer's disease

Senile dementia is classified mainly into cerebrovascular type and Alzheimer type. N-3 fatty acids improve hemodynamics and suppress thrombotic tendency and cerebral bleeding (41). Thus, decreasing the n-6/n-3 balance of dietary fatty acids is recommended for the prevention of senile dementia of cerebrovascular type. Alzheimer’s disease is generally assumed to be due to genetic disorders but environmental factors such as dietary fatty acids may also be involved. Grant (87) reported that fat and total caloric supply had the highest correlation with Alzheimer's disease prevalence rates and fish consumption was negatively correlated. High-fat and high-total caloric consumptions are listed as the primary cause of sporadic Alzheimer's disease and senile dementia. Fatty acids of n-6 type contribute to oxidative damage and inflammatory responses through lipid mediators, and fish oils combat ischemia and inflammation. Consistently, several dietary components and supplements have been found effective in delaying the onset of Alzheimer's disease, including antioxidants, fish oil, and nonsteroidal anti-inflammatory drugs (88). Actually, recent studies have demonstrated that non-steroidal anti-inflammatory drug (aspirin) is effective to delay the onset of Alzheimer’s disease (88, 89). DHA also suppresses stroke-related behavior in SHRSP rats (68, 69).

III-4. Parkinsonism and other behavioral disorders

Oxidative stress may also be associated with Parkinson's disease (90), and increased intake of animal fats and calories was strongly related to the onset of Parkinson's disease, although no significant differences were found in the intake of

antioxidant vitamins such as Vitamin E. The fact that cardiovascular disease is a predominant cause of death among patients with parkinsonism is consistent with the hypothesis that cardiovascular diseases and Parkinsonism share common etiology, e.g., excess intake of n-6 fatty acids and relative n-3 deficiency (41).

Intake of phosphatidylserine derived from brain lipids or prepared by trans-phosphatidylation of soybean lecithin was reported to improve dementia patients and scopolamine-induced amnesia in rats (91, 92). Although phosphatidylserine is associated with calcium-dependent cellular metabolism (such as PKC dependent metabolism), it is unlikely that dietary phosphatidylserine modifies its brain contents.

Attention-deficit hyperactivity disorder (ADHD) is the term used to describe children who are inattentive, impulsive, and hyperactive. Stevens et al. (93) reported that in ADHD patients low concentrations of polyunsaturated fatty acids (20:4n-6, 20:5n-3, 22:6n-3) were demonstrated in the plasma polar lipids. Moreover, in boys aged 6 to 12 with lower total omega-3 fatty acids in plasma phospholipids, more learning, behavior and health problems were found (94). Recently Hamazaki et al. (86) reported in a placebo-controlled double-blind study that DHA intake prevented extraggression from mental stress tests for students. It has been postulated (95) that serotonergic deficits may predispose individuals to poor impulse control, disturbance of glucose metabolism, alcohol abuse, violent behavior and suicide. Aggressive and hostile behavior may be affected by various nutritional factors (vitamins, minerals, heavy metals, amino acids, and hypoglycemia) through modification of serotonergic neuronal functions. In this context, it is noteworthy that n-3 deficiency was reported to elevate serotonine-2 receptor density (60, 96)

These results indicate that long-term effects of dietary fatty acids could enhance and cause brain diseases as well as psychiatric abnormality of human either directly or indirectly. Some psychotic disease may be linked closely to the inflammatory response of neural cells such as microglia, and the onset and development of these diseases may be inhibited by some anti-inflammatory drugs. N-3 fatty acids function like such drugs or hormone to suppress inflammatory or immunoreactive responses by competitively inhibiting the productions of inflammatory lipid mediators related to arachidonic acid (n-6).

IV. Neural networks and cell membranes

The neural system is one of the targets modified by dietary fatty acids. Although changes in fatty acid composition occur ubiquitously in cell membranes, different tissues respond differently. The above briefing indicates that not only learning behavior but also pathophysiological aspects of brain are affected significantly by dietary n-6 and n-3 fatty acids. The precise mechanisms for learning and memory in brain as well as onset of psychotic diseases are not clear, and obviously complicated neural networks are involved.

The difference in the learning performance was observed in the correction performance against 'incorrect responses' (R^- in Fig.3), but not in the performance of 'correct responses' (R^+ in Fig.3) in the brightness-discrimination learning test, suggesting that a part of the network system is affected by the dietary fatty acids more effectively than the other neural systems. Which neural network system in the brain is concerned with the `correction of incorrect responses` ? A specially important circuit from basal ganglia through thalamus, limbic system to cerebral cortex in the brain has

been proposed to play a role in the learning performance. The discrimination learning may involve the "error correction" process and this "error correction" may be done using the so-called "Teacher's Signal" to depress the neural activity which otherwise contributes to error responses (97). However, it has not been realized clearly how and when the "Teacher's Signal" is produced during the learning process. The limbic system might be concerned with the production of this signal (98). The interpretation that the inferior “suppression system” is linked to the behavioral “hyper activity” of laboratory animals and possibly humans under the n-3 fatty acid-deficient dietary conditions is still hypothetical.

The next step of investigation of the effect of dietary fatty acid on learning and memory should be directed to focus on the detailed mechanism of direct and indirect ways; the direct way may be on the important membrane enzymes or receptors and the indirect way may be on some metabolic system (such as glycolipid and glycopeptide or hormonal systems) which would influence functions of synaptic networks.

These basic studies would help understanding the relationship between the neurotic, psychotic changes (major depression, schizophrenia, Alzheimer’s diseases, etc.) and dietary fatty acids.

V. Conclusion

We summarized the physiological and biochemical relationships between dietary n-6 and n-3 fatty acids and neuronal / behavioral changes in laboratory animals and humans. Many lines of evidence have accumulated that dietary fatty acid balance affects membrane enzymes, ion channels, signal transduction systems, and neural network systems. However, it remains entirely to be elucidated how the fatty acid

modifications of membranes modulate the functions of brain cells and their networks related to learning activity and psychotic changes.

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Figure legend:

Fig. 1. Three biosynthetic pathways for major polyunsaturated fatty acids in mammals (desaturation, chain-elongation and chain-shortening steps)

The site of desaturase action is shown as Δ9, Δ6 or Δ5, and the common names are given only for the major polyunsaturated fatty acids found in tissue lipids. Fatty acids are designated by the carbon chains:the number of double bonds, and the position of the first double bond numbered from the methyl terminus is denoted as n-9, n-7, n-6 or n-3. AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Fig. 2. Effect of dietary fatty acids on docosapentaenoate (22:5n-6) and 22:6n-3 (DHA) contents of subcellular fractions of rat brain

Perilla oil diet contained linoleic acid (n-6) (13 % of total fatty acids) and α-linolenic acid (n-3) (64 %) while safflower oil diet contained linoleic acid (78 %) and α-linolenic acid (0.05 %); the proportions of other fatty acids in the diets were relatively similar. Brains from the second generation rats were analyzed. A,synaptosomes; B,plasma membranes; C,myelin; D,microsomes plus synaptic vesicles (*(44)* Tsutsumi T. al, Biol.Pharm.Bull. 18, 664-670 (1995)). n.d., not detectable.

Fig.3 Brightness-discrimination learning test in rats fed α–linolenate-limited safflower oil or α–linolenate-enriched perilla oil through two generations

Numbers of lever-pressing responses under a bright light (R^+) for which diet pellet was reinforced, and those under a dim light (R^-) were recorded. Major differences were

observed in the R- responses and in the correct response ratios ($R^+/(R^+ + R^-)$) of the two groups. After this original schedule, stimuli were reversed; diet pellet was reinforced only for the responses under the dim light. Again, the ability to discriminate the new conditions was inferior in the safflower oil group, in which brain DHA decreased roughly by half (*(99)* Okaniwa et al, Biol.Pharm.Bull. 19, 536-540 (1996); *(100)* Yamamoto et al, J.Gerontol. 46, B17-B22 (1991)).

Fig. 4. Histogram of synaptic vesicle density and typical electron microscopic morphology of brain hippocampus (CA1 region)

Rats fed perilla oil (Per) or safflower oil (Saf) supplemented diet were analyzed after a brightness-discrimination learning test (*(58)* Yoshida et al., J Neurochem 68, 1261-1268 (1997)). NTM, the number of total measurements.