Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 20 (2009) 735–741
Journal of Nutritional Biochemistry

The influence of orally administered docosahexaenoic acid on cognitive ability in aged mice

Li-he Jiang[a,b], Yan Shi[a], Li-sheng Wang[b], Zhi-rong Yang[a,*]

[a]Key Laboratory of Bio-resources and Eco-environment, Ministry of Education, College of Life Sciences, Sichuan University, Chengdu 610064, P.R. China
[b]Guangxi University, Nanning 530004, P.R. China

Received 6 November 2007; received in revised form 19 May 2008; accepted 7 July 2008

Abstract

The purpose of this study was to investigate whether or not the role of docosahexaenoic acid (DHA) supplementation on cognitive capability was related with brain-derived neurotrophic factor (BDNF), nitric oxide (NO) and dopamine (DA) in aged mice. Kunming-line mice were treated with 50 and 100 mg/kg/day of DHA via oral gavage for seven successive weeks. The cognitive ability of mice was assessed by step-through and passageway water maze tests. The levels of NO in hippocampus and striatum tissues were assessed by spectrophotometric method. The levels of DA in hippocampus and striatum tissues were assessed by high-performance liquid chromatography with electrochemical detection. The protein levels of BDNF in hippocampus tissue were assessed by Western blotting. The results showed that the cognitive capability of mice was significantly different between the DHA-treated groups and the control group; the protein level of BDNF was significantly increased in the hippocampus; the levels of NO and DA were significantly increased in hippocampus and striatum tissues. In conclusion, during aging, DHA supplementation can improve the cognitive function in mice and can increase the protein level of BDNF in hippocampus tissue and the levels of NO and DA in hippocampus and striatum tissues. Taken together, our results suggest that DHA supplementation could improve the cognitive dysfunction due to aging, to some extent, and it may have a relationship with increasing the protein level of BDNF and the level of NO and DA.

Keywords: Docosahexaenoic acid; Cognitive ability; Brain-derived neurotrophic factor, Dopamine; Nitric oxide

1. Introduction

Docosahexaenoic acid (DHA) is a polyunsaturated fatty acid composed of 22 carbon atoms and six double bonds. Because the first double bond, as counted from the methyl terminus, is at position 3, DHA belongs to the omega-3 group and is abundant in the brain and retina [1]. There is a high DHA level in the brain and retinal tissues of animals and humans, suggesting that this fatty acid plays an important role in neural function. This has been demonstrated in several animal studies where an increased intake of DHA in the diet influenced brain functions, such as learning ability and emotion [2,3]. Moreover, human studies have indicated that the consumption of DHA may act as a defense against the onset of Alzheimer's disease [4] and improve symptoms of dementia in the elderly [5]. DHA in the brain, similar to arachidonic acid, is known to show an age-dependent decrease [6]; therefore, DHA supplementation is very important to old people.

Nitric oxide (NO) is a gaseous neurotransmitter and was reported to play an important role in several biological systems [7]. In the central nervous system, NO appears to modulate neurotransmitter release in the brain [8]. In addition, the hippocampal administration of NO donors facilitates avoidance learning in the same test [9,10] and the inhibitory avoidance learning has been associated with increased hippocampal NOS activity in rats [11]. Thus, the hippocampus appears to be an important region in which NO synthesis is pivotal for learning and memory processes. Like NO, dopamine (DA) can regulate motivational processes, motor activity and cognition. Several studies

* Corresponding author. Tel.: +86 28 85412943; fax: +86 28 85415300.
E-mail address: au72191@gmail.com (Z. Yang).

0955-2863/$ – see front matter.
doi:10.1016/j.jnutbio.2008.07.003

have shown that striatum DA level is strongly related with behavioral performance [12–14]. A low level of DA is associated with behavioral deficiencies, while a high level of DA leads to better performance in striatum function-related tasks, such as procedural learning and spatial working memory [15].

Neurons die during normal development of the nervous system and if an individual has neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. It has been suggested that chronic neurological diseases as well as acute brain injuries could be treated with trophic factors such as nerve growth factor and brain-derived neurotrophic factor (BDNF). However, poor penetration of the blood–brain barrier and the occurrence of side effects limit the use of exogenous applications of these factors. Therefore, stimulating the local production of trophic factors seems a more attractive approach [16]. Recently, it has been suggested that omega-3 fatty acids, including DHA, can affect BDNF. It has been reported that the anti-inflammatory role of omega-3 fatty acids may influence BDNF in depression [17]. Besides, DHA has the ability to regulate BDNF via a p38 mitogen-activated-protein-kinase-dependent mechanism, which may contribute to DHA's therapeutic efficacy in brain diseases, showing disordered cell survival and neuroplasticity [18]. Wu et al. [19] have also reported that dietary DHA normalizes BDNF levels, reduces oxidative damage and counteracts learning disability after traumatic brain injury in rats. Hence, DHA may be a good drug for stimulating BDNF expression in the brain.

Early tracer experiments suggested that dietary DHA was an efficient source of brain [20]. However, all these studies have only been performed on young animals or infants. Considering that BDNF, NO and DA play important roles in learning and memory performance of rodents, we performed this experiment; this study was designed to evaluate whether or not the role of DHA supplementation on cognitive ability is related with BDNF, NO and DA.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals and reagents

DHA from cod liver oil was purchased from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, USA); DA was from Sigma Chemical Co.; NO assay kit was from Nanjing Jiancheng Institute (Nanjing, China); hydrophobic polyvinylidene difluoride membranes were from Amersham Bioscience (UK); BCA protein assay kit, β-actin, alkaline phosphatase rabbit anti-goat IgG rabbit and BDNF antibody were from WuHan Boster Biological Technology Co. Ltd. (China); protease inhibitor cocktail (PIC Complete) was from Amersham Pharmacia Biotech (used according to the manufacturer's instructions); total protein extraction kit was from Applygen Technologies Co. Ltd. (China); 5% nonfat dry milk was from BD Biosciences (USA). All other chemicals and reagents used were of analytical grade and were made in China.

2.1.2. Animals

Forty-eight 15-month-old Kunming-line female mice (54±3 g, originally introduced from Swiss mice at the Hoffkine Institute, India, in 1944) were purchased from the Animal Experimental Center of Sichuan (Chengdu, China). The mice were first housed in a controlled environment with temperature maintained at 23±2°C and humidity at 42±2% under a 12:12-h light/dark cycle, with free access to water and food. The animals were treated according to the guidelines of the National Institute of Health Guide for the Care and Use of Laboratory Animals and the Animal Ethics Committee of the university.

2.2. Animal treatment

After a week of adaptation, mice were randomly divided into three experimental groups: control group: 15-month-old female mice (n=16) and DHA-treated groups (small-dose group: 15-month-old female mice treated with DHA 50 mg/kg/day; large-dose group: 15-month-old female mice treated with DHA 100 mg/kg/day, n=16). DHA was dissolved in corn oil [21–23] and was administered to the treatment groups via oral gavage for 7 weeks. Control group mice received a similar volume of corn oil alone in order to eliminate the effects of daily gavage.

2.3. Behavioral experiments

Behavioral experiments were carried out on the second day when DHA treatment was finished. The step-through test was performed first, followed by the passageway water maze test.

2.3.1. The step-through test (one trial passive avoidance)

The step-through test was performed in the JZZ94 multifunction passive avoidance apparatus (PA M1 O'Hara and Co. Ltd.). The apparatus consists of two compartments separated by a black wall with a hole in the lower middle part. One of the two chambers is illuminated, and the other is dark. The test was conducted for two consecutive days including one training trial (d1). Each mouse was placed in the illuminated compartment, facing away from the dark compartment for 5 min to be accustomed to the apparatus, and then received the training trial for 1 h. The training trial is similar to the adaptation trial, except that upon entering the dark compartment, the mouse experiences an electric foot shock (DC, 0.3 mA, 30 V, 1 s) through the stainless steel grid floor. In the testing trial (d2), the same procedure was repeated. The interval time between the placement in the illuminated compartment and the entry into the dark compartment was regarded as the memory retention time (in seconds). The entering events per testing trial were recorded as error counts (number of errors). Mice were allowed to stay in the illuminated compartment for 30 s in the training trial and 5 min in the testing trial.

2.3.2. Passageway water maze test

The passageway water maze was provided by the Pharmacological Institute of Chinese Academy of Medical Sciences. The passageway water maze test (62 cm×37 cm×20 cm, width/length/height) was made of gray Plexiglas and consisted of vertical panels inside. There is one safe area with a stairway under the water, three start areas (S1, S2 and S3) with movable guillotine doors and three error areas (E) defined as corners deviated from the correct pathways, which are dead ends in the maze (Fig. 1). The maze, which was filled with water 12 cm deep and kept at 23–25°C, was located in a corner of the room with some cues on the wall. There was a light hung 2 m above the center of the maze.

On Day 1, the guillotine door of S1 was closed; the mouse was individually placed into the start area S1 with its head toward the wall and allowed to acclimatize to the safe area with the underwater stairway for three times. The maximal time for each trial was 2 min. They were returned to their cages after the acclimation.

On Day 2, the guillotine door of S2 was closed, while the door of S1 was open. Each mouse was individually placed into S2 with its head toward the wall. Mice were taken to their cages after they arrived at the safe area. Mice were removed from the cages if they could not find the safe area within 2 min.

On Days 3 to 6, the guillotine doors of S1 and S2 were raised. Each mouse was placed into S3 with its head toward the wall. The training session ended after the mice reached the safe area and climbed out of the water. Latency time for the mouse arriving at the safe area and the number of errors were recorded.

In the training sessions, mice were placed into the exact same position within the testing room. The mice were removed by the experimenter from the maze and placed into their cages if they failed to reach the safe area within 2 min. The maze was cleaned and water was replaced after the experiment every day. It was counted as an error when the mouse head collided with the blind end. Learning and memory abilities were assessed by the number of errors.

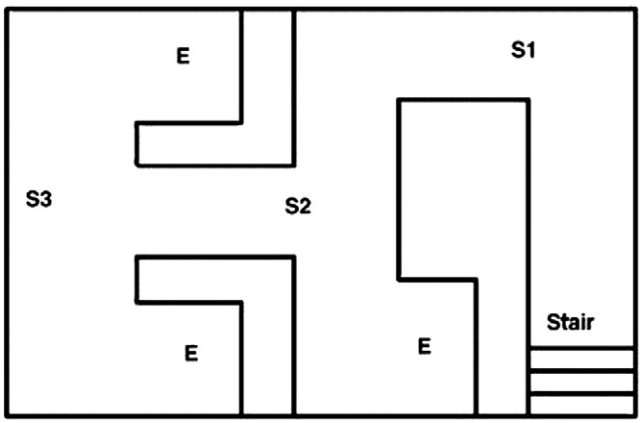

Fig. 1. Passageway water maze. S1, S2 and S3: start areas 1–3. Mice were individually placed into the start area with the head toward the wall during training. They started training from S1 on Day 1, from S2 on Day 2 and from S3 on Days 3–6. E: error area. There are three error areas, which are dead ends in the maze. Stair: the underwater stairway as a safe area where the mice could climb out of the water. The maze is filled with water (23±2°C, 12 cm deep).

2.4. Biochemistry assays

Mice were killed by cervical dislocation without anesthesia just after the behavioral tests. The brain was removed after a rapid dissection of the hippocampus, and striata were isolated and individually stored at −70°C until measurement of NO, DA and BDNF.

2.4.1. Measurement of the levels of NO

The transient and volatile nature of NO makes it unsuitable for most convenient detection methods; however, two stable breakdown products, nitrate (NO^{3-}) and nitrite (NO^{2-}), can be easily detected by photometric means. In this experiment, the levels of NO were determined by the concentration of nitrite accumulated in tissues, which were measured by a spectrophotometric method based on the Greiss reaction [24]. Tissue was homogenized in 10 volumes of 0.1 M phosphate buffer (pH 7.4) and was centrifuged at 5000×*g* at 4°C for 20 min. The supernatant was used for the following biochemical analyses at a wavelength of 550 nm. Protein concentration was determined according to the Lowry method [25]. The NO was expressed as micromoles per milligram of protein.

2.4.2. Measurement of the levels of DA

DA levels in the hippocampus and striatum were measured as described previously [26] using high-performance liquid chromatography with electrochemical detection, with minor modifications. Briefly, each frozen tissue sample was homogenized by ultrasonication in 200 μl of 0.4 M perchloric acid (Solution A). The homogenate was kept on ice for 1 h and then centrifuged at 12,000×*g* (4°C) for 20 min, and the pellet was discarded. An aliquot of 160 μl of supernatant was added to 80 μl of Solution B (containing 0.2 M potassium citrate, 0.3 M dipotassium hydrogen phosphate and 0.2 M EDTA). The mixture was kept on ice for 1 h and then centrifuged at 12,000×*g* (4°C) for 20 min again. Twenty microliters of the resultant supernatant was directly injected into an ESA liquid chromatography system equipped with a reversed-phase C_{18} column (150×4.6 mm I.D., 5 μm) and an electrochemical detector (ESA CoulArray, Chelmsford, MA, USA). The detector potential was set at 50, 100, 200, 300, 400 and 500 mV, respectively. The mobile phase consisted of 125 mM citric acid–sodium citrate (pH 4.3), 0.1 mM EDTA, 1.2 mM sodium octanesulfonate and 16% methanol; the flow rate was 1.0 ml/min. The tissue levels of DA were expressed in terms of nanograms per gram of tissue.

2.5. Western blot analysis of BDNF in hippocampus tissue

Hippocampus tissue lysate was prepared by homogenization in 10 volumes of 0.32 M sucrose, 0.1 M Tris (pH 7.4)

and protease inhibitor cocktail (PIC Complete, Amersham Pharmacia Biotech; used according to the manufacturer's instructions). Equal amounts of protein per lane (50 μg) were loaded onto an 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and then transferred to hydrophobic polyvinylidene difluoride membranes. The membranes were blocked in 5% nonfat dry milk in a PBS buffer containing 0.1% Tween 20 for 1 h at room temperature, followed by an overnight incubation with primary antibody against BDNF (1:400). Mouse anti-β-actin monoclonal antibody (1:2000) was detected on immunoblots as a loading control for protein quantization. Protein levels were normalized to β-actin as a loading control. Relative optical density of protein bands was measured following subtraction of the film background. Protein was determined according to the Bradford method [27], using bovine serum albumin as a standard. The expression of BDNF was determined by calculating the density ratio of each band to β-actin protein.

3. Statistical analysis

The data were analyzed by one-way ANOVA using SPSS program (version 10.0) for all statistical analyses. Expression levels of BDNF protein were analyzed by either the unpaired *t* test or one-way ANOVA. Significance was set at $P<.05$.

4. Results

4.1. The effect of DHA on the performance of mice in the step-through test

The data in Table 1 demonstrate that the retention period was longer and the number of errors significantly decreased for mice in DHA-treated groups when compared with those in the control group. The data showed that the retention time in DHA-treated groups was significantly increased by 28.56% and 45.60%, respectively, when compared with that in the control group. The mean number of errors was reduced from 2.0±0.63 in the control group to 1.20±0.60 and 1.50±0.81, respectively, in DHA-treated groups. Taking all the data into consideration, we may draw the conclusion that DHA can improve the learning and memory abilities of aged mice.

Table 1
The effects of DHA on the memory performance of mice in the step-through test

Groups	Memory performance	
	Retention time (s)	Number of errors
Control group	119.4±13.3	2.0±0.63
Small-dose group	153.5±25.7**	1.20±0.60**
Large-dose group	174.3±31.8**	1.50±0.81**

Data are expressed as means±S.E.M. (n=16).
Values in a row with different superscripts are significantly different ($P<.05$).
The data showed that DHA can significantly increase retention time and decrease number of errors of mice in the step-through test.
** $P<.01$ versus control group.

Table 2
The effects of DHA on the latency period of mice in the passageway water maze

Groups	Latency time (s)			
	Day 3	Day 4	Day 5	Day 6
Control group	95.3±9.9	83.4±11.5	62.2±11.7	54.6±11.3
Small-dose group	73.5±20.2**	64.2±8.2**	46.9±13.2**	38.4±5.9**
Large-dose group	65.6±10.59**	51.5±11.3**	36.7±12.9**	31.3±9.2**

Data are expressed as means±S.E.M. (n=16).
Values in a row with different superscripts are significantly different ($P<.05$).
The data showed that DHA can decrease the latency period of mice in the passageway water maze. From Day 3 to Day 6, the latency period of mice in each group continuously decreased. Moreover, there was a significant difference in the latency period between the DHA groups and the control group.
** $P<.01$ versus control group.

4.2. The effect of DHA on performances of mice in the passageway water maze

The data in Tables 2 and 3 demonstrate that the latency period in the DHA groups was longer and the number of errors significantly decreased when compared with those in the control group. On Days 5 and 6, there was no significant difference between the DHA small-dose group and the control group in the number of errors. All in all, the experimental results showed that DHA treatment can improve the learning and memory abilities of aged mice in the passageway water maze test.

4.3. The effect of DHA on NO in hippocampus and striatum tissues of mice

The data in Table 4 demonstrate that the levels of NO in DHA treatment groups were significantly increased when compared with those in the control group. The levels of NO were increased by 1.23 and 1.45 times in the hippocampus and by 1.16 and 1.54 times in striatum, respectively, when compared with those in the control group.

Table 3
The effects of DHA on the number of errors of mice in the passageway water maze

Groups	Number of errors			
	Day 3	Day 4	Day 5	Day 6
Control group	6.2±0.97	5.6±0.8	4.2±1.2	3.1±0.7
Small-dose group	7.2±2.3	4.8±0.9**	3.7±1.4	2.5±0.8**
Large-dose group	4.9±0.93**	4.5±1.1**	3.3±1.3**	2.2±0.7**

Data are expressed as means±S.E.M. (n=16).
Values in a row with different superscripts are significantly different ($P<.05$).
DHA can decrease the number of errors of mice in the passageway water maze. For Days 5 and 6, there were no significant differences between the DHA small-dose group and the control group.
** $P<.01$ versus control group.

Table 4
The effects of DHA on NO in hippocampus and striatum tissues of mice

Groups	Hippocampus NO (μmol/mg protein)	Striatum NO (μmol/mg protein)
Control group	0.98±0.19	1.58±0.19
Small-dose group	1.24±0.21**	1.84±0.26**
Large-dose group	1.43±0.34**	2.12±0.19**

Data are expressed as means±S.E.M. (n=10).
Values in a row with different superscripts are significantly different (P<.05). The levels of NO in the 50-mg/kg and 100-mg/kg DHA treatment groups were significant (P<.01) compared with the control group. The levels of NO in the hippocampus was increased by about 1.23- and 1.45-fold, respectively, when compared with the control group. The level of NO in the striatum was increased by about 1.16- and 1.54-fold when compared with the control group.

** P<.01 versus control group.

4.4. The effect of DHA on DA in hippocampus and striatum tissues of mice

The data in Table 5 demonstrate that the levels of DA in DHA treatment groups were significantly increased in the hippocampus and striatum when compared with those in the control group. The levels of DA in DHA treatment groups were increased by 1.12 and 1.21 times in the hippocampus and by 1.28 and 1.38 times in striatum, respectively, when compared with those in the control group. Moreover, there was DHA dose dependence in the hippocampus and striatum.

4.5. The effect of DHA on expression of BDNF protein in hippocampus tissue

The Western blotting assay (Fig. 2) demonstrated that levels of BDNF protein in hippocampus tissue of DHA-treated groups were significantly increased when compared with those in the control group. Mice treated with DHA showed a significant increase in BDNF protein level of hippocampus tissue.

5. Discussion

DHA (22:6n-3) is a major component of membrane phospholipids in nerve cells; thus, it is considered to be essential for proper neuronal development and function,

Table 5
The effects of DHA on DA levels in hippocampus and striatum tissue of mice

Groups	Hippocampus DA (ng/g)	Striatum DA (ng/g)
Control group	227.1±18.3	7084.5±912.4
Small-dose group	253.5±18.2*	9120.4±1151.1**
Large-dose group	274.1±24.7**	9799.9±1408.3**

Data are expressed as means±S.E.M. (n=10).
Values in a row with different superscripts are significantly different (P<.05). The DA levels were significantly increased in the hippocampus and striatum relative to the control group, indicating an increase in DA due to DHA treatment.

* P<.05 versus control group.

** P<.01 versus control group.

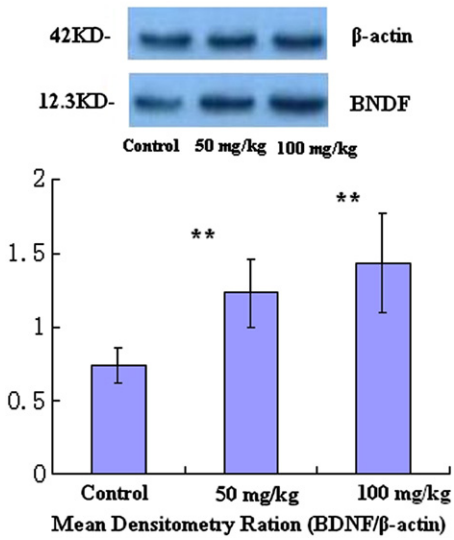

Fig. 2. Western blotting assay of BDNF protein level in hippocampus tissue. The levels of BDNF protein quantified were relative to the density of β-actin and expressed as mean±S.E.M. and were determined by SPSS analysis, followed by either unpaired t test or one-way ANOVA (n=6). **P<.01 versus control group.

which has been well established in brain development and neurological function [28–31].

In the present study, we examined the effect of DHA supplementation on the performance of aged mice in the step-through and passageway water maze tests. The present findings are in agreement with previous studies' results that demonstrate the positive effect of DHA supplementation on learning and memory abilities [2,3].

Our experimental results showed that the beneficial effects of DHA on learning and memory abilities appear to be associated with BDNF, NO and DA. BDNF, as a member of the neurotrophin family of survival-promoting molecules, plays important roles in the growth, development, maintenance and function of several neuronal systems. A decrease in BDNF expression has been associated with neuronal atrophy or death occurring in some neurological disorders [32]. Moreover, BDNF is also associated with brain aging. Novel studies indicate that age-related BDNF expression changes may be restricted to a loss of the activity-dependent component of BDNF [33,34]. In addition, BDNF plays important roles in learning and memory and is critically involved in the consolidation phase of long-term memory due to its involvement in long-term potentiation in the hippocampus [35,36].

BDNF, NO and DA have some mutual relationship. BDNF may contribute to NO-sensitive learning and memory processes, since mutual regulation between BDNF and NO synthesis has been demonstrated in vitro and in vivo [37]. The disruption of spatial learning in the radial arm maze task by treatment with the NO synthase inhibitor 7-nitroindazole was associated with an inhibition of the learning-induced increase in BDNF mRNA and protein level in the hippocampus

[38,39]. Accordingly, the modulation of neuronal plasticity by NO, including leaning and memory, may be mediated, at least in part, through the effects of BDNF.

BDNF in memory formation may be mediated through the modulation of the synaptic release of neurotransmitters such as glutamate and DA [40]. Furthermore, it is likely that BDNF can modulate the release of DA through the activation of tyrosine receptor kinase B [41].

The effect of DHA on NO production and NOS activity is rather unclear. Minami et al. [31] speculated that DHA supplementation might augment NO synthesis in the brain, which prevented neuronal apoptosis and facilitated memory improvement and consolidation. In our study, hippocampus and striatum NO levels significantly increased in DHA-treated mice and show the beneficial effects of NO in the behavioral test. For DA, researches over the past decade had clarified that quantitative changes in n-3 fatty acids in the body affect quantitative changes in the DA concentration in the brain, that their effects differ between the mesocortical DA route and mesolimbic DA route and that DA kinetics parallel changes in n-3 fatty acid levels and dopaminergic activities observed in schizophrenia [42–45]. Our study showed that DHA supplementation could significantly increase the levels of DA in the hippocampus and striatum.

The results from the present study suggested that DHA intake could induce expression of BDNF protein that had a relationship with learning and memory. BDNF might contribute to NO-sensitive learning and memory processes and might modulate the release of DA through the activation of tyrosine receptor kinase B [40,41,43]. On the one hand, DA could regulate motivational processes, motor activity and cognition ability. On the other hand, the interaction of NO and DA actions had been investigated, and bidirectional relations had been shown between NO synthesis and DA release [46,47]. Liu [48] emphasized the significance of NO in prolonging the presence of DA and thereby its efficacy in the synapse. Furthermore, it had been shown that L-arginine induced DA release from the striatum in vivo. NG-nitro-L-arginine, an inhibitor of NOS, markedly reduced the effect of L-arginine on DA release [49]. However, in our study, we found that DHA could increase the levels of NO and DA in the hippocampus and striatum simultaneously. NO–DA dynamics might have the potential to reveal DHA therapeutic strategies to treat various brain disorders. Therefore, the mechanism wherein DHA simultaneously increased the levels of NO and DA in the hippocampus and striatum needs to be further studied.

In conclusion, the present study demonstrated that there was an improvement in cognitive function in aged mice due to DHA supplementation. Furthermore, this work demonstrated that DHA had a positive effect on BDNF, NO and DA. These findings also suggested that DHA supplementation during the aging period might represent a potential means for minimizing certain risk factors that might contribute to the neurochemical alterations associated with neuropsychiatry disorders. Further investigations aimed at clarifying these issues should provide insights into the significance of DHA in neurotrophic factors and NO–DA dynamics.

Acknowledgments

This research was funded by the Sichuan Province Science and Technology Platforms Project, the Cellular and Molecular Biology Experiment Platform Construction Project for Chinese Traditional Medicine and the Sichuan Finance and Education Project (No. 2005172).

The authors thank Mr. Michael Holder for manuscript revision.

References

[1] Hashimoto M, Hossain S, Shimada T, Sugioka K, Yamasaki H, Fujii Y, et al. Docosahexaenoic acid provides protection from impairment of learning ability in Alzheimer's disease model rats. J Neurochem 2002; 81:1084–91.

[2] Catalan J, Moriguchi T, Slotnick B, Murthy M, Greiner RS, Salem Jr N. Cognitive deficits in docosahexaenoic acid-deficient rats. Behav Neurosci 2002;116:1022–31.

[3] Lim SY, Suzuki H. Dose–response effect of docosahexaenoic acid ethyl ester on maze behavior and brain fatty acid composition in adult mice. Int J Vitam Nutr Res 2002;72:77–84.

[4] Kalmijn S, Launer LJ, Ott A, Witteman JC, Hofman A, Breteler MM. Dietary fat intake and the risk of incident dementia in the Rotterdam Study. Ann Neurol 1997;42:776–82.

[5] Suzuki H, Morikawa Y, Takahashi H. Effect of DHA oil supplementation on intelligence and visual acuity in the elderly. World Rev Nutr Diet 2001;88:68–71.

[6] McGahon BM, Martin DS, Horrobin DF, Lynch MA. Age-related changes in synaptic function: analysis of the effect of dietary supplementation with omega-3 fatty acids. J Neurosci 1999;94: 305–14.

[7] Yamada K, Nakayama S, Nakayama S, Komori Y, Sugihara H, Hasegawa T, et al. Role of nitric oxide in learning, and memory and in monoamine metabolism in the rat brain. Br J Pharmacol 1995;115: 852–8.

[8] Prast H, Philippu A. Nitric oxide as modulator of neuronal function. Prog Neurobiol 2001;64:51–68.

[9] Fin C, Dacunha C, Bromberg E, Schmitz PK, Bianchin M, Medina JH, et al. Experiments suggesting a role for nitric oxide in the hippocampus in memory process. Neurobiol Learn Mem 1995;63: 113–5.

[10] Huang AM, Lee EH. Role of hippocampal nitric oxide in memory retention in rats. Pharmacol Biochem Behav 1995;50: 327–32.

[11] Bernabeu R, De Stein MI, Fin C, Izquierdo I, Medina JH. Role of hippocampal NO in the acquisition and consolidation of inhibitory avoidance learning. NeuroReport 1995;6:1498–500.

[12] Wise RA. Dopamine, learning and motivation. Nat Rev Neurosci 2004; 5:483–94.

[13] Stoof JC, Drukarch B, De Boer P, Westerink BHC, Groenewegen HJ. Regulation of the activity of the striatal cholinergic neurons by dopamine. J Neurosci 1992;47:755–70.

[14] Williams GV, Castner SA. Under the curve: critical issues for elucidating D1 receptor function in working memory. J Neurosci 2006;139:263–76.

[15] Luciana M, Collins PF, Depue RA. Opposing roles. modulation of human spatial working memory functions. Cereb Cortex 1998;8: 218–26.

[16] Ciccarelli R, Ballerini P, Sabatino G, Rathbone MP, D'Onofrio M, Caciagli F, et al. Involvement of astrocytes in purine-mediated

reparative processes in the brain. Int J Dev Neurosci 2001;19: 395–414.
[17] Logan AC. Neurobehavioral aspects of omega-3 fatty acids: possible mechanisms and therapeutic value in major depression. Altern Med Rev 2003;8:410–25.
[18] Rao JS, Ertley RN, Lee HJ, DeMar Jr JC, Arnold JT, Rapoport SI, et al. n-3 polyunsaturated fatty acid deprivation in rats decreases striatum BDNF via a p38 MAPK-dependent mechanism. Mol Psychiatry 2007; 12:36–46.
[19] Wu A, Ying Z, Gomez-Pinilla F. Dietary omega-3 fatty acids normalize BDNF levels, reduce oxidative damage, and counteract learning disability after traumatic brain injury in rats. J Neurotrauma 2004;21: 1457–67.
[20] Su HM, Bernardo L, Mirmiran M, Ma XH, Corso TN, Nathanielsz PW, et al. Dietary 18:3n-3 and 22:6n-3 as sources of 22:6n-3 accretion in neonatal baboon brain and associated organs. Lipids 1999;34: S347–50.
[21] Kremer JM, Lawrence DA, Jubitz W, Digiacomo R, Ryans R, Bartholomex LE, et al. Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis. Arthritis Rheum 1990;33:810–20.
[22] Simopoulos AP. Summary of the NATO advanced research workshop on dietary omega 3 and omega 6 fatty acids: biological effects and nutritional essentiality. J Nutr 1989;119:521–8.
[23] Hacioglu G, Kose O, Aslan M, Agar A. Beneficial effects of docosahexaenoic acid on active avoidance performance in 1K–1C hypertensive rats. Neurobiol Learn Mem 2007;87:159–65.
[24] Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite and [15N] nitrate in fluids. Anal Biochem 1982;126:131–8.
[25] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
[26] Nitta A, Furukawa Y, Hayashi K, Hiramatsu M, Kameyama T, Hasegawa T, et al. Denervation of dopaminergic neurons with 6-hydroxydopamine increases nerve growth factor content in rat brain. Neurosci Lett 1992;144:152–6.
[27] Bradford MM. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem 1976;72:248–54.
[28] Akbar M, Calderon F, Wen Z, Kim HY. Docosahexaenoic acid: a positive modulator of Akt signaling in neuronal survival. Proc Natl Acad Sci U S A 2005;102:10858–63.
[29] Glomset JA. Role of docosahexaenoic acid in neuronal plasma membranes. Sci STKE 2006;321:1–6.
[30] Salem Jr N, Litman B, Kim HY, Gawrisch K. Mechanisms of action of docosahexaenoic acid in the nervous system. Lipids 2001;36:945–59.
[31] Minami M, Kimura S, Endo T, Hamaue N, Hirafuji M, Togashi H, et al. Dietary docosahexaenoic acid increases cerebral acetylcholine levels and improves passive avoidance performance in stroke-prone spontaneously hypertensive rats. Pharmacol Biochem Behav 1997;58: 1123–9.
[32] Murer MG, Yan Q, Raisman-Vozari R. Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. Prog Neurobiol 2001;63:71–124.
[33] Adlard PA, Cotman CW. Voluntary exercise protects against stress-induced decreases in brain-derived neurotrophic factor protein expression. J Neurosci 2004;124:985–92.
[34] Adlard PA, Perreau VM, Cotman CW. The exercise-induced expression of BDNF within the hippocampus varies across life-span. Neurobiol Aging 2005;26:511–20.
[35] Pang PT, Teng HK, Zaitsev E, Woo NT, Sakata K, Zhen S, et al. Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. Science 2004;306:487–91.
[36] Zafra F, Castren E, Thoenen H, Lindholm D. Interplay between glutamate and gamma-aminobutyric acid transmitter systems in the physiological regulation of brain-derived neurotrophic factor and nerve growth factor synthesis in hippocampal neurons. Proc Natl Acad Sci U S A 1991;88:10037–41.
[37] Xiong H, Yamada K, Han D, Nabeshima T, Enikolopov G, Carnahan J, et al. Mutual regulation between the intercellular messengers nitric oxide and brain-derived neurotrophic factor in rodent neocortical neurons. Eur J Neurosci 1999;11:1567–76.
[38] Mizuno M, Yamada K, Olariu A, Nawa H, Nabeshima T. Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. J Neurosci 2000;20: 2122–30.
[39] Han D, Yamada K, Senzaki K, Xiong H, Nawa H, Nabeshima T. Involvement of nitric oxide in pentylenetetrazol-induced kindling in rats. J Neurochem 2000;74:792–8.
[40] Paredes D, Granholm ACh, Bickford PC. Effects of NGF and BDNF on baseline glutamate and dopamine release in the hippocampal formation of the adult rat. Brain Res 2007;1141:56–64.
[41] Blochl A, Sirrenberg C. Neurotrophins stimulate the release of dopamine from rat mesencephalic neurons via Trk and $p75^{Lntr}$ receptors. J Biol Chem 1996;271:21100–7.
[42] Chalon S, Delion-Vancassel S, Belzung C, Guilloteau D, Leguisquet AM, Besnard JC, et al. Dietary fish oil affects monoaminergic neurotransmission and behavior in rats. J Nutr 1998;128:2512–9.
[43] Chalon S, Vancassel S, Zimmer L, Guilloteau D, Durand G. Polyunsaturated fatty acids and cerebral function: focus on monoaminergic neurotransmission. Lipids 2001;36:937–44.
[44] Delion S, Chalon S, Hérault J, Guilloteau D, Besnard JC, Durand G. Chronic dietary α-linolenic acid deficiency alters dopaminergic and serotoninergic neurotransmission in rats. J Nutr 1994;124:2466–76.
[45] Zimmer L, Delion-Vancassel S, Durand G, Guilloteau D, Bodard S, Besnard JC, et al. Modification of dopamine neurotransmission in the nucleus accumbens of rats deficient in n-3 polyunsaturated fatty acids. J Lipid Res 2000;41:32–40.
[46] Fujiyama F, Masuko S. Association of dopaminergic terminals and neurons releasing nitric oxide in the rat striatum: an electron microscopic study using NADPH-diaphorase histochemistry and tyrosine hydroxylase immunohistochemistry. Brain Res Bull 1996; 40:121–7.
[47] Hidaka S, Totterdell S. Ultrastructural features of the nitric oxide synthase-containing interneurons in the nucleus accumbens and their relationship with tyrosine hydroxylase-containing terminals. J Comp Neurol 2001;431:139–54.
[48] Liu Y. Nitric oxide influences dopaminergic processes. Adv Neuroimmunol 1996;6:259–64.
[49] Strasser A, McCarron RM, Ishii H, Stanimirovic D, Spatz M. L-Arginine induces dopamine release from the striatum in vivo. NeuroReport 1994;5:2298–300.