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Molecular mechanism of polyunsaturated fatty acids improving spatial learning and memory in developmental rat

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Abstract

Study on the effects and mechanisms of different oils on spatial learning and memory in developing rats. Fifty-six Sprague Dawley (SD) rats with primary weaning were randomly divided into the 7 groups, DHA, walnut oil, perilla oil, safflower seed oil, α-linoleic acid, and Essential fatty acid (EFA)-deficient and negative control. Morris water maze behavioral test was performed after 8 weeks of continuous feeding. Real-time fluorescence quantification and immunoblotting were performed to evaluate changes in the expression of NR1, CREB and c-Fos in rat hippocampus, qPCR detected the expression in hippocampal cells. The results showed that the rats fed various oils significant improvement in the Morris water maze test. The mRNA expression of NR1, CREB and c-Fos in the hippocampus of rats fed with various oils were significantly up-regulated ($P<0.05$ and $P<0.01$), and CREB and c-Fos proteins expression were up-regulated ($P<0.05$). The expression of genes and proteins in hippocampus of EFA-deficient control was not significantly different from negative control. It is suggested that polyunsaturated fatty acids could significantly improve the learning and memory ability of rats, which may be through regulating the mRNA expression of cfos, CREB and NR1 in rat hippocampus and the synthesis of CREB and c-Fos proteins.

Keywords: polyunsaturated fatty acids; spatial learning and memory; qPCR; western blot.

Practical Application: DHA, perilla oil, walnut oil, safflower oil and α-linoleic acid were used to feed the developing rats. Morris water maze experiment was used to investigate the effects of various oils on learning and memory in rats. RT-PCR and western-bolt was used to detected expression of CREB and c-Fos genen and proteins.The explore of mechanism of PUFAs to improve learning and memory in rats from the molecular level, its providing a direction for further research on the mechanism of PUFAs to improve learning and memory.

1 Introduction

Polyunsaturated Fatty Acids (PUFAs) are linear fatty acids containing two or more double bonds and having a carbon chain length of 18-22 carbon atoms. PUFAs are classified into n-3, n-6, n-7 and n-9 series according to their structural characteristics and metabolic transformation methods in humans. Among them, n-3 and n-6 series of PUFAs play a central role in the function and normal development of the brain and central nervous system (Bo et al., 2017). DHA and perilla oil are representative of n-3 PUFAs, and walnut oil, safflower oil, α-linoleic acid are representative of n-6 PUFAs.

The brain has highest lipid content in the human body, and 35% of the brain composition is PUFAs. n-3 and n-6 PUFAs play a very important role in the normal functioning of the normal development and function of the brain and central nervous system. The intake of PUFAs is insufficient or unbalanced, it will have a serious impact on brain function during the growth and development stage (van der Wurff et al., 2020). The supplementation of PUFAs is necessary to maintain the normal function of the adult brain, especially learning and memory (Fan et al., 2022). The supplementation of PUFAs in infants and young children can promote brain growth and development in children (Zou et al., 2021; Mun et al., 2019), and delay the decline of brain memory function in middle-aged and old age (Alex et al., 2020) and reduced risk of Alzheimer's disease (Wood et al., 2022; Sala-Vila et al., 2021). Hippocampus, as a structure in the brain that regulates learning and emotions, is a good research target for many neuroscientists. The long-term potentiation (LTP) of the hippocampus is a synaptic model of memory by activating a post-synaptic glutamate receptor subtype NMD receptor (N-methyl-D-aspartate receptors). The supplementation with DHA can enhance synaptic plasticity and spatial learning and memory (Pérez et al., 2018).

The explore of mechanism of PUFAs to improve learning and memory in rats from the molecular level, its providing a direction for further research on the mechanism of PUFAs to improve learning and memory in the future. In the present study, DHA, perilla oil, walnut oil, safflower oil and α-linoleic acid were used to feed the developing rats, and Carboxy methyl cellulose sodium (CMC) was used as a control. Morris water maze experiment was used to investigate the effects of various oils on learning and memory in rats. The expression of immediate early genes c-Fos, cAMP response element binding protein (CREB) and NMDA

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receptor subunit (NR1) mRNA in hippocampus was detected by RT-PCR. The expression of CREB and c-Fos proteins in rat hippocampus were detected by western-bolt technique. The cell model was constructed by cultured hippocampal neurons in vitro, and the hippocampal neurons were detected by RT-PCR mRNA expression of c-Fos, CREB and NR1.

2 Materials and methods

2.1 Materials

DHA (purity 35%) was purchased from Hubei Fuxing Biotechnology Co, Ltd. perilla oil (purity 77.1%), and walnut oil (purity 79.5%) were purchased from Hubei Li Shizhen Health Oil Co, Ltd. safflower seed (purity 83.2%) was purchased from Xinjiang Tacheng Honghuayuan Technology Co, Ltd. α-Asiaoleic acid (purity 100%) waspurchased from Zhengzhou Zhongxin Chemical Products Co, Ltd. sodium carboxy methyl cellulose (CMC) was purchased from Lianyungang Youjin Food Additive Technology Development Co, Ltd. Ordinary feed (formula: corn 45%, 35% powder, 25% bran, 5% fish meal, 10% soybean meal, 4% bone meal, 0.5% salt, 0.5% multidimensional) was purchased from Tongji Medical College, Huazhong University of Science and Technology. Special feed (deducted from unsaturated fatty acids, formula: corn 18%, fish meal 4%, bran 12%, flour 33%, soybean meal 31.5%, bone meal 1%, salt 0.5%) purchased from Hubei Province experiment Animal Research Center.

Clean-grade primary weaning SD rats weighing (80 ± 10) g, provided by Hubei Experimental Animal Research Center, license number: SCXK (E) 2018-0005. This study was approved by the Research Ethics Committee of Wuhan Polytechnic University [No. WHPU20181107]. TRIzol Reagent (total RNA extraction kit)Invitrogen; All-in-One TM First-Strand cDNA Synthesis Kit, All-in-One TM qPCRMix (Sybr Green Fluorescent Dye Quantitative PCR) GeneCopoeia TM; NR1, CREB, c-Fos and GAPDH primers were synthesized by Beijing Dingguo Biotechnology Co, Ltd. Other chemicals used were of analytical reagent grade.

2.2 Methods

Animal grouping and feeding

Fifty-six Male Sprague-Dawley rats (8-9 weeks old weighing 180-200 g) were purchased from Tonji Medical College of Huazhong University of Science and Technology (Wuhan, China). The rats were housed five per cage and maintained in a 12-hour day/night cycle and kept under standard laboratory conditions with a room temperature of 22 °C ± 1, relative humidity 60% ± 10%, and 20 air changes per hour. After acclimatized on laboratory conditions for 1 week before the experiment and randomly allocated to seven groups of 8 animals each fed for 16 weeks: negative control group (general feed), EFA deletion (feed of PUFA), DHA (300 mg/kg·d), perilla oil (2.445 mL/kg·d), walnut oil (2.445 mL/kg·d), safflower seed oil (1.6503 g/kg·d), α-linoleic acid (1.3203 g/kg·d). This dosage is based on the recommended consumption of edible oil in the Dietary Balance Pagoda of the Chinese Nutrition Society for 25 g per person per day, which is converted into the equivalent dose of the rat by the corresponding conversion, and then refer to the previous experimental results (Liang et al., 2011; Wang et al., 2013). The oral dose of perilla oil and walnut oil was determined to be 2.445 mL/kg·d, and then the equivalent α-linoleic acid group and safflower were determined according to the content of α-linoleic acid in walnut oil. The intragastric dose of the seed oil and the intragastric dose of the DHA are the best doses determined according to the experimental results of the previous period.

Each group of rats was given the same amount of feed per day, and they were given free drinking water. At the same time, each group of rats was orally administered. The experimental group required to administer the test substance required 5% carboxymethyl cellulose for the test substance. The sodium solution was diluted so that the test substance was better absorbed in the rats, the negative control and the EFA-deficient were intragastrically administered with a corresponding dose of 5% carboxymethy lcellulose solution. The stomach was administered for 8 weeks every three days. The body weight of the rats was weighed to change the dose administered.

Morris water maze test

The Morris water maze consists of a stainless steel circular pool, automatic videorecording and computer analysis processing system. The pool is 150 cm in diameter and 50 cm high, and the water temperature is controlled at 22 ± 1 °C. A camera with a display system is installed above the pool to simultaneously record the movement of the rat in the pool. The circular pool is divided into four different quadrants, and the platform is placed 2 cm below the second quadrant horizontal plane, with the intermediate points of the four quadrants as the water inlet points. During the whole experiment, the reference material around the water maze was kept unchanged, and the surrounding environment was kept quiet while the experimenter was not in the field of view of the rat. On the 1st day before the experiment, the rats were allowed to swim freely for 2 minutes in the pool to adapt to the environment. The experiment was divided into two parts: positioning navigation experiment and space search experiment.

Positioning navigation experiment, the rats were placed in the water from the water inlet points of the four quadrants in the pool wall, and the time from the water entering to the platform was recorded, that is escape latency. After the rat climbed the platform, let it stay on the platform for 15 s. If the rat still does not find the platform within 90 s, it is towed to the platform and allowed to stay on the platform for 15 s. The escape latency is recorded as 90 s, with a training interval of 15 s. After training for 5 days, the average of the five quadrants to escape the incubation period was used as the escape latency of the day, and the escape latency of the 5th was the final score to test the learning ability of the rats.

Space exploration experiment, on the 6th day, the space exploration experiment was carried out. The platform was removed, and the rats were placed into the water from the quadrant (fourth quadrant) farther from the original platform position (second quadrant), and the rats were recorded to cross the platform within 90s. The number of positions and the effective

time ratio (i.e, the ratio of the swimming time of the rat in the quadrant of the original platform to the total swimming time) to detect the memory ability of the rat.

Gene expression analysis of NR1, CREB and c-Fos

Total RNA from rat hippocampus tissue was extracted according to the TRIzol extraction kit instructions. Reverse transcription synthesis of cDNA was carried out according to the reverse transcription kit method. Finally, the real-time PCR was performed according to the SybrGreen kit. The expression of the target gene was detected by three-step RT-PCR. The primer design of the gene is shown in Table 1. RT-PCR conditions were as follows: pre-denaturation at 95 °C for 10 min; denaturation at 95 °C for 10 s, annealing at 58 °C for 20 s, extension at 72 °C for 20 s, where fluorescence was collected, cycled 40 times; finally incubated at 16 °C for 30 min.

Western blot detection of CREB and c-Fos proteins expression in rat hippocampus

Equal amounts of protein (20-30 μg) were separated on 5-12% SDS-PAGE gels and transferred to polyvinylidene fluoride membranes. After blocking in a gelatin-NET solution, blots were incubated with Rabbit CREB antibody(1:800) (Epitomics Inc.), Rabbit c-Fos antibody(1:800) (Epitomics Inc.), Mouse GAPDH antibody(1:1000) (Epitomics Inc.)at 4 °C overnight. Then, bands were incubated with specific horse radish peroxidase (HRP)-conjugated secondary antibodies (1:1000) at room temperature for 1 h and visualized by enhanced chemiluminescence (ECL) substrate with UVP Auto Chemi Image system (UVP Inc, Upland, CA, USA). Protein loading was evaluated by anti-GADPH antibody (1:1000). Band intensities were quantified using UVP LabWork 4.5 software (UVP Inc, Upland, CA, USA).

Primary culture of hippocampal neuronal cells

The newborn rats within 24 hours, after disinfection in 75% alcohol, as for the sterile ice pack, directly broke the head, quickly dissected the meninges and removed the hippocampus tissue under the dissecting microscope, then used the pre-cooled D-hanks solution and washed twice. It was cut to a size of 0.5 mm 3 (suspended state), then an equal volume of 0.25% trypsin was added to maintain a final trypsin concentration of 0.125%, digested at 37 °C for 10 min, and gently shaken once every 3 min during digestion. After the end of digestion, added an equal volume of planting medium to terminated the digestion, pipet several times, filtered on 200 mesh cell sieve, centrifuged at 1000r/min for 5min, discarded the supernatant, left the precipitate (precipitation is the cell), and finally used the planting medium to suspend. The cells were transferred to an ornithine-treated cell culture flask and cultured at 37 °C in a 5% CO_2 incubator.

Grouping of hippocampal neuronal cells

After primary culture, hippocampal neurons were divided into six groups: negative control group, DHA, perilla oil, α-linoleic acid, walnut oil and safflower seed oil. After primary cultured hippocampal neurons were cultured for 24 hours, the fresh oil medium was exchanged, and the above five oil stock solutions were added. The amount of the addition was based on the study of Pan Jianping and the concentration of DHA was determined to be 50 umol/L. The remaining fats were converted according to the concentration of DHA. The negative control group was added with an equal volume of medium, and after 24 hours, the cells were collected and RNA was extracted.

2.3 Statistical analysis

The experimental results were expressed by x ± s, and statistical analysis was performed using SPSS19.0 statistical software and prism6.0.1 software. The water maze data were statistically analyzed by one-way ANOVA and LSD test, and the quantitative PCR data were statistically analyzed by T test. *Compared with the negative control group, there was a significant difference ($p<0.05$); **Compared with the negative control group, there was a significant difference ($p<0.01$); #Compared with the negative deletion group, significant Differences ($p < 0.05$); ##Compared with the negative deletion group, there was a significant difference ($p <0.01$).

3 Results

3.1 Determination of spatial learning and memory ability of Morris water maze

After 5 days of directional navigation test, the daily escape latency gradually decreased. The decreasing trend of escape latency was different in each group, including DHA group, safflower seed oil group, walnut oil group, perilla oil group and α-linoleic acid group. The change trend of negative control group was more consistent with that of EFA deficient control group (Figure 1).

In the water maze experiment (Table 2), the escape latency of each group was significantly shorter than that of the negative control group ($p < 0. 05$). The shortest escape latency was (10.01 ± 3.65) s in safflower seed oil group and (9.96 ± 4.69) s in DHA group. The effective time ratio of target quadrant in all groups was significantly higher than that of negative control

Table 1. Primers used in qPCR.

Gene	Forward Primer(5'- 3')	Reverse Primer(5'- 3')
CREB	ACCCAGGGAGGAGCAATACA	GGTGCTGTGCGAATCTGGTAT
c-fos	CGGTCAAGAACATTAGCAACAT	AGGAACCAGACAGGTCCACAT
NR1	AAGCTGCACGCCTTTATCTG	TCTCATGGGACTTGAGTATGGA
GAPDH	CGCTAACATCAAATGGGGTG	TTGCTGACAATCTTGAGGGAG

Note: GAPDH is reference gene.

Table 2. Effect of different grease matter on the ablilty of learning and memory of mice in the morris water maze test (x ± s, n=8).

Groups	Escape latency(s)	Target quadrant time ratio	Correct annulus crossings(number)
DHA	9.96 ± 4.69*##	0.34 ± 0.06**##	3.38 ± 1.30*
perilla oil	10.12 ± 3.93*##	0.30 ± 0.05*	3.25 ± 1.39*
α-linoleic acid	12.20 ± 3.59*#	0.29 ± 0.03*	2.57 ± 2.07
walnut oil	10.88 ± 1.51*#	0.31 ± 0.10*	3.25 ± 1.04*
safflower seed oil	10.01 ± 3.65*##	0.30 ± 0.03*#	3.38 ± 1.30*
negative control	15.81 ± 3.75	0.23 ± 0.23	1.50 ± 1.38
Essential fatty acid (EFA)-deficient	15.75 ± 4.96	0.25 ± 0.04	2.13 ± 1.55

Note: *Compared with the negative control group, there was a significant difference ($p < 0.05$); **Compared with the negative control group, there was a significant difference ($p < 0.01$); #Compared with the negative deletion group, there was a significant difference ($p < 0.05$); ##Compared with the negative deletion group, there was a very significant difference ($p < 0.01$). There was no significant difference between the two groups.

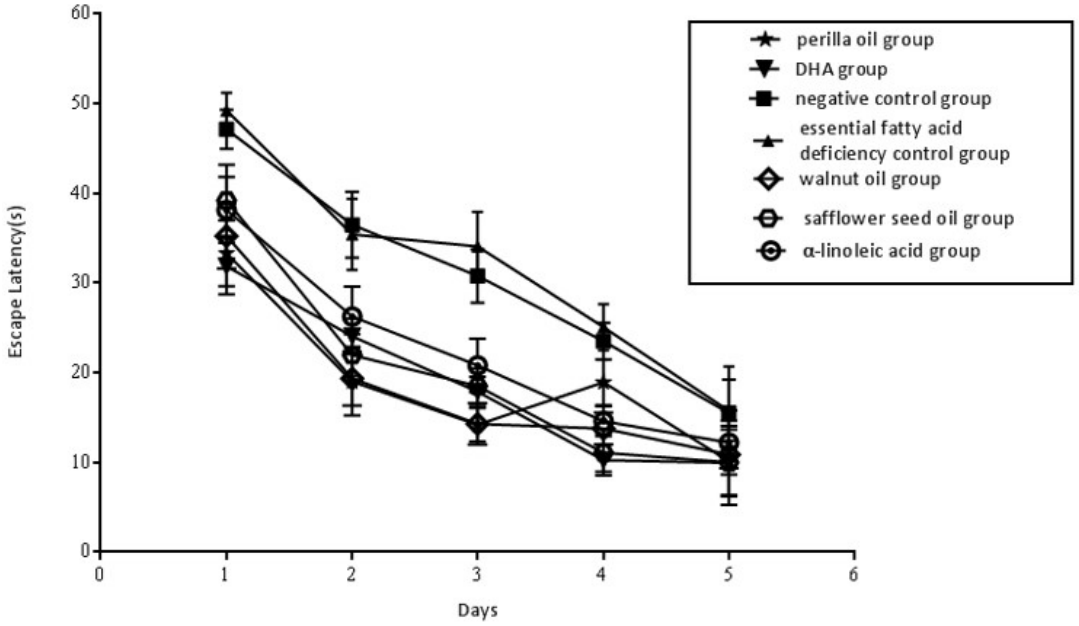

Figure 1. Escape latency variation tendency of rats in five days.

group ($p < 0.05$). DHA group was significantly higher than that of negative control group ($p < 0.01$). The highest target quadrant effective time ratio was in walnut oil group and DHA group, which were 0.31 ± 0.10 and 0.34 ± 0.06, respectively. The crossing times of DHA, walnut oil, perilla oil group and safflower seed oil group were significantly higher than that of negative control group ($p < 0.05$), but there was no significant difference between α -linoleic acid group and negative control group. DHA group (3.38 ± 1.30) and safflower seed oil group (3.38 ± 1.30) times traversing the platform were the most frequent ones. These results suggest that the PUFAs could improving the ability of learning and memory in rats, but the degree and focus are different. And the loss of PUFAs in daily diet can reduce the learning and memory ability of rats, but the degree of reduction is not very obvious.

The Morris water maze experiment mainly tests the effect of experimental PUFAs on learning and memory from the perspective of animal behavior. Then, from the perspective of genetics, the expression of genes in the hippocampus of mice was detected. Changes to explore the mechanism of PUFAs in improving spatial learning and memory in the rats.

3.2 Effects of different PUFAs on the expression of NR1, CREB and c-Fos mRNA in rat hippocampus

Compared with the negative control, the mRNA expression of NR1 in each sample group was significantly increased ($p<0.05$), and the relative expression of NR1 in the hippocampus of DHA and walnut oil was higher than those of other groups ($P<0.01$). There was no significant difference in the mRNA expression of NR1 in the EFA-deficient (Figure 2a).

The mRNA expression of CREB in all five groups was significantly higher than that in the negative control ($P<0.05$ and $P<0.01$) . Both of the DHA and the perilla oil were larger.

The mRNA expression of CREB in the EFA-deficient was not significantly different with the negative control (Figure 2b).

The mRNA expression levels of c-Fos in DHA, perilla oil and the α-linoleic acid in the hippocampus were significantly increased (P<0.05 and P<0.01) . The mRNA expression of c-Fos in hippocampus of DHA group and perilla oil group was slightly higher than that in other groups. There was no significant difference between EFA-deficient and negative control (Figure 2c).

3.3 Effects of different PUFAs on the expression of CREB and c-Fos protein in rat hippocampus

The relative expression levels of CREB protein of negative control, EFA-deficient control, walnut oil, safflower oil, α-linoleic acid, perilla oil and DHA were 1 ± 0, 1.03 ± 0.68, 2.68 ± 1.01, 2.97 ± 1.46, 3.39 ± 1.43, 4.19 ± 1.57, 4.04 ± 1.71 (Figure 3). All five measured variables for relative expression of CREB protein in the hippocampus was significant compared with negative control and EFA-deficient control. Upward floating (P<0.05). There was no significant difference in the relative expression of CREB protein in rat hippocampus with negative control and EFA-deficient control.

The relative expression levels of c-Fos protein (Figure 4) of negative control, EFA-deficient control, walnut oil, safflower oil, α-linoleic acid, perilla oil and DHA were 1 ± 0, 0.96 ± 0.76, 4.16 ± 1.77, 5.07 ± 1.53, 4.17 ± 1.50, 5.43 ± 1.91, 5.94 ± 1.61. The relative expression of c-Fos protein in of the five groups was significantly up-regulated (P<0.05) compared with negative

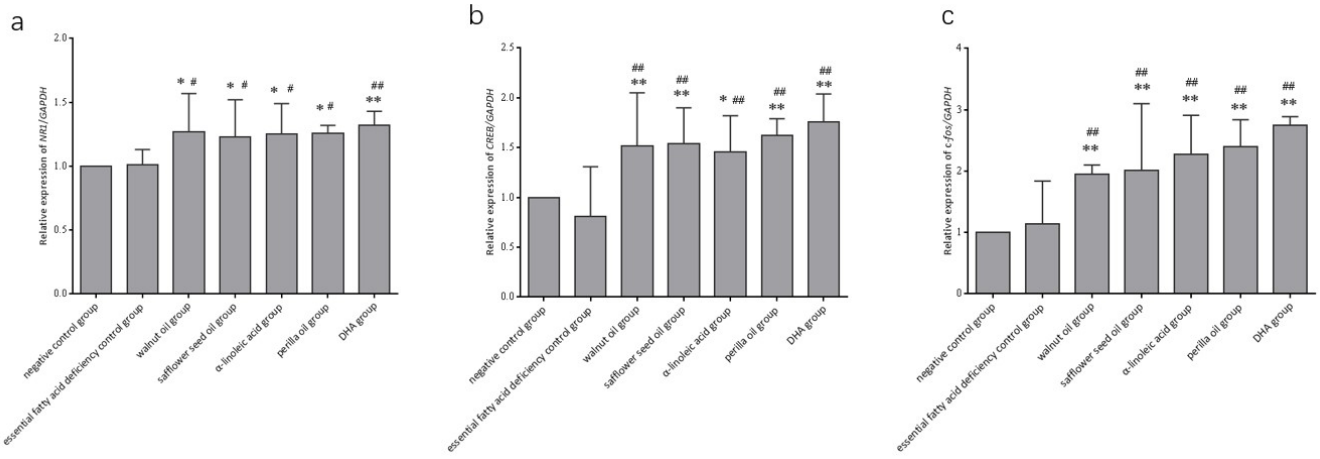

Figure 2. The mRNA expression of hippocampus of rats. (a) NR1; (b) CREB; (c) c-Fos.

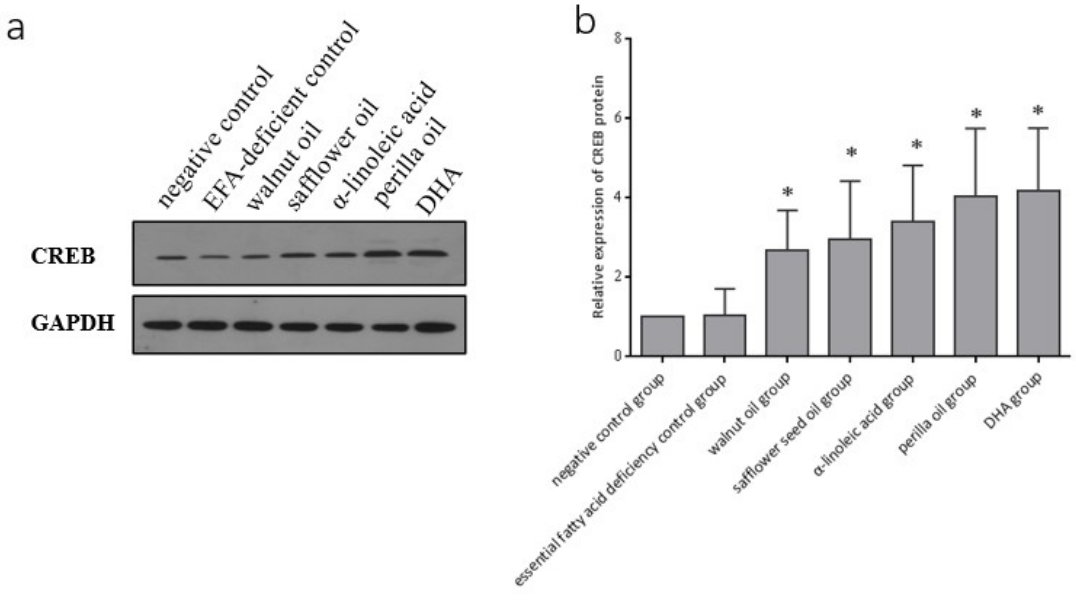

Figure 3. The relative protein expression of CREB in hippocampus of rats. (a) Western blotting of CREB treated with different oil. (b)The average of protein band grayscale.

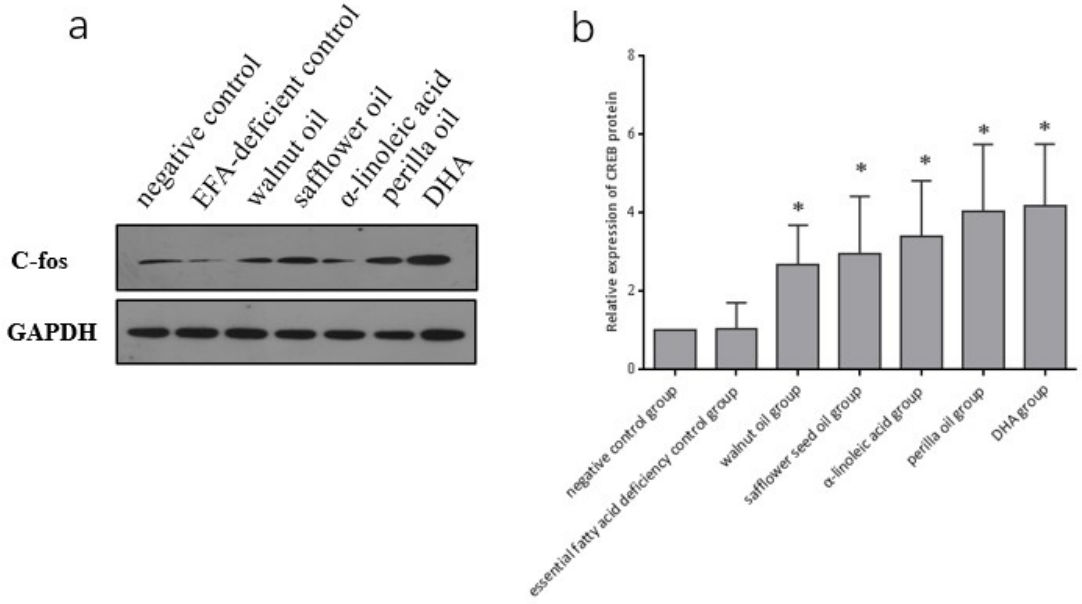

Figure 4. The relative protein expression of c-Fos in hippocampus of rats. (a) Western blotting of c-Fos treated with different oil. (b) The average of protein band grayscale.

control and EFA-deficient control. No significant differences were found among the relative expression of c-Fos protein of negative control and EFA-deficient control.

3.4 Expression of NR1, CREB and c-Fos in hippocampal neurons

After inoculation, hippocampal neurons were individually floating in the cell culture medium. The cells were small, round and translucent. After then (1 h of culture), the cells began to adhere. Within 8 h-12 h, a few hippocampal neurons began to grow. Then (24 h of culture), most of the cells were completely adherent, and some of the cells were extended. Short protrusions, at this time, the hippocampal neurons were mostly fusiform, small, refractive, and there is halo around the cells. At 48 h of culture, the volume of the cells increased slightly, and the protrusion increased further and became more obvious, the cultured mature hippocampal neurons are pleomorphic. The cells were mostly pyramidal, triangular, and fusiform, but mostly pyramidal. The volume of the cells was obviously larger, and the diameter could reach 6-12μm.

Five kinds of stock solutions of DHA, perilla oil, α-linoleic acid, walnut oil and safflower seed oil were added to the medium. After 24 hours, NR1, CREB and c-Fos in all groups of hippocampal neurons were observed. Expressed and presented in a floating trend. It can be seen from the results of RT- PCR that the NR1, CREB and c-Fos genes of hippocampal neurons in the five medium stock solution were expressed in different degrees. The relative expression levels of NR1 gene in DHA, perilla oil, α-linoleic acid, walnut oil and safflower oil were 1.22 ± 0.22, 1.23 ± 0.23, 1.18 ± 0.17, 1.19 ± 0.15, 1.20 ± 0.18 (Figure 5a). The relative expression levels of CREB gene in DHA, perilla oil, α-linoleic acid, walnut oil and safflower oil were 1.64 ± 0.28, 1.55 ± 0.25, 1.49 ± 0.31, 1.42 ± 0.24, 1.54 ± 0.36 (Figure 5b). The relative expression levels of c-Fos gene in DHA, perilla oil, α-linoleic acid, walnut oil and safflower oil were 1.33 ± 0.23, 1.31 ± 0.29, 1.29 ± 0.23, 1.30 ± 0.28, 1.31 ± 0.25 (Figure 5c). The results showed that the relative expression of NR1, CREB and c-Fos in hippocampal neurons were significantly higher than that in the negative control ($P<0.05$), and the relative expression of NR1, CREB and c-Fos in the perilla oil and DHA were higher than that in other groups.

4 Discussion

The NR1 subunit is an important component of the N-methyl-D-aspartate (NMDA) receptor and belongs to the postsynaptic membrane protein involved in NMDA receptor-dependent LTP. Formation is closely related to synaptic plasticity and learning and memory functions of the hippocampus. An L mention that the reduced expression of NR1 related to the impairment of formation of long-term spatial memory, particularly consolidation memory (An & Sun, 2018). Lyu et al. (2020) reported that the inhibition of NR1 subunit transcription and protein phosphorylation can lead to significant deficits in learning and memory function and the impairment of hippocampal LTP. The cAMP response element binding protein (CREB) is an important regulator in the nucleus of eukaryotic cells (Bartolotti & Lazarov, 2019). CREB plays a wide range of physiological functions in the body, especially in the nervous system (Belgacem & Borodinsky, 2017). CREB can regulate synaptic plasticity and learning and memory activities as well as participate in neuronal regeneration, and is closely related to the formation of LTP (Nakagawasai et al., 2020; Meyer Zu Reckendorf et al., 2022). The transcriptional activity of CREB is regulated by autophosphorylation. Phosphorylated CREB can bind to the target gene CRE in the promoter region of the gene and enhance the transcription of downstream genes, such as the immediate early gene c-Fos (Gandolfi et al., 2017), brain-

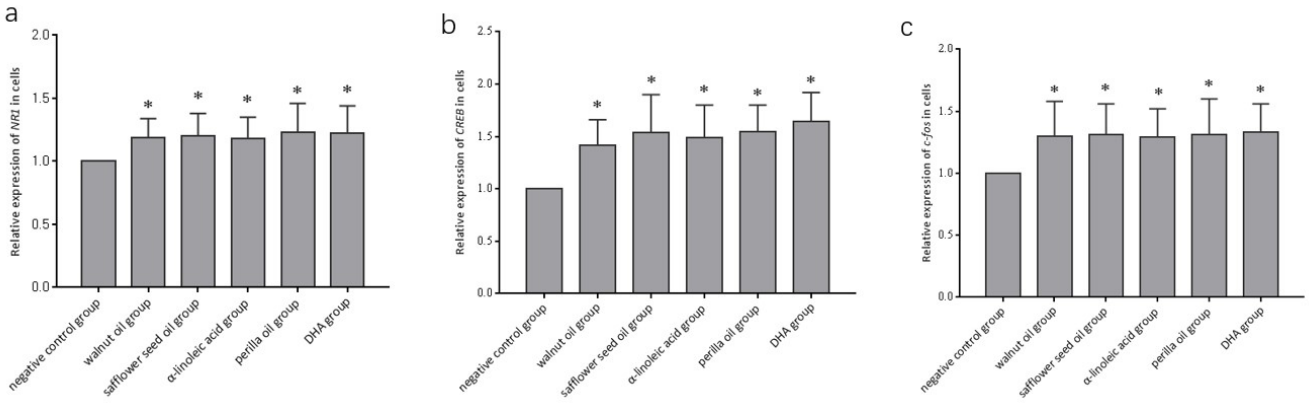

Figure 5. The relative gene expression of in hippocampal neurons treated with different oil after 24 hours. (a) NR1; (b) CREB; (c) c-Fos.

derived neurotrophic factor. The immediate early gene c-Fos is closely related to the central nervous system and learning and memory, and its mRNA expression is related to the production of LTP. Observing the intrinsic relationship of NR1, CREB and c-Fos, we found that both are present in hippocampus and are associated with LTP.

Hippocampus is a structure closely related to learning and memory in the brain. It plays an important role in the acquisition and consolidation of learning and memory (Cui et al., 2022). At present, LTP and hippocampal synaptic plasticity have been recognized as the material basis of learning and memory (Yang & Liu, 2022; Chen et al., 2022). The formation of LTP is undergoing two stages of induction and maintenance. The formation of LTP in hippocampus is affected by both the release of presynaptic neurotransmitters and the NMDA receptor channel in the postsynaptic membrane, both of which are at the synapse (Watson et al., 2016). As one of the subunits of the NMDA receptor, NR1 is involved in the formation of NMDA receptor-dependent LTP. Normally, NMDA receptor-regulated voltage-gated channels are blocked by Mg^{2+}. At resting potential, NMDA is inhibited by Mg^{2+}. When the postsynaptic membrane depolarized, the Mg^{2+} will separated from the channel. At this time, the receptor and the transmitter were combined, the channel opened, and Ca^{2+} influx enters the cell. Ca^{2+} ions as a second messenger activate cAMP-dependent protein kinase, Ca^{2+}/calmodulin-dependent protein kinase signaling pathway (Ca^{2+}/CAMKs), and the like. At this time, the phosphorylated CREB binds to a DNA regulatory sequence CRE (Luo et al., 2017), thereby activating the transcriptional function of CREB and promoting various downstream genes. Transcription, such as the proto-oncogene c-Fos, which is closely related to learning and memory. The c-Fos gene can encode the c-Fos protein, and the c-Fos protein binds to another proto-oncogene Jun to form a heterodimer, which then binds to the AP1 locus and affects the expression of the target gene (Alfonso-Gonzalez & Riesgo-Escovar, 2018). The extracellular signal of action is coupled to long-term changes in intracellular function, ultimately affecting the formation of LTP.

Based on the results of the Morris water maze test, the RT-PCR test of hippocampal tissue and hippocampal neurons, and the western-bolt experiment of hippocampus, we found that supplementing the n-3 series of DHA, perilla oil and n-6 series α-linoleic acid, walnut oil and safflower seed oil increased the learning and memory of rats, and the expression of NR1, CREB and c-Fos protein increased with the up-regulation of NR1, CREB and c-Fos mRNA expression. Our findings in this study hypothesized that the PUFAs improves learning and memory in rats by up-regulating the mRNA expression of NR1, CREB, c-Fos, and relative expression of CREB and c-Fos proteins.

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References

Alex, A., Abbott, K. A., McEvoy, M., Schofield, P. W., & Garg, M. L. (2020). Long-chain omega-3 polyunsaturated fatty acids and cognitive decline in non-demented adults: a systematic review and meta-analysis. *Nutrition Reviews*, 78(7), 563-578. http://dx.doi.org/10.1093/nutrit/nuz073. PMid:31841161.

Alfonso-Gonzalez, C., & Riesgo-Escovar, J. R. (2018). Fos metamorphoses: lessons from mutants in model organisms. *Mechanisms of Development*, 154, 73-81. http://dx.doi.org/10.1016/j.mod.2018.05.006. PMid:29753813.

An, L., & Sun, W. (2018). Acute melamine affects spatial memory consolidation via inhibiting hippocampal NMDAR-dependent LTD in rats. *Toxicological Sciences*, 163(2), 385-396. http://dx.doi.org/10.1093/toxsci/kfx039. PMid:28206646.

Bartolotti, N., & Lazarov, O. (2019). CREB signals as PBMC-based biomarkers of cognitive dysfunction: a novel perspective of the brain-immune axis. *Brain, Behavior, and Immunity*, 78, 9-20. http://dx.doi.org/10.1016/j.bbi.2019.01.004. PMid:30641141.

Belgacem, Y. H., & Borodinsky, L. N. (2017). CREB at the crossroads of activity-dependent regulation of nervous system development and function. *Advances in Experimental Medicine and Biology*, 1015, 19-39. http://dx.doi.org/10.1007/978-3-319-62817-2_2. PMid:29080019.

Bo, Y., Zhang, X., Wang, Y., You, J., Cui, H., Zhu, Y., Pang, W., Liu, W., Jiang, Y., & Lu, Q. (2017). The n-3 polyunsaturated fatty acids

supplementation improved the cognitive function in the chinese elderly with mild cognitive impairment: a double-blind randomized controlled trial. *Nutrients*, 9(1), 54. http://dx.doi.org/10.3390/nu9010054. PMid:28075381.

Chen, L., Wang, H., Cha, S., Li, J., Zhang, J., Wu, J., Guo, G., & Zhang, J. (2022). Phosphorylation of spastin promotes the surface delivery and synaptic function of AMPA receptors. *Frontiers in Cellular Neuroscience*, 16, 809934. http://dx.doi.org/10.3389/fncel.2022.809934. PMid:35418834.

Liang, C., Wang, L., Guo, Y., Xiao, N., Tao, X., Liu, Z., Liu, D., & Liu, L. (2011). Effects of walnut oil, perilla oil, α-linolenic acid and linoleic acid on learning and memory in rats. *Chinese Oil*, 36(10), 33-37.

Cui, X., Zhang, R., Yang, Y., Wu, E., Tang, Y., Zhao, Z., Li, C., Yang, L., Teng, X., Ye, Y., Cui, Y., Xu, F., Su, Z., Wang, D., Zhang, D., Yang, Y., Sun, J., Luo, J., Zhang, S., Chen, R., & Xi, J. J. (2022). Identification and characterization of long non-coding RNA Carip in modulating spatial learning and memory. *Cell Reports*, 38(8), 110398. http://dx.doi.org/10.1016/j.celrep.2022.110398. PMid:35196493.

Fan, R., Hua, Y., Shen, J., Xiao, R., & Ma, W. (2022). Dietary fatty acids affect learning and memory ability via regulating inflammatory factors in obese mice. *The Journal of Nutritional Biochemistry*, 103, 108959. http://dx.doi.org/10.1016/j.jnutbio.2022.108959. PMid:35158028.

Gandolfi, D., Cerri, S., Mapelli, J., Polimeni, M., Tritto, S., Fuzzati-Armentero, M. T., Bigiani, A., Blandini, F., Mapelli, L., & D'Angelo, E. (2017). Activation of the CREB/c-Fos pathway during long-term synaptic plasticity in the cerebellum granular layer. *Frontiers in Cellular Neuroscience*, 11, 184. http://dx.doi.org/10.3389/fncel.2017.00184. PMid:28701927.

Luo, Y., Kuang, S., Li, H., Ran, D., & Yang, J. (2017). cAMP/PKA-CREB-BDNF signaling pathway in hippocampus mediates cyclooxygenase 2-induced learning/memory deficits of rats subjected to chronic unpredictable mild stress. *Oncotarget*, 8(22), 35558-35572. http://dx.doi.org/10.18632/oncotarget.16009. PMid:28415673.

Lyu, Y., Ren, X. K., Zhang, H. F., Tian, F. J., Mu, J. B., & Zheng, J. P. (2020). Sub-chronic administration of benzo[a]pyrene disrupts hippocampal long-term potentiation via inhibiting CaMK II/PKC/PKA-ERK-CREB signaling in rats. *Environmental Toxicology*, 35(9), 961-970. http://dx.doi.org/10.1002/tox.22932. PMid:32255272.

Meyer Zu Reckendorf, S., Moser, D., Blechschmidt, A., Joga, V. N., Sinske, D., Hegler, J., Deininger, S., Catanese, A., Vettorazzi, S., Antoniadis, G., Boeckers, T., & Knöll, B. (2022). Motoneuron-specific PTEN deletion in mice induces neuronal hypertrophy and also regeneration after facial nerve injury. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, 42(12), 2474-2491. http://dx.doi.org/10.1523/JNEUROSCI.1305-21.2022. PMid:35149515.

Mun, J. G., Legette, L. L., Ikonte, C. J., & Mitmesser, S. H. (2019). Choline and DHA in maternal and infant nutrition: synergistic implications in Brain and Eye Health. *Nutrients*, 11(5), 1125. http://dx.doi.org/10.3390/nu11051125. PMid:31117180.

Nakagawasai, O., Lin, J. R., Odaira, T., Takahashi, K., Nemoto, W., Moriguchi, S., Yabuki, Y., Kobayakawa, Y., Fukunaga, K., Nakada, M., & Tan-No, K. (2020). Scabronine G methyl ester improves memory-related behavior and enhances hippocampal cell proliferation and long-term potentiation via the BDNF-CREB pathway in olfactory bulbectomized mice. *Frontiers in Pharmacology*, 11, 583291. http://dx.doi.org/10.3389/fphar.2020.583291. PMid:33281604.

Pérez, M. Á., Peñaloza-Sancho, V., Ahumada, J., Fuenzalida, M., & Dagnino-Subiabre, A. (2018). n-3 polyunsaturated fatty acid supplementation restored impaired memory and GABAergic synaptic efficacy in the hippocampus of stressed rats. *Nutritional Neuroscience*, 21(8), 556-569. http://dx.doi.org/10.1080/1028415X.2017.1323609. PMid:28482757.

Sala-Vila, A., Arenaza-Urquijo, E. M., Sánchez-Benavides, G., Suárez-Calvet, M., Milà-Alomà, M., Grau-Rivera, O., González-de-Echávarri, J. M., Crous-Bou, M., Minguillón, C., Fauria, K., Operto, G., Falcón, C., Salvadó, G., Cacciaglia, R., Ingala, S., Barkhof, F., Schröder, H., Scarmeas, N., Gispert, J. D., Molinuevo, J. L., Beteta, A., Brugulat-Serrat, A., Cañas, A., Carranza, N., Deulofeu, C., Dominguez, R., Emilio, M., Hernandez, L., Huesa, G., Huguet, J., Knezevic, I., Marne, P., Menchón, T., Polo, A., Pradas, S., Shekari, M., Soteras, A., Vilanova, M., & Vilor-Tejedor, N. (2021). ALFA study. DHA intake relates to better cerebrovascular and neurodegeneration neuroimaging phenotypes in middle-aged adults at increased genetic risk of Alzheimer disease. *The American Journal of Clinical Nutrition*, 113(6), 1627-1635. http://dx.doi.org/10.1093/ajcn/nqab016. PMid:33733657.

van der Wurff, I. S. M., Meyer, B. J., & de Groot, R. H. M. (2020). Effect of omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) Supplementation on cognition in children and adolescents: a systematic literature review with a focus on n-3 LCPUFA blood values and dose of DHA and EPA. *Nutrients*, 12(10), 3115. http://dx.doi.org/10.3390/nu12103115. PMid:33053843.

Wang, L., Ye, C., Guo, Y., Xiao, N., Ma, L., Liu, Z., & Liu, L. (2013). Effects of DHA oil and other four oils on spatial learning and memory, lipid peroxidation and lipid levels in rats. *Chinese Oil*, 38(3), 34-38.

Watson, D. J., Ostroff, L., Cao, G., Parker, P. H., Smith, H., & Harris, K. M. (2016). LTP enhances synaptogenesis in the developing hippocampus. *Hippocampus*, 26(5), 560-576. http://dx.doi.org/10.1002/hipo.22536. PMid:26418237.

Wood, A. H. R., Chappell, H. F., & Zulyniak, M. A. (2022). Dietary and supplemental long-chain omega-3 fatty acids as moderators of cognitive impairment and Alzheimer's disease. *European Journal of Nutrition*, 61(2), 589-604. http://dx.doi.org/10.1007/s00394-021-02655-4. PMid:34392394.

Yang, Y., & Liu, J. J. (2022). Structural LTP: signal transduction, actin cytoskeleton reorganization, and membrane remodeling of dendritic spines. *Current Opinion in Neurobiology*, 74, 102534. http://dx.doi.org/10.1016/j.conb.2022.102534. PMid:35398661.

Zou, R., El Marroun, H., Voortman, T., Hillegers, M., White, T., & Tiemeier, H. (2021). Maternal polyunsaturated fatty acids during pregnancy and offspring brain development in childhood. *The American Journal of Clinical Nutrition*, 114(1), 124-133. http://dx.doi.org/10.1093/ajcn/nqab049. PMid:33742211.