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The Salutary Effects of DHA Dietary Supplementation on Cognition, Neuroplasticity, and Membrane Homeostasis after Brain Trauma

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Abstract

The pathology of traumatic brain injury (TBI) is characterized by the decreased capacity of neurons to metabolize energy and sustain synaptic function, likely resulting in cognitive and emotional disorders. Based on the broad nature of the pathology, we have assessed the potential of the omega-3 fatty acid docosahexaenoic acid (DHA) to counteract the effects of concussive injury on important aspects of neuronal function and cognition. Fluid percussion injury (FPI) or sham injury was performed, and rats were then maintained on a diet high in DHA (1.2% DHA) for 12 days. We found that DHA supplementation, which elevates brain DHA content, normalized levels of brain-derived neurotrophic factor (BDNF), synapsin I (Syn-1), cAMP-responsive element-binding protein (CREB), and calcium/calmodulin-dependent kinase II (CaMKII), and improved learning ability in FPI rats. It is known that BDNF facilitates synaptic transmission and learning ability by modulating Syn-I, CREB, and CaMKII signaling. The DHA diet also counteracted the FPI-reduced manganese superoxide dismutase (SOD) and Sir2 (a NAD+-dependent deacetylase). Given the involvement of SOD and Sir2 in promoting metabolic homeostasis, DHA may help the injured brain by providing resistance to oxidative stress. Furthermore, DHA normalized levels of calcium-independent phospholipase A2 (iPLA2) and syntaxin-3, which may help preserve membrane homeostasis and function after FPI. The overall results emphasize the potential of dietary DHA to counteract broad and fundamental aspects of TBI pathology that may translate into preserved cognitive capacity.

Key words: brain-derived neurotrophic factor; plasticity; Sir2; superoxide dismutase; traumatic brain injury

Introduction

Traumatic brain injury (TBI) is one of the most common causes of death and disability in the United States, with 220,000 hospitalizations, 52,000 deaths from head trauma, and 80,000–90,000 patients suffering from permanent disability each year. The total costs of TBI in the U.S. are about $44 billion each year (Sosin et al., 1995; Waxweiler et al., 1995). Even though over 30 major clinical trials have been done, no efficient treatment for TBI has been found to date. It is well known that TBI results in long-lasting consequences for the cognitive ability of patients. Animal studies show that the pathology of TBI is characterized by a wide range of cellular and molecular effects, including membrane damage, oxidative stress, and synaptic dysfunction. These in turn likely reduce the capacity of the brain to process information crucial for supporting cognitive function. We have previously shown that dietary supplementation with fish oil (an enriched source of docosahexaenoic acid [DHA]; C22: 6n-3) before injury onset can protect the brain from the deleterious effects of TBI on cognition and plasticity (Wu et al., 2004). However, whether DHA exerts protection after TBI occurs is poorly understood. DHA, a crucial omega-3 fatty acid abundant in brain, is important for brain development and plasticity, and has been shown to support learning and memory in animal models of neurodegenerative disorders such as Alzheimer's disease (Hashimoto et al., 2002; Lim et al., 2005). A recent report showed that DHA supplementation for 30 days reduced axonal injury in brainstem white matter tracts in a rodent head acceleration injury model (Bailes and Mills, 2010). In the current study, we investigated the healing capacity of DHA dietary supplementation when provided immediately after a concussive injury.

Given the important role of DHA in supporting membrane integrity and fluidity, we assessed levels of molecules related

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to the metabolism of phospholipids in the plasma membrane, such as calcium-independent phospholipase A2 (iPLA2) and syntaxin-3 (STX-3). For example, iPLA2 has been shown to cleave membrane phospholipids, resulting in a free fatty acid and a lysophospholipid (Farooqui et al., 2004). It has been reported that iPLA2 plays an important role in long-term potentiation and certain aspects of cognitive function (Wolf et al., 1995). STX-3, which is a structural component of synaptic membranes, has been reported to be under the regulation of DHA, which has important implications for synaptic function. It has been reported that supplementation of DHA increases synaptic STX-3 levels (Cansev and Wurtman, 2007), thereby contributing to neurite outgrowth and membrane expansion (Darios and Davletov, 2006).

The function of brain-derived neurotrophic factor (BDNF) has been linked to regulation of synaptic plasticity and cognitive function (Adasme et al., 2011; Kang et al., 1997; Sajikumar and Korte, 2011; Tanaka et al., 2008), which is compromised after TBI (Wu et al., 2003). In turn, our previous studies have shown that omega-3 fatty acid supplementation counteracted the reduction of BDNF seen after TBI (Wu et al., 2004). Synapsin I, which can be activated by BDNF (Jovanovic et al., 1996,2000), plays an important role in neural development (Fornasiero et al., 2010), synaptic transmission (Cesca et al., 2010), and neurite outgrowth (Wang et al., 2011). BDNF also activates cAMP-responsive element-binding protein (CREB), a transcription factor involved in learning and memory (Finkbeiner, 2000). By acting on the trkB receptor, the BDNF-mediated calcium/calmodulin-dependent kinase II (CaMKII) signaling system is required for learning and memory (Elgersma et al., 2004). In this study, we investigated the altered BDNF and its downstream effectors that may mediate the beneficial effects of DHA on cognition and plasticity.

Methods

Experimental design and tissue preparation

Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing between 200 and 240 g were housed in cages and maintained in environmentally-controlled rooms (22–24°C) with a 12-h light/dark cycle. After acclimatization for 1 week on standard rat chow, the rats were subjected to fluid percussion injury (FPI) or sham surgery. Then they were maintained on a DHA-enriched diet (1.2% DHA; Nordic Naturals, Santa Cruz, CA) or a regular diet for 12 days. The rats were divided into three groups: (1) RD-sham (regular diet), (2) RD-FPI, and (3) DHA-FPI; a RD-sham group was used as a control. At 1 week post-surgery the rats ($n = 12$ in each group) were tested in the Morris water maze (MWM) for learning ability. The rats, still on the diet, were then killed by decapitation; the fresh tissues including the hippocampus were dissected, frozen in dry ice, and stored at −70°C until use for biochemical analyses. The diets, fed *ad libitum*, were provided in powder form. The average food intake per day was: RD-sham (34.4 ± 2.1 g), FPI-RD group (33.2 ± 3.1 g), and FPI-DHA group (32.4 ± 1.9 g). There was no difference in food intake in all groups. All experiments were performed in accordance with the United States National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, and animal suffering was minimized.

Fluid percussion injury

The injury was performed as previously described (Wu et al., 2003). In brief, with the aid of a microscope (Wild, Heerburg, Switzerland) a 3.0-mm-diameter craniotomy was made 3.0 mm posterior to the bregma and 6.0 mm lateral (left) to the midline with a high-speed drill. A plastic injury cap was placed over the craniotomy with silicone adhesive and dental cement. When the dental cement hardened, the cap was filled with 0.9% saline solution. Anesthesia was discontinued and the injury cap was attached to the fluid percussion device. At the first sign of hindlimb withdrawal to a paw pinch, a mild fluid percussion pulse (1.5 atm) was administered. Sham animals underwent identical preparation with the exception of the FPI. Immediately upon responding to a paw pinch, anesthesia was restored and the skull was sutured. Neomycin was applied to the sutures and the rats were placed in a heated recovery chamber for approximately 1 h before being returned to their cages. There is evidence indicating that there are effects of mild FPI on learning performance in water maze testing. For example, it has been reported that mild FPI results in significant cognitive deficits as assessed by water maze testing (Hicks et al., 1993; Pillay et al., 2005).

Morris water maze

Cognitive function was tested in a MWM by referring to previously-described reports (Vaynman et al., 2007; Wu et al., 2004). The MWM is a well-accepted test to assess hippocampus-dependent spatial learning (Kenney and Gould, 2008; Morris et al., 1982; Riedel et al., 1999). A swimming pool 130 cm in diameter and 50 cm high was divided into four quadrants, which represent separate zones. The target zone is the quadrant where the escape platform (12 cm in diameter) was located in a fixed position 2 cm under the water's surface. The other three quadrants were the left, right, and opposite zones. The water was kept at a steady 22 ± 2°C. The rats were trained in the water maze with 2 consecutive trials per day for 5 days as previously described (Vaynman et al., 2007). The rats were placed in the tank facing the wall in one of the equally-spaced start locations that were randomly changed after every trial. Each trial lasted until the rat found the platform or for a maximum of 1 min. If the rat failed to find the platform in the allocated time, it was gently placed on the platform and it stayed there for 1 min. The escape latencies were recorded.

Western blot

Hippocampal tissue from the left hemisphere was homogenized in lysis buffer containing 137 mM NaCl, 20 mM Tris-HCl (pH 8.0), 1% NP40, 10% glycerol, 1 mM PMSF, 10 μg/mL aprotinin, 0.1 mM benzethonium chloride, and 0.5 mM sodium vanadate. The homogenates were then centrifuged, the supernatants were collected, and total protein concentration was determined according to the MicroBCA procedure (Pierce, Rockford, IL), using bovine serum albumin as a standard. Levels of BDNF, Syn-I, CREB, CaMKII, SOD, Sir2, 4-hydroxynonenal (4-HNE), iPLA2, and STX-3 were analyzed by Western blot. Briefly, protein samples were separated by electrophoresis on an 8% polyacrylamide gel and electrotransferred to a nitrocellulose membrane. Non-

specific binding sites were blocked in TBS overnight at 4°C, with 2% BSA and 0.1% Tween-20. The membranes were rinsed for 10 min in buffer (0.1% Tween-20 in TBS), and then incubated with anti-actin or anti-BDNF, Syn-I, CREB, CaMKII, SOD, 4-HNE, iPLA2, and STX-3 (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA). or Sir2 (1:1000; Upstate, Billerica, MA), followed by anti-rabbit IgG horseradish peroxidase-conjugate (Santa Cruz Biotechnology). After rinsing with buffer, the immunocomplexes were visualized by chemiluminescence using an ECL kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ) according to the manufacturer's instructions. The film signals were digitally scanned and then quantified using NIH ImageJ software. Actin was used as an internal control for Western blot such that data were standardized according to actin values.

Lipid analysis

Total lipids were extracted from cerebral cortical tissues with a lysis solution of chloroform-methanol (2:1 vol:vol) containing 0.005% butylated hydroxytoluene. The ratio of sample to lysis solution was 1:20 (i.e., 100 mg tissue + 2 mL lysis solution). After centrifugation at 9000 rpm for 10 min, the chloroform layer was transferred to a 15-mL tube, and then mixed with 0.9% saline. After centrifugation again, the chloroform layer was transferred to another 15-mL tube and dried under nitrogen. The dried sample of total lipids was dissolved in hexane and methylated by methanol in the presence of BF3 at 90°C for lipid analysis. Fatty acid profiles were determined using gas chromatography (GC). The system consisted of a Clarus 500 gas chromatograph (PerkinElmer, Waltham, MA) with a built-in autosampler. An Elite-WAX column (60 m, 0.32-mm internal diameter; PerkinElmer) was used, with hydrogen as the carrier gas. The GC oven temperature was initially held at 140°C for 2 min, and it was raised with a gradient of 5°C min^{-1} up to 250°C, and held there for 10 min. The total run time was 34 min. The injector and detector were maintained at 250° and 300°C, respectively. A 1-μL sample of fatty acid methyl esters (FAME) was injected in split injection mode with a 100:1 split ratio. Peaks of resolved FAME were identified and quantified by comparison with standards (Supelco® 37-component FAME mix; Sigma-Aldrich, Carlsbad, CA).

Statistical analysis

For the Western blots, the values were expressed as a ratio of actin value, and then converted to percentages of RD-sham as presented in the figures, and are represented as the mean ± standard error of the mean (SEM). All statistical analyses were performed by SPSS 16.0 software. The MWM data were analyzed by repeated-measures analysis of variance (ANOVA). The Western blot data were analyzed by one-way ANOVA followed by Bonferroni's *post-hoc* test. Statistical differences were considered significant at $p < 0.05$.

Results

DHA content in the brain

DHA content in the brain was measured by GC. The results showed that there was a significant group effect for DHA content in the brain by ANOVA ($F_{2,33} = 7.27$; $p < 0.05$). The DHA content was significantly higher in the DHA-fed FPI group (14%) than in the RD-fed FPI group (12.6%, $p < 0.05$), and RD-fed sham group (12.9%, $p < 0.05$). Fatty acid contents are shown in Table 1.

Table 1. Fatty Acid Composition in Rat Brain[a]

Fatty acid	*Sham-RD*	*FPI-RD*	*FPI-DHA*
C14:0	0.26 ± 0.10	0.23 ± 0.06	0.28 ± 0.07
C16:0	19.94 ± 1.01	19.67 ± 1.28	21.14 ± 1.08
C16:1	0.21 ± 0.02	0.20 ± 0.03	0.22 ± 0.02
C18:0	19.88 ± 0.80	19.00 ± 1.13	19.94 ± 1.33
C18:1	15.89 ± 1.12	14.66 ± 0.67	15.29 ± 0.97
C18:2n-6	0.47 ± 0.04	0.44 ± 0.07	0.49 ± 0.10
C20:0	0.34 ± 0.03	0.28 ± 0.03	0.27 ± 0.04*
C20:3n-6	0.26 ± 0.03	0.25 ± 0.02	0.30 ± 0.07
C20:4n-6	9.06 ± 0.56	8.70 ± 0.97	8.93 ± 0.85
C22:0	0.40 ± 0.06	0.35 ± 0.04	0.31 ± 0.04*
C22:5n-6	0.44 ± 0.04	0.40 ± 0.12	0.43 ± 0.13
C22:6n-3 (DHA)	12.90 ± 0.29	12.63 ± 0.71	14.04 ± 0.69*,**
C24:0	0.76 ± 0.04	0.68 ± 0.07	0.60 ± 0.09*
C24:1n-9	1.43 ± 0.14	1.19 ± 0.21	1.18 ± 0.24

*$p < 0.05$ versus sham-RD animals.
**$p < 0.05$ versus FPI-RD animals.
[a]Wt% of total fatty acids.
RD, regular diet; FPI, fluid percussion injury; DHA, docosahexaenoic acid.

Cognitive testing

To determine whether DHA can counteract the detrimental effects of TBI on learning performance, we evaluated the effects of dietary DHA supplementation in rats subjected to TBI. Rats were exposed to a regular or DHA-enriched diet for 7 days, followed by the learning test (MWM) for 5 days. Repeated-measures ANOVA showed a significant group effect on learning ($F_{2,33} = 7.7$, $p < 0.05$). The results demonstrated that the rats with TBI performed worse, as evidenced by higher escape latency than the sham rats ($p < 0.05$; Fig. 1). DHA dietary supplementation counteracted learning disability after TBI compared to TBI rats without DHA supplementation ($p < 0.05$; Fig. 1).

Effects on BDNF-related plasticity: BDNF and CaMKII, Syn-I and CREB

The results showed that there was a significant group effect on BDNF levels ($F_{2,33} = 23.9$, $p < 0.05$ by ANOVA). TBI reduced BDNF (70% of RD-sham animals, $p < 0.05$; Fig. 2A) compared to rats fed a regular diet. DHA supplementation counteracted the decrease in BDNF (103% of RD-sham animals, $p > 0.05$; Fig. 2A) in TBI rats. We measured CaMKII levels based on its involvement in hippocampal learning and memory (Vaynman et al., 2007), and a significant group effect on CaMKII levels was seen ($F_{2,33} = 19.2$, $p < 0.01$ by ANOVA). TBI reduced CaMKII levels (76% of RD-sham animals, $p < 0.05$; Fig. 2B) compared to rats fed a regular diet. DHA supplementation increased levels of CaMKII to near-normal values (98% of RD-sham animals; Fig. 2B).

To evaluate the effects of DHA dietary supplementation on the molecular systems associated with the effects of BDNF on synaptic plasticity, we assessed the protein levels of Syn-I and CREB in all groups. The results showed a significant group

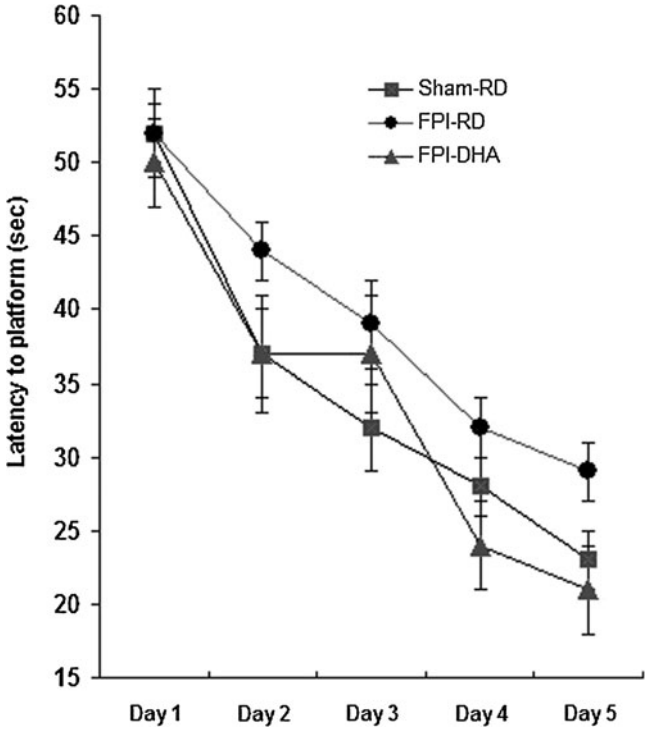

FIG. 1. Docosahexaenoic acid (DHA) supplementation provides protection against cognitive disability in fluid-percussion-injured (FPI) rats. Learning performance was scored as average escape latency to locate the platform in the Morris water maze. The escape latencies were longer in FPI rats compared to sham animals. FPI rats receiving DHA supplementation had lower latencies than untreated FPI rats (on days 4 and 5; RD, regular diet).

effect on Syn-I levels ($F_{2,33} = 11.2$, $p < 0.01$ by ANOVA). TBI resulted in reduced Syn-I levels (74% of RD-sham animals, $p < 0.05$; Fig. 3A) in rats fed a regular diet, and DHA supplementation counteracted the TBI-reduced levels of Syn-I (96% of RD-sham animals; Fig. 3A). We also found that there was a significant group effect on CREB levels ($F_{2,33} = 15.8$, $p < 0.01$ by ANOVA). TBI reduced CREB (78% of RD-sham animals, $p < 0.05$; Fig. 3B) in rats fed a regular diet, and DHA supplementation normalized levels of CREB (104% of RD-sham animals; Fig. 3B).

Effects on oxidative stress: 4-HNE, SOD, and Sir2

To determine the potential of TBI to increase membrane lipid peroxidation and neuronal signaling, we measured hippocampal levels of 4-HNE, which is produced by free radicals reacting with double bands of phospholipids. The multiple bands of HNE-bound proteins were grouped and quantified as previously described (Sharma et al., 2010). There was a significant group effect on 4-HNE ($F_{2,33} = 23.8$, $p < 0.01$ by ANOVA). TBI increased levels of 4-HNE (133% of RD-sham animals, $p < 0.05$; Fig. 4A) in rats fed a regular diet. DHA supplementation significantly reduced levels of 4-HNE (85% of RD-sham animals) in TBI rats compared to untreated TBI rats ($p < 0.05$; Fig. 4A). In addition, we found a significant positive correlation between levels of 4-HNE and latency to find the platform in the MWM task, using the last day of water maze testing data ($r = 0.52$, $p < 0.05$; Fig. 4B), meaning that rats with more 4-HNE performed worse.

We measured SOD levels based on its scavenger capacity, and the fact that FPI elevates levels of oxidative stress (Wu et al., 2004). There was a significant group effect on SOD levels ($F_{2,33} = 16.5$, $p < 0.01$ by ANOVA). TBI reduced SOD levels (73% of RD-sham animals, $p < 0.05$; Fig. 5A), but DHA supplementation normalized the levels of SOD (92% of RD-sham animals; Fig. 5A). We also measured Sir2 levels, since Sir2 has

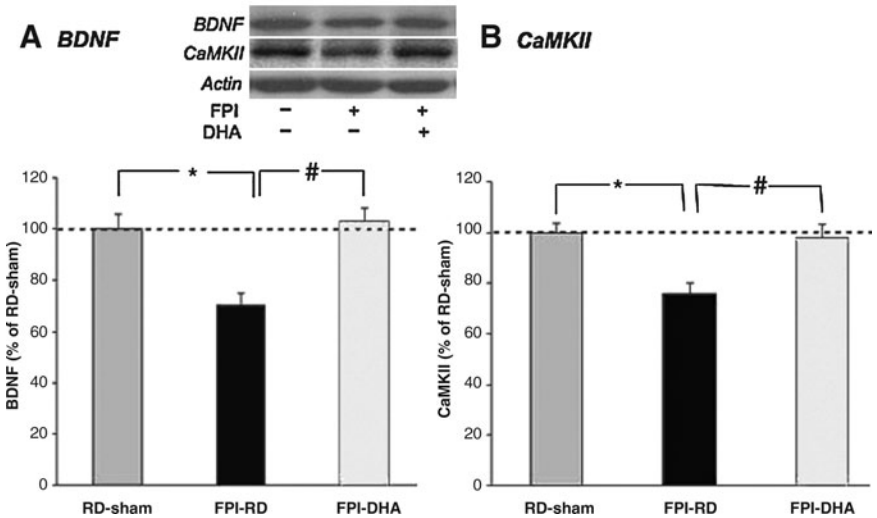

FIG. 2. Effects of docosahexaenoic acid (DHA) supplementation on brain-derived neurotrophic factor (BDNF; **A**) and calcium/calmodulin-dependent kinase II (CaMKII; **B**) levels in the hippocampus of fluid-percussion-injured (FPI) rats. FPI resulted in reductions of BDNF and CaMKII. DHA increased levels of both in FPI rats compared to untreated FPI rats. The values were converted to percentages of RD-sham animals (mean ± standard error of the mean; $^{*}p < 0.05$ versus RD-sham; $^{\#}p < 0.05$ versus untreated FPI animals; RD, regular diet).

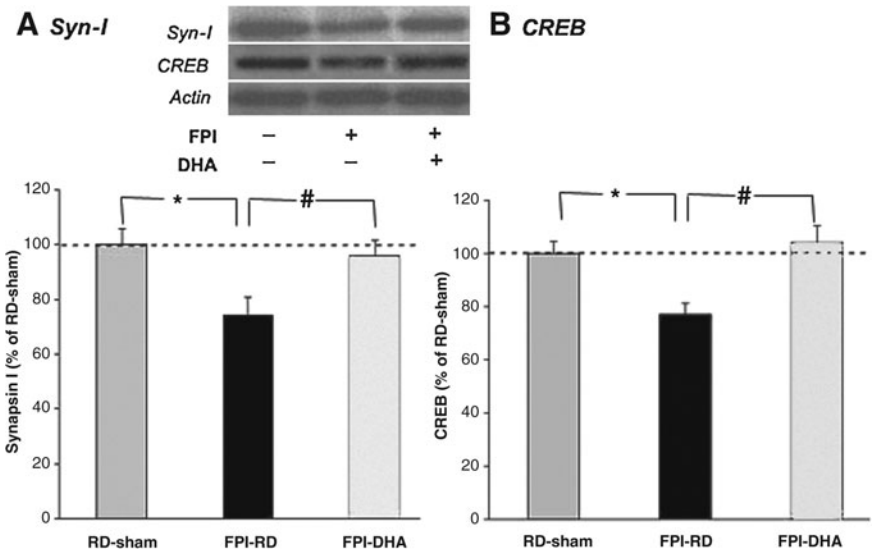

FIG. 3. Effects of docosahexaenoic acid (DHA) supplementation on synapsin I (Syn-I, **A**) and cAMP-responsive element-binding protein (CREB; **B**) levels in the hippocampi of fluid-percussion-injured (FPI) rats. FPI resulted in reductions in Syn-I and CREB. DHA supplementation increased Syn-I and CREB levels in FPI rats compared to untreated FPI rats. The values were converted to percentages of RD-sham animals (mean ± standard error of the mean; $^{*}p < 0.05$ versus RD-sham animals; $^{\#}p < 0.05$ versus untreated FPI animals; RD, regular diet).

been shown to mediate the elevation of the antioxidant system involving SOD, thereby playing an important role in stress resistance and synaptic plasticity. There was a significant group effect on Sir2 levels ($F_{2,33} = 29.5$, $p < 0.05$ by ANOVA). TBI reduced Sir2 (64% of RD-sham animals, $p < 0.05$; Fig. 5B), and this was reversed by DHA supplementation (90% of RD-sham animals; Fig. 5B).

Effects on membrane-related systems: iPLA2 and STX-3

We measured iPLA2 levels based on its involvement in membrane homeostasis, which may influence neuronal signaling and learning and memory. We found a significant group effect on iPLA2 levels ($F_{2,33} = 36.6$, $p < 0.01$ by ANOVA). TBI reduced iPLA2 (65% of RD-sham animals, $p < 0.05$; Fig. 6A), while DHA supplementation increased the levels of iPLA2 (104% of RD-sham animals) compared to untreated TBI rats ($p < 0.05$; Fig. 6A). We also measured syntaxin-3 (STX-3) levels based on its involvement in membrane expansion and synaptic plasticity. There was a significant group effect on STX-3 levels ($F_{2,33} = 27.1$, $p < 0.01$ by ANOVA). TBI reduced STX-3 levels (74% of RD-sham animals, $p < 0.05$; Fig. 6B), but DHA supplementation normalized the levels of STX-3 (97% of RD-sham animals) compared to untreated TBI rats ($p < 0.05$; Fig. 6B).

Discussion

In this study, we provide evidence for the homeostatic effects of DHA dietary supplementation when provided immediately after TBI. A short period of DHA supplementation

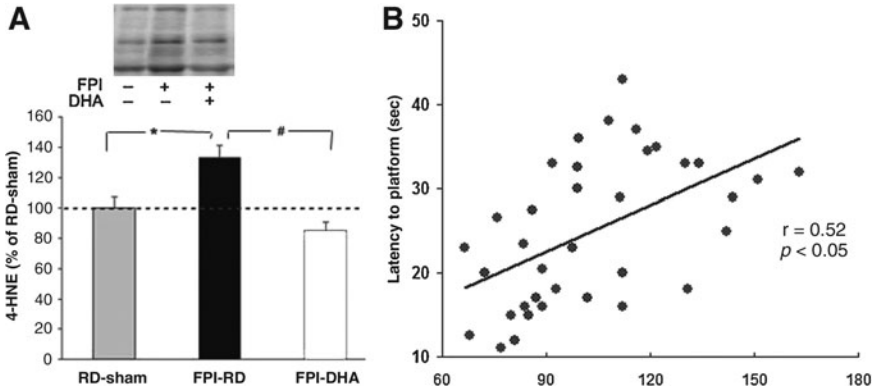

FIG. 4. Effects of docosahexaenoic acid (DHA) supplementation on 4-hydroxynonenal (4-HNE) levels in the hippocampus of fluid-percussion-injured (FPI) rats. (**A**) FPI resulted in an increase in 4-HNE. DHA supplementation reduced 4-HNE levels in FPI rats compared to untreated FPI rats. The values were converted to percentages of RD-sham animals (mean ± standard error of the mean; $^{*}p < 0.05$ versus RD-sham animals; $^{\#}p < 0.05$ versus untreated FPI animals; RD, regular diet). (**B**) Correlation analysis between 4-HNE and latency of the last day of testing in the Morris water maze. Each dot represents a single animal.

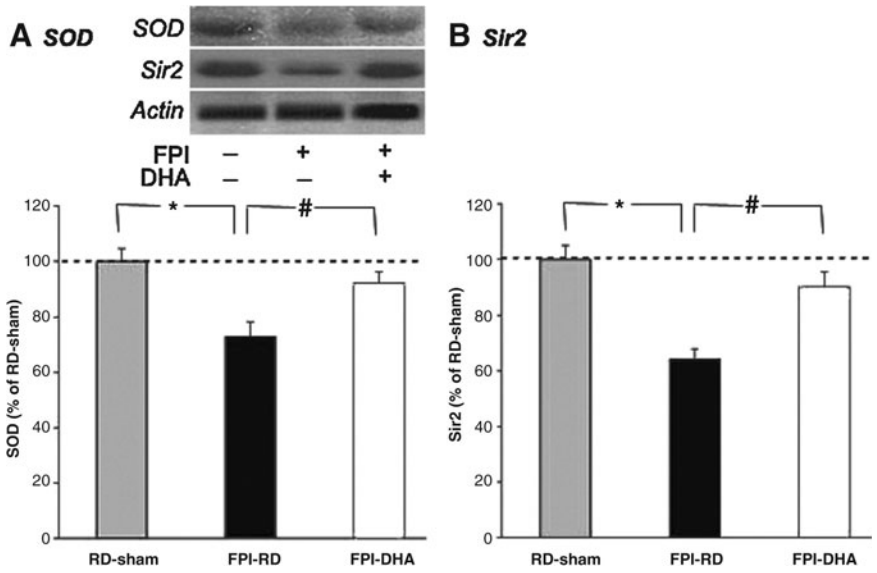

FIG. 5. Effects of docosahexaenoic acid (DHA) supplementation on superoxide dismutase (SOD; **A**) and Sir2 (**B**) levels in the hippocampi of fluid-percussion-injured (FPI) rats. FPI resulted in reductions of SOD and Sir2. DHA supplementation increased SOD and Sir2 in FPI rats compared to untreated FPI rats. The values were converted to percentages of RD-sham animals (mean ± standard error of the mean; $^{*}p < 0.05$ versus RD-sham animals; $^{\#}p < 0.05$ versus untreated FPI animals; RD, regular diet).

elevated DHA content in the brain, and significantly counteracted the negative effects of injury on cognitive function, neuronal signaling (BDNF, Syn-I, CaMKII, and CREB), and membrane homeostasis (4-HNE, SOD, iPLA2, STX-3, and Sir2). These results indicate that DHA supplementation can provide broad protection, which is important for counteracting the effects of TBI. Furthermore, given the role of DHA in membrane homeostasis and neuronal signaling, these findings implicate dietary DHA as a potential candidate for counteracting the adverse effects of TBI on synaptic plasticity and cognition (Fig. 7).

A DHA diet may protect membrane homeostasis after TBI

It is known that proper membrane function is crucial for supporting the neuronal signaling events underlying synaptic plasticity and cognition, such that a loss of membrane

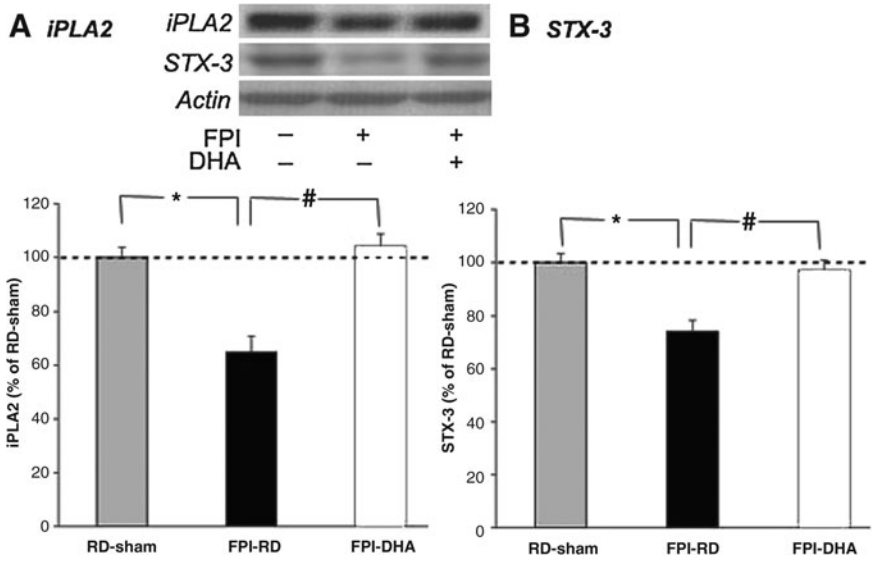

FIG. 6. Effects of docosahexaenoic acid (DHA) supplementation on calcium-independent phospholipase A2 (iPLA2; **A**) and syntaxin-3 (STX-3; **B**) levels in the hippocampi of fluid-percussion-injured (FPI) rats. FPI resulted in reductions of iPLA2 and STX-3. DHA supplementation increased levels of both in FPI rats compared to untreated FPI rats. The values were converted to percentages of RD-sham animals (mean ± standard error of the mean; $^{*}p < 0.05$ versus RD-sham animals; $^{\#}p < 0.05$ versus untreated FPI animals; RD, regular diet).

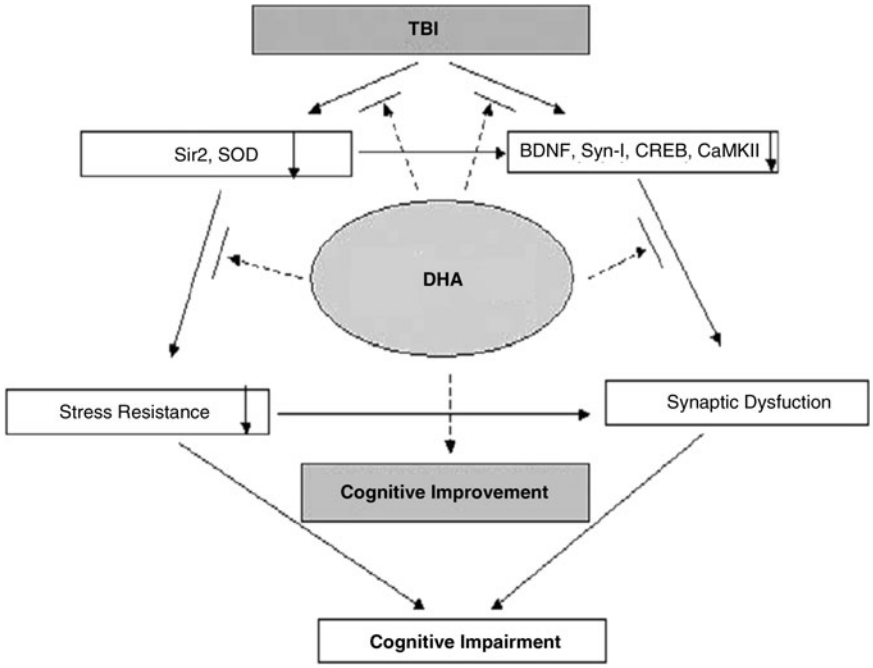

FIG. 7. Possible mechanisms underlying the beneficial effects of docosahexaenoic acid (DHA) dietary supplementation on the injured brain. Sir2 and its induced superoxide dismutase (SOD) may provide protection against insults to the brain, and therefore a reduction of Sir2 and subsequently SOD levels as a consequence of brain injury may reduce its resistance to insults. In turn, dietary supplementation with DHA may protect the brain from trauma by maintaining levels of Sir2 and the antioxidant SOD. Sir2 may provide neuroprotection via mechanisms that promote stress resistance, such as induction of antioxidant gene expression and maintenance of energy homeostasis. Brain-derived neurotrophic factor (BDNF), its downstream effectors synapsin I (Syn-I) and cAMP-responsive element-binding protein (CREB), and calcium/calmodulin-dependent kinase II (CaMKII) play important roles in synaptic plasticity. Reductions of these molecules may result in synaptic dysfunction and subsequent cognitive impairment. Compensation for those reductions via DHA supplementation can reduce the dysfunction of plasticity, thereby leading to cognitive improvement.

function may be responsible for the cognitive impairments observed after TBI (Sharma et al., 2010). We assessed levels of the lipid peroxidation marker 4-hydroxyneoenal (4-HNE) in order to estimate the damage done to plasma membranes, and found that TBI increased hippocampal levels of 4-HNE. 4-HNE can bind to proteins, compromising their structure and function (Neely et al., 1999), and thereby may play an important role in the oxidative damage seen after TBI (Hall et al., 2004). The fact that DHA normalized levels of 4-HNE in TBI rats may be indicative of the protective action of DHA on the membrane. This possibility is supported by our results showing that dietary DHA affected levels of membrane-related iPLA2 and STX-3.

Our results showed that DHA in the diet counteracted the reduction in hippocampal iPLA2 levels observed after TBI. Given the role of iPLA2 in stimulating phospholipid turnover in the membrane, the reductions of iPLA2 may be related to mechanisms that preserve membrane phospholipids. The fact that these changes were accompanied by improvements in learning ability in DHA rats, suggests a relationship between membrane stability and cognitive function. Selective blocking of iPLA2 in rodents has been shown to impair cognitive function (Sato et al., 2007), and to prevent long-term potentiation (Martel et al., 2006). Therefore, our results suggest that a DHA diet may work in conjunction with iPLA2 to preserve learning and memory performance after TBI. DHA treatment counteracted the TBI-related reduction in STX-3 levels, with potential implications for synaptic function. STX-3 is a plasma membrane-bound protein, which is found in neuronal growth cones and is susceptible to the influence of DHA on membrane expansion (Darios and Davletov, 2006). Supplementation of DHA in intact animals has been shown to increase hippocampal levels of STX-3 (Chytrova et al., 2010). In the current study, we found that dietary DHA counteracted the reduction in STX-3 seen after TBI. Therefore, it is possible that the effects of DHA on iPLA2 and STX-3 may help reduce TBI pathology. However, the mechanisms underlying DHA-elevated iPLA2 and STX-3 levels remain to be further explored.

Membrane damage as a result of TBI is reflected in peroxidation of phospholipids, which is evidenced by increases in 4-HNE levels. We found a significant association between hippocampal 4-HNE levels and the latency that rats spent locating the platform (i.e., rats with more 4-HNE performed worse than rats with less 4-HNE). The potential mechanisms linking 4-HNE to the physiopathology of TBI may include: (1) 4-HNE reduces cellular ATP levels and mitochondrial function that may worsen the energy crisis seen after TBI; (2) 4-HNE may lead to a reduction in membrane-bound syntaxin-3 and the NR2B subunit of the NMDA receptor, which is involved in cognition (Sharma et al., 2010); and (3) 4-HNE prevents neurite outgrowth and changes in the neuronal cytoskeleton structure, which in turn contributes to neural dysfunction. Our results showing that DHA counteracted the

changes seen in 4-HNE, iPLA2, and STX-3 elicited by TBI, suggest that DHA's protection may be achieved by promoting membrane homeostasis and synaptic plasticity. It should be noted that changes in 4-HNE were observed 12 days after FPI, which is in contrast with the rapid and transient changes in ROS seen after injury. ROS has an extremely short half-life, which makes direct measurement of ROS virtually impossible (Hillered et al., 2005). Instead of measuring ROS directly, we detected 4-HNE, which is a reliable marker of lipid peroxidation (Deng et al., 2007) as discussed above. We also measured SOD and Sir2, which both play important roles in antioxidant systems.

A DHA diet may increase stress resistance by upregulating SOD and Sir2 after TBI

Our results showed that short-time feeding with DHA significantly upregulated molecules with recognized antioxidant capacity, such as SOD and Sir2. The function of SOD has been associated with the regulatory action of the NAD+-dependent deacetylase Sir2 (Brunet et al., 2004). Emerging evidence shows that Sir2 not only regulates genomic stability and metabolic homeostasis, but that it also plays an important role in synaptic plasticity and cognition. For example, loss of Sir2 function downregulates expression of CREB and BDNF, with subsequent impairment of synaptic plasticity and cognition (Gao et al., 2010). Accordingly, our results showing that DHA counteracts reductions of BDNF and CREB after TBI, in conjunction with the changes seen in Sir2, suggest that these events may contribute to the improvements seen in cognition in TBI animals.

The BDNF system is involved in the effects of the DHA diet on the brain after TBI

The current results indicate that the effects of DHA enrichment on spatial learning performance are accompanied by changes in BDNF-related plasticity. BDNF can facilitate synaptic transmission and mediate signaling pathways implicated in cognition and plasticity. In particular, BDNF can regulate activation of its downstream effectors Syn-I and CREB, both of which play important roles in plasticity and cognition (Finkbeiner, 2000; Jovanovic et al., 1996; Wang et al., 2011; Ying et al., 2002). BDNF regulates the expression and activation of Syn-I, which can affect neural development (Fornasiero et al., 2010), synaptic transmission (Cesca et al., 2010), and neurite outgrowth (Wang et al., 2011). BDNF also regulates activation of CREB, a transcription factor involved in learning and memory (Finkbeiner, 2000). We found that DHA normalized the levels of both Syn-I and CREB in the hippocampus after TBI. Based on the fact that BDNF and its downstream effectors Syn-I and CREB are involved in learning events, our findings suggest that supplementation of DHA in the diet may provide protection against the learning disability seen after TBI via upregulation of BDNF-mediated plasticity (Fig. 7). Our findings also indicate that the DHA-enriched diet upregulates hippocampal CaMKII after TBI. DHA dietary supplementation has been shown to prevent the decrease in CaMKII seen in a transgenic mouse model of Alzheimer's disease (Calon et al., 2005). CaMKII is a signaling system whose action is critical for learning and memory ability (Elgersma et al., 2004), and plays a role in hippocampal-dependent cognitive enhancement (Vaynman et al., 2007).

DHA supplementation increases DHA content in the brain, which may help preserve membrane fluidity and integrity and enhance cognitive function

Our results showed that DHA supplementation significantly increased DHA content in the brains of TBI rats. In turn, the brains of TBI rats showed a trend toward a reduction in fatty acids, including DHA (Table 1), which is consistent with the loss of fatty acids from membrane phospholipids after TBI. It is possible that the increase in DHA content may help maintain membrane fluidity, thereby preserving cognitive function in TBI animals. Elevated levels of DHA have been shown to enhance hippocampus-dependent learning processes (Hashimoto and Hossain, 2011). The question is how increased DHA levels can benefit the TBI brain. It is well known that TBI causes degradation of membrane phospholipids (Homayoun et al., 1997; Marklund et al., 1997), resulting in disturbances in cellular membrane functions, and contributing to secondary neuronal injury (Farooqui and Horrocks, 1994). Synaptic membrane phospholipids are preferentially enriched in omega-3 fatty acids, especially DHA. Increased DHA content may help prevent the loss of DHA from membrane lipids, and thus reduce the oxidative damage to the plasma membrane. Thus our findings suggest that supplementation with DHA may help the TBI brain preserve synaptic membrane integrity and fluidity, thereby enhancing membrane-related cellular functions and subsequent cognitive improvements.

Conclusions

Our results demonstrate that DHA dietary supplementation applied immediately after TBI counteracts the related cognitive decay. These effects may be achieved by involving the actions of proteins important for maintaining membrane function, in conjunction with synaptic and neuronal signaling modulators (Fig. 7). For example, DHA upregulates SOD and Sir2, which may contribute to counteracting the oxidative damage done to plasma membranes after TBI. A recent report indicates that Sir2 is required for BDNF-mediated plasticity and cognition (Gao et al., 2010). This suggests that elevation of Sir2 following DHA supplementation may influence BDNF and related molecules involved in synaptic plasticity and cognitive function. The overall body of evidence emphasizes the therapeutic potential of dietary DHA supplementation to reduce the effects of the pathobiology of TBI.

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Author Disclosure Statement

No competing financial interests exist.

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