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Mechanisms of n-3 fatty acid-mediated development and maintenance of learning memory performance

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Abstract

Docosahexaenoic acid (DHA, 22:6n-3) is specifically enriched in the brain and mainly anchored in the neuronal membrane, where it is involved in the maintenance of normal neurological function. Most DHA accumulation in the brain takes place during brain development in the perinatal period. However, hippocampal DHA levels decrease with age and in the brain disorder Alzheimer's disease (AD), and this decrease is associated with reduced hippocampal-dependent spatial learning memory ability. A potential mechanism is proposed by which the n-3 fatty acids DHA and eicosapentaenoic acid (20:5n-3) aid the development and maintenance of spatial learning memory performance. The developing brain or hippocampal neurons can synthesize and take up DHA and incorporate it into membrane phospholipids, especially phosphatidylethanolamine, resulting in enhanced neurite outgrowth, synaptogenesis and neurogenesis. Exposure to n-3 fatty acids enhances synaptic plasticity by increasing long-term potentiation and synaptic protein expression to increase the dendritic spine density, number of c-Fos-positive neurons and neurogenesis in the hippocampus for learning memory processing. In aged rats, n-3 fatty acid supplementation reverses age-related changes and maintains learning memory performance. n-3 fatty acids have anti-oxidative stress, anti-inflammation, and anti-apoptosis effects, leading to neuron protection in the aged, damaged, and AD brain. Retinoid signaling may be involved in the effects of DHA on learning memory performance. Estrogen has similar effects to n-3 fatty acids on hippocampal function. It would be interesting to know if there is any interaction between DHA and estrogen so as to provide a better strategy for the development and maintenance of learning memory.

Keywords: Docosahexaenoic acid; Eicosapentaenoic acid; n-3 fatty acids; Learning memory; Hippocampus; Synaptic plasticity; Neurogenesis; Retinoid signaling; Estrogen; Brain; Aged; Alzheimer's disease

1. Introduction

Docosahexaenoic acid (DHA, 22:6n-3) is mainly enriched in the brain and is essential for normal neurological function [1]. In the mammalian brain, lipids make up 10% of the fresh weight and 50% of the dry weight, and the major brain lipid class is phospholipids, of which DHA and arachidonic acid (20:4n-6) are the major polyunsaturated fatty acids [2]. DHA is mainly found in the phosphatidylethanolamine (PE) and phosphatidylserine (PS) fractions [3–7], and PE and phosphatidylcholine (PC) constitute the major neuronal membrane phospholipid fractions [3,8].

Most DHA accumulation in the brain takes place during brain development in the perinatal period from the beginning

Abbreviations: AD, Alzheimer's disease; AGPATs, 1-acyl-sn-glycerol-3-phosphate acyltransferases; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors; 20:4n-6, arachidonic acid; BDNF, brain-derived neurotrophic factor; CaMKII, calcium-calmodulin-dependent protein kinase II; CREB, cyclic AMP response element binding protein; DHA, docosahexaenoic acid (22:6n-3), 22:5n-3 or 22:5n-6, docosapentaenoic acid; 20:5n-3, eicosapentaenoic acid; EPSP, excitatory postsynaptic potential; GAP-43, growth-associated protein-43; HRT, hormone replacement therapy; 18:3n-3, α-linolenic acid; LPAATs, lysophosphatidate acyltransferases; LPEAT2, lyso-PE acyltransferase 2; LPLATs, lysophospholipid acyltransferases; LTP, long-term potentiation; MHC II, major histocompatibility complex molecule II; NMDA, N-methyl-D-aspartate receptors 18:1n-9, oleic acid; 16:0, palmitic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; TG, triacylglycerol; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

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of the third trimester of gestation to 2 years after birth in humans and from prenatal Day 7 to postnatal Day 21 in rats [9–11]. However, hippocampal DHA levels decrease with age in rats [12,13] and are reduced in the human brain disorder Alzheimer's disease (AD) [14–16]. The main function of the hippocampus is the linking of short-term memory to the learning process and the storing of spatial information [17–19]. Hippocampal DHA deficiency is therefore associated with reduced learning memory ability in rats [20] and with memory loss in AD patients [21].

This review will focus primarily on DHA-mediated hippocampal-dependent spatial learning memory performance, which is usually evaluated using the Morris water maze [22] or sometimes the arm radial maze [1]. It is proposed that the n-3 fatty acid-mediated development and maintenance of learning memory performance results from DHA biosynthesis and incorporation into PE in the brain, where it is involved in neuronal morphology and synaptic plasticity.

2. DHA biosynthesis and incorporation into PE in the brain

2.1. DHA biosynthesis in the developing fetus and adult

DHA contains 22 carbon atoms and 6 double bonds at carbon 3, 6, 9, 12, 15 and 18, counting from the methyl end of the carbon chain. It is either formed from its precursor, α-linolenic acid (18:3n-3), or obtained as preformed DHA or from fish oil containing eicosapentaenoic acid (20:5n-3) and DHA. 18:3n-3 is an essential fatty acid which must be obtained from the diet and is found in canola oil, soybean oil, linseed oil, and flaxseed oil [23]. 18:3n-3 and 20:5n-3 can be converted to DHA by sequential desaturation and elongation in the endoplasmic reticulum, followed by peroxisomal β-oxidation (Fig. 1) [24]. Although the liver is the major site of DHA biosynthesis [25], DHA can also be synthesized locally in the developing brain [26,27].

Studies in which ^{14}C-labeled 18:3n-3 was injected intracranially into the fetal rat brain or ^{13}C-labeled 18:3n-3 was injected into the fetal baboon jugular artery showed that the fetal brain can take up, desaturate, and elongate 18:3n-3 to DHA and incorporate DHA into membrane phospholipids [26,27]. Moreover, the activity of brain Δ6 desaturase, the rate-limiting step in DHA biosynthesis, is much higher during the perinatal period than after brain development in both rats [28] and mice [29]. The efficiency with which dietary preformed DHA is incorporated into the fetal or newborn baboon brain is seven- to eightfold higher than that of 18:3n-3-derived DHA [26,30], indicating that uptake of preformed DHA in the brain is effective in brain DHA accumulation during brain development. Increasing the level of maternal dietary 18:3n-3 from 0.6% of the energy source to 7% did not alter DHA levels in neuronal cells in the 2-week-old rat pup brain [31]. Exposure of rats to 18:3n-3 at levels greater than 200 mg/100 g diet from in utero to lactation via maternal intake, then as an adult (2 months old) fed the same diet, did not alter brain DHA levels [32]. These last two studies suggest that the DHA content of the brain is well controlled and that the n-3 fatty acid dietary requirement is 0.4% of the energy source [32].

A study using ^{2}H-labeled 18:3n-3 showed that human infants are able to synthesize DHA [33] and another using ^{11}C-labeled DHA showed that the human adult is able to take up DHA and incorporate it into the brain [34]. However, although the human adult can convert ^{13}C-labeled 18:3n-3 into 20:5n-3 and, to a lesser extent, docosapentaenoic acid (22:5n-3), very little is converted to DHA (summarized in the review [35]). Maternal milk or plasma

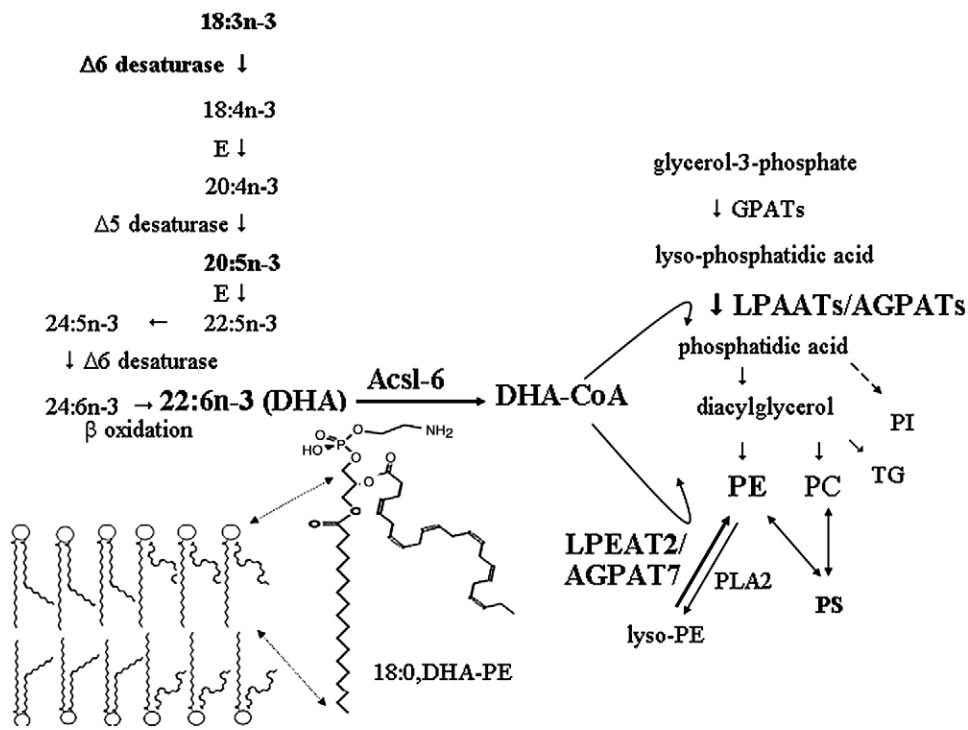

Fig. 1. Pathway for DHA biosynthesis and incorporation into neuronal membrane phospholipids. DHA is either formed from its precursor 18:3n-3, the limiting step being Δ6 desaturase, or is provided by preformed DHA or fish oil containing 20:5n-3 and DHA. DHA is converted to DHA-Co-A by Acsl-6, then incorporated into lyso-phosphatidic acid or lyso-PE at the *sn*-2 position by LPAATs in de novo synthesis or by LPEAT2 in the deacylation-reacylation process.

levels of 18:3n-3, 20:5n-3, and 22:5n-3, but not DHA, are increased in lactating women supplemented with 18:3n-3 (10.7 g/day for 4 weeks) from flaxseed oil [36], and blood levels of 20:5n-3, but not DHA, are increased in healthy humans supplemented with 18:3n-3 (2–40 g/day for 3–26 weeks) or 20:5n-3 (1–4 g/day for 4–12 weeks) [37]. These findings indicate that the developing brain has the ability to synthesize DHA, while in the adult, DHA synthesis is low, and it is not known whether the aged brain can synthesize DHA. Thus, preformed DHA derived from the food, may be the best source of DHA for maintaining brain DHA levels in the adult.

2.2. DHA incorporation into PE in the brain

DHA is converted into DHA-Co-A by acyl-CoA synthetases then incorporated into lyso-phosphatidate or lyso-phospholipids at the *sn*-2 position by lysophosphatidate acyltransferases (LPAATs) (also known as 1-acyl-*sn*-glycerol-3-phosphate acyltransferases, AGPATs) in de novo synthesis or by lysophospholipid acyltransferases in the deacylation-reacylation process (Fig. 1) [38].

LPAAT/AGPAT mRNA expression is tissue-specific. Levels of mRNAs for LPAATδ (also known as AGPAT4) and lyso-PE acyltransferase 2 (LPEAT2, previously named AGPAT7 or LPAATη) are much higher in the brain than in other tissues [38,39]. Recently, it was reported that mRNA for LPEAT2, which acts mainly to acylate 1-acyl-lyso-PE or 1-alkenyl-lyso-PE to form PE and plasmalogen, is mainly expressed in the brain in the human or mouse [40], suggesting that LPEAT2 is important in maintaining high levels of brain PE and plasmalogen. PE and plasmalogen levels are reduced in the hippocampus in aged rats (12 vs. 2 months old), aged humans (>70 vs 33–36 years old) and AD patients [13,14,41–43]. It would be interesting to know whether DHA is the preferred substrate for LPEAT2 and whether LPEAT2 activity is responsible for DHA incorporation into PE and plasmalogen during brain development and for the decrease in hippocampal DHA and PE levels in the aged or AD brain.

3. n-3 fatty acids are involved in neuronal development and function

3.1. n-3 fatty acids supplementation increases PE levels and neurite outgrowth

DHA and PE are the major neuronal membrane components, and the formation of neurons requires membrane structures. Neurons in the hippocampus can synthesize DHA from 18:3n-3 and incorporate DHA mainly into the PE fraction [44]. In PC12 cells (a neuronal cell model) overexpressing long-chain acyl-CoA synthetase 6 (Acsl-6, previously named acyl-CoA synthetase 2), DHA is converted to DHA-Co-A, but not 20:4n-6 or oleic acid (18:1n-9) (Fig. 1), DHA uptake into cells is specifically increased; levels of phospholipid, especially PE, are increased and neurite outgrowth is enhanced [45,46]. In human neuroblastoma SH-SY5Y cells, DHA and 20:5n-3 but not 18:3n-3, 20:4n-6, linoleic acid (18:2n-6), erucic acid (22:1n-9) or behenic acid (22:0) increase neuron growth-associated protein-43 (GAP-43) mRNA levels, and DHA increases expression of GAP-43 protein, a marker of axonal growth essentially for neurite outgrowth, to promote neurite outgrowth [47]. In addition, DHA, but not 20:4n-6, docosapentaenoic acid (22:5n-6) or 18:1n-9, promotes neurite growth in rat primary hippocampal neurons [48,49], in cortical neurons [50] and differentiated human mesenchymal stem cells [51]. DHA also enhances the differentiation of G-olig2 embryonic stem cells into neuronal cells and increases neurite outgrowth, including neurite length, neurite number and number of branches per neuron [52]. In addition, DHA, but not 20:4n-6, 22:5n-6, or 18:1n-9, promotes synaptogenesis in rat primary hippocampal neurons, as shown by the number of synapsin puncta [49]. Moreover, a single intracerebroventricular injection of 30 nmol of 20:5n-3 stimulates myelin protein expression in the neonatal rat brain [53]. Furthermore, neuron size in the hippocampus is decreased in DHA-deficient young rats (21 days old) [54]. These studies show that DHA increases membrane PE levels, GAP-43 protein expression, neurite outgrowth, neuronal differentiation and synaptogenesis during neuronal development. It would be interesting to know whether Ascl-6 plays an important role in brain DHA accumulation during brain development and whether it is responsible for the decrease in brain DHA levels in aged or AD patients.

3.2. Optimal DHA levels for neuronal development

A study on hippocampal neurons showed that supplementation with 5–10 μM DHA, which resulted in hippocampal neuron DHA levels of 12–16% of the total fatty acids in the total lipid fraction, is optimal for rat primary hippocampal neuronal survival during development, while supplementation with a concentration higher than 50 μM resulted in decreased neuronal survival [55], suggesting that an appropriate DHA concentration is important for hippocampal neuronal development. In addition, supplementation with 5 μM DHA enhances the differentiation of neuron cells from G-olig2 embryonic stem cells [52]. The n-3 fatty acid concentration in rat plasma or brain is reported to be 10.6 μM or 1.3 nmol/g brain, respectively [56], and hippocampal DHA levels in normal rats or primates are 12–14% of the total fatty acids in the total lipid fraction [20,57]. These studies indicate that n-3 fatty acids help in the development of hippocampal neurons by maintaining appropriate DHA levels.

3.3. Effects of DHA levels on learning memory performance

In general, brain DHA deficiency is induced by feeding an n-3 fatty acid-deficient diet in utero (via the maternal intake) and throughout life for two to three generations [58]. A reduction in brain DHA levels down to 3–5% of the total fatty acids in the total lipid fraction is associated with poor water-maze learning memory performance [58,59], and recovery of brain DHA levels to 8–12% of the total fatty acids in the total lipid fraction in DHA-deficient rats leads to recovery of water-maze learning memory [60]. In addition, in young adult (2 months old) DHA-deficient rats fed a diet supplemented with n-3 fatty acid-enriched fish oil (18 mg of 20:5n-3+12 mg of DHA/day, accounting for 0.3% of the energy source) for about 12 weeks, hippocampal DHA levels recovered from 5% to 11% of the total fatty acids in the total lipid fraction and water-maze learning memory performance was improved [20]. Moreover, n-3 fatty acid supplementation during brain development and during 80 days of adulthood of chow diet-fed rats induced an increase in DHA levels in the hippocampus from 12% to 15% of the total fatty acids in the total lipid fraction and water-maze learning memory performance was enhanced [20]. These studies indicate that hippocampal DHA levels play an important role in mediating learning memory performance.

4. n-3 fatty acids maintain learning memory performance by strengthening synaptic plasticity

Synaptic plasticity is the remodelling and reinforcement of connections between neurons. Long-term potentiation (LTP) in the hippocampus is an experimental model of activity-dependent synaptic plasticity used to study synaptic efficiency in learning memory formation. LTP is triggered by activation of postsynaptic *N*-methyl-D-aspartate (NMDA) receptors via strong postsynaptic

depolarization followed by activation of calcium-calmodulin-dependent protein kinases II (CaMKII) and cyclic adenosine monophosphate (AMP) response element binding protein (CREB) in postsynaptic dendritic spines, resulting in an increased strength of synaptic transmission [61,62]. It is triggered by tetanic electric stimulation, which causes a long-lasting potential recorded as excitatory postsynaptic potential in the activated synapse that is maintained for hours in in vitro hippocampal slice preparations and for days for the maintenance of memory in animals [63].

4.1. n-3 fatty acids enhance LTP and dendritic spine formation in the hippocampus

20:5n-3 enhances CREB activity in PC12 cells [64] and DHA supplementation shows longer-lasting phases of LTP in rat hippocampal slices [65]. In rat hippocampal slices prepared from young mice (18 days old) exposed to an n-3 deficient diet compared to an n-3 adequate diet via the maternal intake, the expression of the NMDA receptor subunits NR1, NR2A, NR2B was reduced in the hippocampus, LTP was impaired and hippocampal DHA levels were reduced from 12% to 3% of total fatty acids in the total lipid fraction [49]. DHA acts synergistically with the effect of exercise on synaptic plasticity and water-maze learning memory performance by increasing levels of CaMKII, CREB, brain-derived neurotrophic factor (BDNF) and synapsin 1 in the hippocampus in adult rats given a DHA-enriched diet (1.25% DHA and 0.25% 20:5n-3 w/w in the control chow diet for 12 days) [66]. LTP in the hippocampus was enhanced in adult rats (1.5 months old) supplemented with 20:5n-3 (1 mg/day for 8 weeks) [64]. Adult normal gerbils given DHA supplementation (300 mg/kg/day for 4 weeks) showed an increase in phospholipids [PE, PS, and phosphatidylinositol (PI), but not PC] and in postsynaptic dendritic spine density in the hippocampus, while 20:4n-6 had no such effect [67]. In addition, memory performance in the radial-maze test was improved and c-Fos-positive neurons, a marker of neuronal activity, were increased in the hippocampus in adult rats (5 weeks old) given DHA supplementation (300 mg/kg per day for 12 weeks) [68]. These findings suggest that n-3 fatty acids increase CaMKII and CREB levels to enhance LTP promoting dendritic spine formation, BDNF secretion, and the number of c-Fos-positive neurons to strengthen synaptic plasticity for spatial learning memory formation.

4.2. n-3 fatty acids increase synaptic protein expression and synaptogenesis

In rat primary hippocampal neurons, DHA, but not 20:4n-6 promotes synaptogenesis, synaptic activity, synapsin-1 expression and the expression of glutamate receptors α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor subunit GluR1, GluR2 and NMDA receptor subunits NR1, NR2A, NR2B [49]. In adult (12-week-old) fat-1 transgenic mice with high endogenous DHA levels compared to the wild-type mice, expression of synaptic genes including those coding for synapsin-1, GAP-43, post-synaptic density protein-95 (PSD-95), GluR1 and cytoskeleton protein F-actin in the hippocampus is up-regulated, and water-maze learning memory performance improved [52]. In adult gerbils (4–6 months old, weighing about 60–80 g), DHA or 20:5n-3 supplementation (300 mg/kg per day for 4 weeks) of the normal chow resulted in an increase in brain PE, PS, and PI levels and synaptic protein expression, while 20:4n-6 had no effect [69]. Levels of synaptic proteins, including pre-synaptic protein synapsin-1 and syntaxin-3, post-synaptic protein PSD-95 and cytoskeleton protein F-actin, were increased in the hippocampus of adult gerbils given DHA supplementation (300 mg/kg per day for 4 weeks) [67,70]. Levels of DHA and PSD-95 in the cortex were increased and water-maze learning memory performance improved in aged (17 months old) AD mice fed an n-3 fatty acid-deficient diet with DHA supplementation (0.6% w/w in the n-3 deficient diet for 103 days) [71]. These studies show that synapsin-1 and GAP43, markers of synapses and axons, respectively, in the hippocampus is increased in n-3 fatty acid-supplemented adult rats, suggesting that n-3 fatty acids increase synaptic gene and protein expression, thus increasing synaptogenesis and neurite outgrowth to maintain learning memory function.

5. n-3 fatty acids promote neurogenesis

In adult rodents, the hippocampus and olfactory bulb are the only two brain regions in which continuous neurogenesis is seen [72,73], while in monkeys [74] and humans [75], neurogenesis occurs only in the hippocampus. LTP enhances neurogenesis [76]. Hippocampal-dependent memory performance is correlated with hippocampal neurogenesis in aged rats (21 months old) [77] and adult rats [78]. While neurogenesis demonstrated by immunohistochemical staining for 5-bromo-2'-deoxyuridine, an analogue of thymidine, is decreased in the DHA-deficient embryonic rat brain [79], it is increased in the hippocampus of adult (12-week-old) fat-1 transgenic mice with high endogenous DHA levels [52] and in the hippocampus of aged rats (18 months old) supplemented with DHA (300 mg/kg per day for 2 weeks) [80], suggesting that n-3 fatty acids promote neurogenesis in the developing, adult and aged brain. These findings indicate a potential mechanism by which n-3 fatty acids help in the development and maintenance of learning memory performance by generating new hippocampal neurons for better neural networking properties and computational capability.

6. n-3 fatty acids in neuron protection

6.1. Specific effect of n-3 fatty acids in neuron protection

Evidence is accumulating that neuron protection is provided by n-3 fatty acids, but not other fatty acids. At the concentration of 25 μM, DHA, but not 22:5n-6, 20:4n-6, or 18:1n-9, protects mouse neuroblastoma Neuro 2A cells against apoptosis induced by 2-day serum starvation [81,82], and DHA, but not 20:4n-6 or 18:1n-9, prevents oxidative stress-induced apoptosis of Neuro 2A cells [83] as shown by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) or DNA fragmentation assay. At 6.7 μM, DHA, but not 20:4n-6, 18:1n-9 or palmitic acid (16:0), prevents apoptosis of rat retina photoreceptor cells during development or induced by oxidative stress, as shown by cell counting [84,85]. At the concentration of 10 μM, 18:3n-3, but not 16:0, provides protection against ischemia-induced hippocampal cell death and prevents kainic acid-induced seizures in rats as shown by the TUNEL or lactate dehydrogenase assay [86]. These findings indicate a specific effect of n-3 fatty acids in neuron protection.

6.2. n-3 fatty acids overcome oxidative stress in the damaged brain

Levels of reactive oxygen species and the number of apoptotic neurons in the hippocampus are reduced, LTP maintained, and water-maze learning memory performance improved in γ-irradiation-damaged rats given 20:5n-3 supplementation (2% v/w in the chow diet for 4 weeks) [87] or in rats with cerebral ischemia given fish oil supplementation (400 mg of n-3 fatty acids/kg per

day for 2 weeks) [88]. BDNF levels are normalized, oxidative damage reduced, and water-maze learning memory improved in traumatic brain injury rats fed an n-3 fatty acid-enriched diet [89]. These finding indicate that n-3 fatty acids have an antioxidative stress effect, thus protecting neurons and maintaining learning memory performance.

7. n-3 fatty acids reverse age-related changes

7.1. n-3 fatty acids reverse age-related synaptic plasticity changes

An age-related decrease in levels of DHA and of the GluR2 and NR2B glutamate receptor subunits in the hippocampus is reversed to the same levels as in adult rats (3–4 months old), in aged rats (24 months old) given n-3 fatty acid supplementation (160 mg of 20:5n-3+110 mg of DHA/kg per day for 12 weeks) [90]. DHA levels, KCl-stimulated glutamate release, and LTP in the hippocampus are decreased in aged rats (22 months old) compared to adult rats (4 months old), and these effects are reversed in aged rats (22 months old) supplemented with DHA (10 mg/day for 8 weeks) or 20:5n-3 (12 mg/day for 8 weeks) [91,92]. Hippocampal neurogenesis is reduced in aged rats (10- or 20 months old) compared to adult rats (3 months old) [93], and is increased in aged rats (18 months old) supplemented with DHA (300 mg/kg per day for 2 weeks) [80]. A decreased spine density in the hippocampus is seen in aged rats (20–24 vs 3–5 months old) and humans (>50 vs. ≤50 years old) [94–96], while DHA supplementation (300 mg/kg per day for 4 weeks) results in an increase in spine density in adult normal gerbils [67]. These studies indicate a potential mechanism by which n-3 fatty acids help in the maintenance of learning memory performance by preventing age-related synaptic plasticity changes.

7.2. n-3 fatty acids reverse age-related inflammation changes

Levels of mRNAs coding for major histocompatibility complex molecule II (MHC II) and CD40, markers of microglial cell activation indicating neuronal inflammation, and protein levels of interferon-γ and interleukin-1β are increased and LTP reduced in the hippocampus in aged (22 months old) compared to young rats (4 months old), and these effects in aged rats are overcome by supplementation with 20:5n-3 (125 mg/kg/day for 4 weeks) [97]. These findings indicate a potential mechanism by which n-3 fatty acids help in the maintenance of learning memory performance by reversing age-related inflammation changes.

7.3. n-3 fatty acids reverse the age-related reduction in hippocampal DHA levels and learning memory performance

DHA levels in the normal adult rat brain appeared not to be affected by feeding an n-3 fatty acid-deficient diet for 7 months from the age of 2 months [98]. In contrast, DHA levels in the hippocampus are reduced by 37% from 18% to 11% of total fatty acids in the PE fraction in aged rats (18 months old) compared to young adult rats (2 months old) fed the same normal diet [12,99] and water-maze learning memory is impaired in aged rats (17 months old) compared to younger adults (5 months old) [100]. In addition, brain DHA levels are reduced and water-maze learning memory impaired in aged rats (24 months old) compared to young adult rats (2 months old) fed normal chow diet [101]. In contrast, in aged rats (24 months old) given n-3 fatty acid supplementation (160 mg of 20:5n-3+110 mg of DHA/kg/day for 12 weeks), the age-related reduction in hippocampal DHA levels is reversed to the levels seen in adult rats (4 months old) [90] and radial-maze learning memory is improved in aged rats (23 months old) given 300 mg DHA/kg/day for 5 weeks [102]. These studies indicate that the age-related reduction in hippocampal DHA levels is prevented and learning memory function improved by n-3 fatty acid consumption.

8. n-3 fatty acids in AD prevention

AD is a progressive neurodegenerative disease characterized by dementia. The main pathology of AD is extracellular deposits of fibrillar aggregated amyloid β peptide (Aβ) as plaques and of intracellular phosphorylated tau protein as tangles, which cause

Table 1
Reported effects of n-3 fatty acids on the hippocampus and learning memory performance

Molecular effect	Cellular effect	Integrative effect	Reference
Signaling			
↑ CaMKII	↑ Long-term potentiation	↑ Synaptic plasticity	[49,65,66,90–92]
↑ CREB		↑ Spatial learning memory	
↑ Glutamate receptors		Reversal of age-related changes	
↑ Glutamate release			
↑ BDNF			
Structure			
↑ PE	↑ Neuronal differentiation	↑ Synaptic plasticity	[49,52,53,67,68,70,80]
↑ GAP-43	↑ Neurite outgrowth	↑ Spatial learning memory	
↑ Myelin	↑ Synaptogenesis	↑ Neuron protection	
↑ Synapsin-1	↑ Neurogenesis	Reversal of age-related changes	
↑ Syntaxin-3	↑ Dendritic spine density		
↑ PSD-95	↑ c-Fos-positive neurons		
↑ F-actin			
↑ Tyrosine tubulin			
↑ Acetylated tubulin			
Protection			
↓ MHC II	Anti-oxidative stress	↑ Spatial learning memory	[87,88,108,110,112,116]
↓ CD40	Anti-inflammation	↑ Neuron protection	
↓ Interferon-γ	Anti-apoptosis	Reversal of age-related changes	
↓ Interleukin-1β		↑ AD prevention	
↓ Proinflammatory genes		↓ Cognitive decline	
↑ Anti-apoptotic genes			
↓ Aβ levels			
↓ Phosphorylated tau protein			

neuronal death [103,104]. In AD patients, the hippocampus is one of the first brain regions to suffer damage [105,106].

8.1. n-3 fatty acids prevent amyloid β peptide-induced neuronal death

In studies on hippocampal neurons, DHA supplementation increased levels of cytoskeleton protein tyrosine tubulin and acetylated tubulin and attenuated Aβ-induced neurotoxicity [55], while Aβ-induced cytoskeleton perturbation in primary cortical neurons was prevented by DHA [107]. These studies suggest that the increase in cytoskeleton protein levels caused by DHA may make the cells more resistant to Aβ-induced neurotoxicity and thus prevent AD. Neuroprotectin D1, a DHA-derived 10, 17S-docosatriene, up-regulates the expression of anti-apoptotic genes and down-regulates the expression of proinflammatory genes to prevent Aβ-induced neuronal death [16]. In rat primary cortical neurons, DHA or 20:5n-3 prevents neuronal apoptosis induced by soluble Aβ oligomers by inhibiting caspase activation and enhancing ERK signaling [107]. These findings indicate that n-3 fatty acids prevent Aβ-induced neurotoxicity by protecting neurons from inflammation and apoptosis.

8.2. n-3 fatty acids decrease AD pathology

In studies of n-3 fatty acid supplementation in an AD animal model, Aβ plaques in the hippocampus were reduced in aged (22.5 months old) AD mice fed a DHA-enriched diet (0.6% w/w in chow diet) for about 103 days [108], DHA levels were increased, and soluble Aβ levels reduced, and levels of phosphorylated tau protein decreased in the brain in adult (3 months old) AD mice fed a DHA-enriched diet (1.3% w/w in control diet) for 3–9 months [109], while reactive oxygen species levels and the number of apoptotic neurons in the hippocampus were decreased, hippocampal DHA levels increased, and radial-maze learning memory performance improved in Aβ-infused adult rats supplemented with DHA or 20:5n-3 (300 mg/kg per day for 12 weeks) [110–112]. Phosphorylation of the anti-apoptotic protein Bcl-2 associated death promotor (BAD) is increased and levels of oxidized proteins decreased in the cortex and water-maze learning memory performance is improved in aged (17 months old) AD mice fed an n-3 fatty acid-deficient diet when the diet is supplemented for 103 days with DHA (0.6% w/w in the n-3 deficient diet) [71]. These findings indicate that n-3 fatty acids decrease Aβ levels and have antioxidative stress and antiapoptosis effects, leading to neuron protection and maintenance of learning memory ability.

8.3. n-3 fatty acids delay cognitive decline

In humans, DHA levels do not change in the healthy hippocampus between the ages of 33–90 years [14], but in AD patients, they are reduced by 53% to 8% of total fatty acids in the PE fraction compared to 17% in healthy age-matched controls [14], and in patients with mild AD, fish oil supplementation (600 mg of 20:5n-3 +1700 mg of DHA/day for 6–12 months) delays cognitive decline [113]. In addition, serum DHA levels in AD patients gradually decrease with the severity of clinical dementia compared to healthy age matched controls [21], while AD risk is reduced and cognitive decline is delayed by higher DHA levels in blood [114,115] or n-3 fatty acid consumption (summarized in the review [116]). These studies indicate that n-3 fatty acids play an important role in the maintenance of cognitive performance.

9. Effects of estrogen on DHA levels and learning memory performance

Plasma DHA levels are higher in healthy adult women than in healthy adult men [117–119]. The conversion of 18:3n-3 to

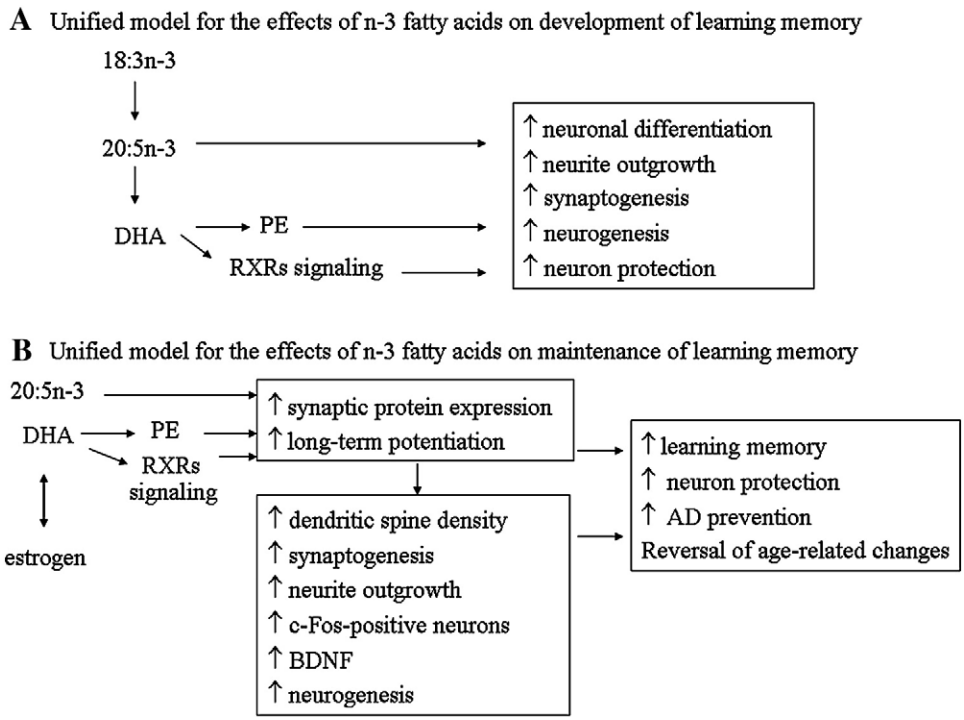

Fig. 2. An unified model of the effects of n-3 fatty acids on the development and maintenance of learning memory performance. (A) Unified model for effects on the development of learning memory. It is proposed that the developing brain can carry out DHA biosynthesis and incorporated it into PE. n-3 fatty acids or DHA-RXRs signaling is involved in brain development. (B) Unified model for effects on maintenance of learning memory. It is proposed that the mature brain can take up DHA and incorporate it into neuron membrane PE fractions, where n-3 fatty acids or DHA-RXRs signaling strengthen synaptic plasticity, increase neuron protection and reverse age-related changes. There may be an interaction between the effects of DHA and estrogen.

20:5n-3, 22:5n-3, and DHA is greater in adult women than in adult men [120,121]. In addition, postmenopausal women with hormone replacement therapy (HRT) have higher plasma DHA levels than those without HRT [122,123]. Furthermore, plasma DHA levels are increased in male-to-female transsexual subjects receiving estradiol and decreased in female-to-male transsexual subjects who have undergone ovariectomy and are receiving testosterone [119]. These studies suggest that DHA biosynthesis is higher in women than in men because of sex hormones, especially estrogen.

Liver Δ6 and Δ5 desaturase expression is significantly higher in 8-week-old female rats than in male rats of the same age [124]. Erythrocyte DHA levels are higher in adult female rats than in male rats of the same age [125] and DHA levels in erythrocytes and the hippocampus, but not in the frontal cortex, hypothalamus or midbrain, are decreased in ovariectomized female rats compared to sham operated rats [126]. These studies indicate that estrogen may affect DHA levels.

Estrogen plays an important role in hippocampal function by maintaining LTP, enhancing neurite outgrowth, increasing spine density, strengthening synaptic plasticity and improving spatial learning memory performance [127–130]. It would be interesting to know whether there is any interaction between DHA and estrogen in learning memory performance.

10. Summary of the effects of n-3 fatty acids on the development and maintenance of learning memory performance

10.1. Effects of n-3 fatty acids on the hippocampus

The reported effects of n-3 fatty acids on the hippocampus and learning memory performance are summarized in Table 1. Molecular signaling effects are the increased expression of CaMKII, CREB and glutamate receptors and increased glutamate release which promote LTP to strengthen synaptic plasticity for spatial learning memory formation. Molecular structuring effects are increased PE levels and synaptic and cytoskeleton protein expression, which promote the cellular effects of increased neurite outgrowth, synaptogenesis, dendritic spine density and neurogenesis and strengthen synaptic plasticity. n-3 fatty acids have anti-oxidative stress, anti-inflammation and anti-apoptosis effects, which result in neuron protection. In addition, n-3 fatty acids reverse age-related changes and prevent AD. It is interesting to note that DHA may act through retinoid signaling.

10.2. Retinoid signaling may be involved in the effect of DHA on learning memory performance

DHA is a ligand for the retinoid X receptors (RXRs) [131], ligand-activated transcription factors that control the expression of genes involved in brain development, neuronal differentiation, LTP, neurite outgrowth and neurogenesis [132–136]. Retinoid signaling has been shown to play an important role in neuron protection, modulation of inflammation, neurotrophin regulation and decreasing the pathology of AD [137,138].

10.3. A proposed unified model for the effects of n-3 fatty acids on the development and maintenance of learning memory performance

In the unified model of the effects of n-3 fatty acids on the development of learning memory (Fig. 2A), it is proposed that the developing brain can carry out DHA biosynthesis and take up DHA to optimal levels. DHA is mainly incorporated into PE and n-3 fatty acids or DHA-RXRs signaling helps neuronal differentiation, neurite outgrowth, synaptogenesis, neurogenesis and neuron protection during brain development. In terms of maintenance of learning memory (Fig. 2B), it is proposed that the mature brain can take up DHA and incorporated it into neuron membrane PE fractions, where n-3 fatty acids or DHA-RXRs signaling increase synaptic protein expression and LTP, resulting in increased dendritic spine formation, neurite outgrowth, synaptogenesis, number of c-Fos-positive neurons, BDNF secretion and neurogenesis to strengthen hippocampal synaptic plasticity, protect neurons, prevent AD and reverse age-related changes in the hippocampus. As noted in this review, estrogen has a similar effect to DHA, and it would be interesting to know if there is any interaction between the effects of DHA and estrogen on learning memory function.

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