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Research report

Chronic administration of DHA and UMP improves the impaired memory of environmentally impoverished rats

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ABSTRACT

Living in an enriched environment (EC) during development enhances memory function in adulthood; living in an impoverished environment (IC) impairs memory function. Compounds previously demonstrated to improve memory among IC rats include CDP-choline and uridine monophosphate (UMP). Brain phosphatidylcholine (PC) synthesis utilizes both the uridine formed from the metabolism of exogenous CDP-choline and UMP, and the choline formed from that of CDP-choline. It also uses the polyunsaturated fatty acid (PUFA) DHA, a precursor for the diacylglycerol incorporated into PC. DHA administration also improves cognition in young and aged rodents and humans; its effects on cognitively impaired IC rats have not been characterized. We have thus examined the consequences of administering DHA (300 mg/kg) by gavage, UMP (0.5% in the diet), or both compounds on hippocampal- and striatal-dependent forms of memory among rats exposed to EC or IC conditions for 1 month starting at weaning, and consuming a choline-containing diet. We observe that giving IC rats either dietary UMP or gavaged DHA improves performance on the hidden version of the Morris water maze (all $P < 0.05$), a hippocampal-dependent task; co-administration of both phosphatide precursors further enhances the IC rats' performance on this task ($P < 0.001$). Neither UMP nor DHA, nor giving both compounds, affects the performance of EC rats on the hidden version of the Morris water maze ($P > 0.05$), nor the performance by IC or EC rats on the visible version of the Morris water maze (all $P > 0.05$), a striatal-dependent task. We confirm that co-administration of UMP and DHA to rats increases brain levels of the phosphatides PC, PE, SM, PS, PI, and total brain phospholipid levels (all $P < 0.05$), and show that rearing animals in an enriched environment also elevates brain PC, PS, and PI levels (all $P < 0.01$) and total brain phospholipids ($P < 0.01$) compared with their levels in animals reared in an IC environment. These findings suggest that giving DHA plus UMP can ameliorate memory deficits associated with rearing under impoverished conditions, and that this effect may be mediated in part through enhanced synthesis of brain membrane phosphatides.

1. Introduction

Changes in an animal's environment, and the ways in which that animal reacts to those changes, can have significant long-term effects on the brain and behavior [1]. Laboratory rodents are normally reared under standard conditions (SC), during which they are housed individually and exposed to few novel objects. If instead the animals are reared under environmentally enriched (EC) or impoverished conditions (IC), memory functions are improved in the former state [2] and impaired in the latter [3]. Rearing under EC conditions typically involves being housed in larger cages, with more cage mates, and novel objects; rearing under IC conditions typically involves being housed in smaller cages, isolation from other rodents, and exposure only to essential objects [4].

Environmental enrichment is known to enhance memory performance in various learning tasks, including performance by rodents on a water maze test of spatial memory [5,6]; by rats on a T-maze [7]; on tests of spatial learning by adult and aged rats [8]; and of learning by rats with traumatic brain injury (TBI) [9]. The hippocampus, which is essential in learning and memory [1], is the brain region most profoundly affected by rearing in an enriched or impoverished environment [10].

Environmental impoverishment has been shown to impair memory function in a number of learning tasks, and various compounds reportedly protect rodents from these impairments. IC rats demonstrated deficits when tested on a T-maze [3] or using a water maze [11], and various hippocampal-dependent memory deficits [4]. Compounds previously demonstrated to improve memory in IC rats include CDP-choline and uridine mono-phosphate (UMP). Long-term dietary supplementation with either CDP-choline, a source of choline and circulating cytidine (in rats) or uridine (in

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humans), prevented memory impairment in IC rats tested on a hippocampal-dependent spatial task [12,13].

PC synthesis requires the use of three circulating compounds: choline, a pyrimidine such as uridine, and a polyunsaturated fatty acid (PUFA) such as docosahexaenoic acid (DHA) [14]. Consumption of DHA during development [15] or by aged rats [16] can improve learning and memory, and chronic administration of either the uridine source UMP, or especially, of DHA, can increase brain phosphatide levels [14].

Administration of these compounds or choline increases phosphatide synthesis by enhancing the substrate-saturation of low-affinity brain enzymes that catalyze their use in the Kennedy cycle. The first of these, choline kinase (CK), phosphorylates choline, to form phosphocholine. Uridine is phosphorylated by uridine–cytidine kinase (UCK) to form uridine triphosphate (UTP) [17], which is transformed in brain to cytidine triphosphate (CTP) by the enzyme CTP synthetase [18]. DHA is acylated by fatty acyl-CoA synthetase [19]. Phosphocholine and CTP combine to form cytidine-5′-diphosphocholine (CDP-choline), which then combines with diacylglycerol (DAG) to form phosphatidylcholine (PC) [20]. DAG species containing polyunsaturated fatty acids (PUFA) such as DHA are preferentially incorporated into PC [19,21]. All of these enzymes have high K_m's for their substrates relative to normal plasma and brain levels [18,22,23]. Uridine conversion to UTP and CTP is enhanced in PC12 cells [24] and rodent brain [14] when the saturation of UCK [25–30] has been increased by pyrimidine administration [17]. Also, the concentration of CTP in brain is insufficient to saturate CTP: phosphocholine cytidylyltransferase (CT) [31,32]. Giving animals DHA, choline and uridine increases enzyme saturation by all three of these precursors, and thus maximally potentiates synthesis of PC and other phosphatides [14].

The increases in brain phosphatides caused by administering DHA alone or, especially DHA plus UMP are associated with increases in pre- and post-synaptic proteins [14], as well as in hippocampal dendritic spines [41], and probably, synapses. These additional hippocampal synapses could underlie enhancements in hippocampal types of memory. We thus examined the effects of DHA and UMP on hippocampal- and striatal-dependent forms of memory among rats that had been exposed to EC or IC for 1 month starting at weaning, using the hidden and visible versions of the Morris water maze. We also subsequently analyzed brain samples for their phospholipid contents and correlated the extent of each treatment on phosphatide levels and on performance on the water maze task.

2. Materials and methods

2.1. Rats and diet

Male Sprague Dawley rats, 4 weeks of age, purchased from Charles River Laboratories, Wilmington, MA, were housed in a climate-controlled area and exposed to a 12:12 h light cycle (lights on at 7:00 h). The rats were matched according to body weight and assigned to either the IC or EC group. Subgroups of IC rats and EC rats were given either the control diet; this diet supplemented with 0.5% UMP; this diet supplemented with 300 mg/kg DHA daily by gavage; or both the 0.5% UMP-containing diet and the gavaged DHA. All diets also contained choline chloride. Rats were weighed weekly to ensure that animals exposed to each of the treatments were eating equivalent amounts of food. Efforts were made to minimize animal suffering, according to NIH guidelines. Protocols were approved by the Massachusetts Institute of Technology Institutional Animal Care and Use Committee (IACUC).

2.2. Environmental conditions

Rats were housed in the same rack in plastic cages with wire lids. Bedding and water were regularly changed, and rats had ad libitum access to food and water. EC rats were housed in groups of two, in cages containing plastic toys (blocks, balls, PVC tubing, etc.). Toys were rotated between groups twice a week; new toys were introduced weekly. EC rats were exposed to a "playroom" measuring 5 ft × 5 ft every other day for 45 min, and handled daily. The "playroom" contained several toys including plastic tubing, small balls, plastic boxes, wire brushes, and paper towels to shred. The IC rats were housed individually, without toys; handled 3 times/week; and allowed to exercise only 3 times/week for 15 min in an empty room measuring 5 ft × 5 ft, with only the experimenter present.

2.3. Water maze apparatus

The water maze was a galvanized circular tank, 6 ft in diameter and 2 ft in height. The tank was filled with water maintained at room temperature to a depth of 1 ft, and located in a dimly lit room containing several extramaze cues. Four starting positions were spaced around the perimeter of the tank to divide the pool into four equal quadrants. For the visible platform version of the water maze, a white flag attached to the top of the submerged platform protruded above the water surface. A video camera mounted directly above the maze was linked to a computer with video tracking software (HVS Image) to automatically record the escape latency (time to reach the platform), distance traveled (length of swim path taken to find the platform), and swim speed of all rats.

2.4. Behavioral test

All behavioral training was carried out as described previously [12,13] between 14:00 and 18:00 h, and each experiment was repeated at least 3 times and by at least two different experimenters who were blind to the treatments. Briefly, rats were given four trials per day for 4 days to locate the hidden platform (1 cm below the water surface), which, for each rat, remained in the same position (within one of the four quadrants). If a rat did not find the platform within 90 s, it was guided to the escape platform by the experimenter. After mounting the platform, rats were allowed to remain on it for 20 s. Following each trial, rats were removed from the maze and placed in a holding cage for a 30 s intertrial interval. On the fifth day of testing rats were given a probe test; the platform was removed, and the swim path and time spent in the quadrant of the pool that had previously contained the platform were measured over 60 s.

In the visible version of the Morris water maze [33] rats were given four trials per day for 4 days to locate the visible platform, which was placed in a different quadrant on each of the four trials. If a rat did not escape within 90 s, it was manually guided to the escape platform by the experimenter. After mounting the platform, rats remained on it 20 s. Following each trial, rats were removed from the maze and placed in a holding cage for a 30 s intertrial interval.

2.5. Rotarod apparatus and testing

The rotarod apparatus consisted of a 3.2-cm-diameter rod (RRAC-3002; O'Hara & Company, Tokyo, Japan). The rotarod test was performed according to the procedure described previously [34]. Rats were tested on the rotarod following the completion of all water maze testing. During the training period, rats were placed on the rod rotating at 4 rpm, and this speed was gradually accelerated to 40 rpm at an acceleration of 0.15 rpm/s. The latency to fall (retention time) was measured with a cutoff time of 4 min. Rats were trained for 3 consecutive days, receiving four trials per day with 1 h intertrial interval.

2.6. Sample collection

Rat brains were obtained immediately following the conclusion of each behavioral test; animals were anesthetized using CO_2; decapitated; and their brains dissected, weighed, and homogenized in distilled water such that each sample contained the same ratio of tissue to water; i.e., 20 mg tissue:1 mL water. Samples were stored at −80 °C for further analysis.

2.7. Total DNA assay

To determine the total DNA in each sample of homogenized brain tissue, previously described techniques [35] were used. DNA readings were compared with those of standards. Briefly, known standards were diluted 50 μg/mL in DNA buffer (50 mM KPO_4, 2 mM EDTA, 250 mM NaCl, pH 7.4); 10 μL of standards and of homogenized tissue were then placed in well plates (Falcon MicroTest 96-well Assay Plate, optilux Black). Hoechst solution was diluted to 1 μg/μL in DNA buffer, and 200 μL was added to standard- and sample-containing wells. Following a 30-min incubation at room temperature, the plate was read and analyzed on Thermo Labsystem Fluoroskan Ascent using Microplate Manager software.

2.8. Total protein assay

To determine the total protein content of each sample, previously described techniques [35] were used. Readings of homogenized brain tissues were compared with those of known BSA standards. Briefly, BSA standards and samples were added to well plates (clear Falcon Pro-Bind 96 well assay plate); $CuSO_4$ solution was diluted 1:49 in bichinconinic acid and added to all standard- and sample-containing wells. Following a 30-min incubation at room temperature and in the dark, the plate was read and analyzed on a Bio Rad microplate reader, model 550, using Ascent Software.

2.9. Total phospholipid assay

Total phospholipid contents were determined by comparing the phosphorus levels in samples with those in potassium phosphate standards. Phosphatides were extracted using previously described methods [36]: 1 mL homogenates were mixed with 3 mL of chloroform and methanol mixture (2:1, v/v) and vortexed for 30 s. After cooling on ice for 1 h, the mixture was added to 1 mL deionized water, and then to 3 mL of chloroform and methanol (2:1, v/v). After remaining at −4 °C for 18–20 h, the mixture was separated by centrifugation at 3500 rpm for 15 min at 4 °C; 100 μL aliquots of the bottom phase was dried in a savant lyopholizer and then digested in 70% perchloric acid for 1.5 h at 150 °C. Phosphatides were measured as described previously [37]: 300 μL of 15% ascorbic acid and 200 μL of 5% ammonium molybdate were added to samples and standards. These remained at room temperature for 30 min, and were then read on a PerkinElmer Lambda 3B UV/VIS spectrophotometer. The absorbency reading of each sample was compared to the absorbency readings of the standards to determine the phospholipid content in samples. This value was then expressed per DNA or protein content.

2.10. Phospholipid separation

To measure the amounts of individual phospholipids, previously described methods [38] were used. Digested samples were separated using thin layer chromatography (TLC); 30 μL of each sample was spotted onto Alltech silica gel G channeled plates, placed in running buffer (30 mL chloroform, 34 mL ethanol, 30 mL triethylamine, and 8 mL water) for 1.5 h, and visualized by spraying plates with petroleum ether containing 1,6-diphenyl-1,3,5-hexatriene and viewing under UV light. Bands corresponding to individual phospholipids were scraped, reconstituted in methanol, and dried overnight in a savant lyopholizer. Following this initial separation step, procedures used to measure each sample's phosphorous contents were the same as those described for the total phospholipid assay above.

2.11. Data analysis

For all tests comparing two groups, two-tailed *t*-tests were used. For comparisons involving more than one factor, or comparing more than two groups, factorial ANOVA was used.

3. Results

3.1. Body weight

Body weight did not differ among animals in the various control or experimental groups (data not shown), indicating that rats were probably eating equivalent amounts of their diets.

3.2. Effects of UMP, DHA, and environmental conditions on performance on a hippocampal-dependent water maze test

All groups were able to learn the hidden version of the Morris water maze to some degree, showing a decrease in the number of errors recorded over time (Fig. 1A and B) and indicated by a significant main effect of day (block of four training trials per day) ($P < 0.001$). Values are mean ± S.E.M., $n = 12$ for each group. Main effects of environment ($P < 0.001$) and of an environment × diet interaction were also observed ($P < 0.05$). IC rats treated with UMP; DHA; or UMP plus DHA exhibited decreased escape latencies compared with those of IC control rats; the largest decrease was observed in IC rats receiving both UMP and DHA; UMP ($F(1,12) = 7.563$, $P < 0.042$), DHA ($F(1,12) = 13.253$, $P < 0.035$), and UMP × DHA ($F(1,12) = 27.635$, $P < 0.001$) (Fig. 1A). EC rats treated with UMP; DHA; or UMP plus DHA did not acquire the task at a faster rate then did control rats ($P > 0.05$) (Fig. 1B). These results indicate that long-term dietary treatment with UMP, DHA or, especially UMP plus DHA improves the spatial memory deficits associated with impoverished conditions but does not affect this memory function in rats reared in an enriched environment.

The results of the 60 s probe test indicated that all experimental groups spent more time in the quadrant that had originally contained the platform, suggesting that the rats used spatial cues to locate the hidden platform (Fig. 1C). The percentage of swim time each group spent was affected by whether it had been reared

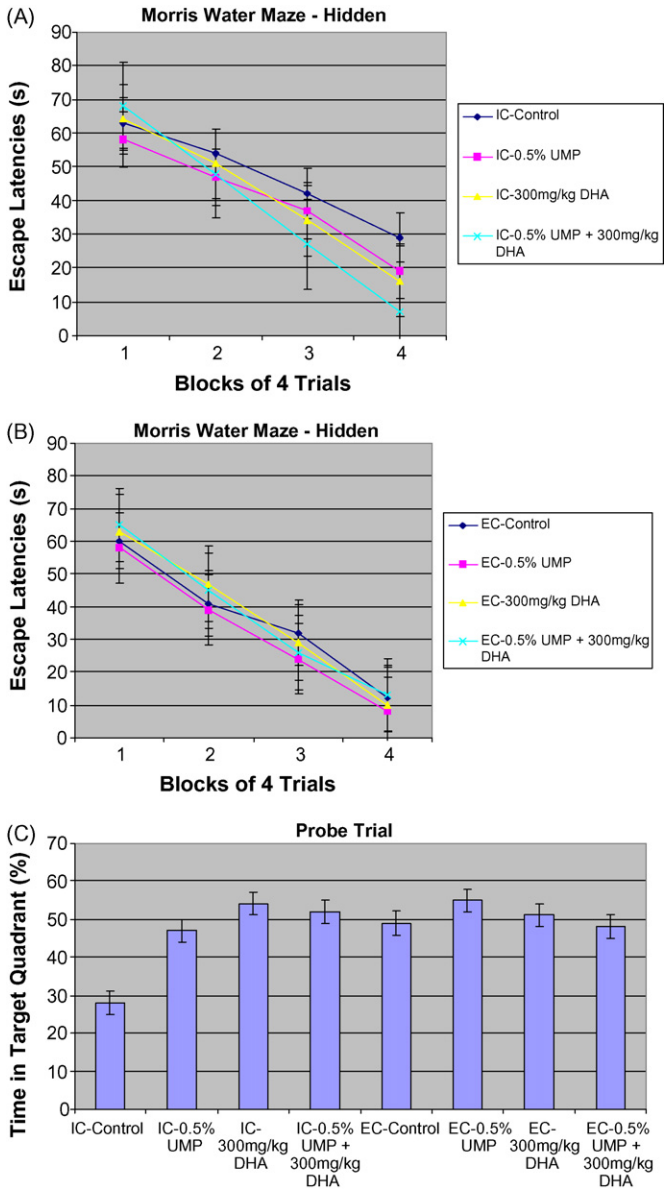

Fig. 1. The effects of environment, UMP, and DHA administration on memory for a hippocampal-dependent hidden platform water maze in rats reared under EC or IC conditions for 1 month immediately postweaning. Values are means ± S.E.M., $n = 12$. (A) IC rats administered UMP, DHA, or UMP and DHA had decreased escape latencies compared to the IC control rats (all $P < 0.05$). (B) EC rats administered UMP, DHA, or UMP and DHA did not have decreased escape latencies compared to the EC control rats (all $P > 0.05$). (C) The 60 s probe test was affected by environment ($P < 0.042$), quadrant ($P < 0.001$), and diet × environment interaction ($P < 0.05$).

in an impoverished or exposed environment ($P < 0.042$); quadrant ($P < 0.001$); and diet × environment interaction ($P < 0.05$). IC rats treated with UMP, DHA, or UMP plus DHA spent more time in the correct quadrant than did IC control rats not receiving either compound, UMP ($F(1,12) = 7.845$, $P < 0.025$), DHA ($F(1,12) = 12.374$, $P < 0.021$), UMP × DHA ($F(1,12) = 22.428$, $P < 0.001$) (Fig. 1C). In contrast, EC rats treated with UMP, DHA, or UMP and DHA did not spend more time in the correct quadrant than EC control rats ($P > 0.05$).

3.3. Effects of UMP, DHA, and environmental conditions on performance on a striatal-dependent water maze test

All groups were able to learn the visible version of the Morris water maze to some degree, showing a decrease in the number

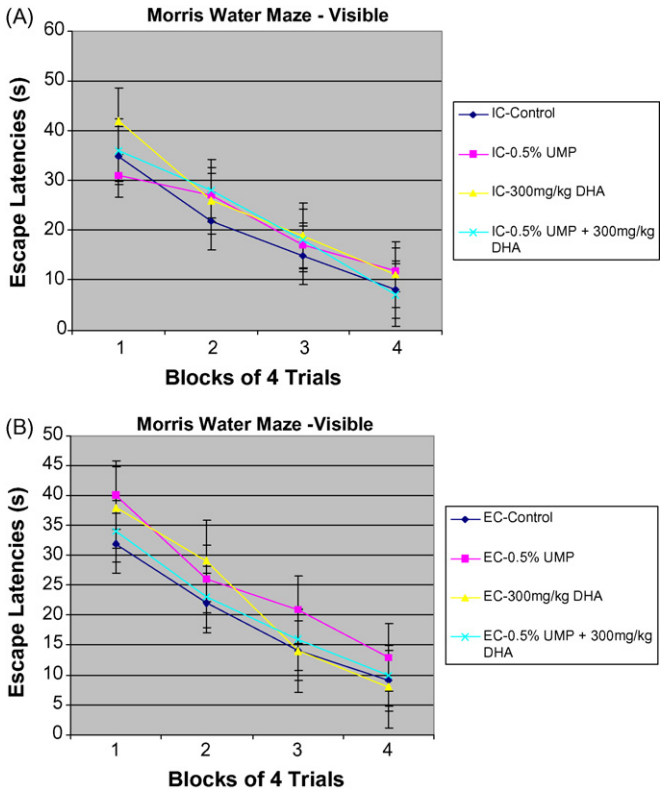

Fig. 2. The effects of environment, UMP, and DHA administration on memory for a striatal-dependent visible platform water maze in rats reared under EC or IC conditions for 1 month immediately postweaning. Values are means ± S.E.M., $n = 12$. (A) IC rats administered UMP, DHA, or UMP and DHA did not have decreased escape latencies compared to the IC control rats (all $P > 0.05$). (B) EC rats administered UMP, DHA, or UMP and DHA did not have decreased escape latencies compared to the EC control rats (all $P > 0.05$).

of errors recorded over time (Fig. 2A and B) and a main effect of day (block of four training trials per day) ($P < 0.001$). Values are means ± S.E.M., $n = 12$. No other significant effects were observed, suggesting that environment or treatment with UMP or DHA have no effect on striatal-dependent learning and memory.

3.4. Effects of UMP and DHA supplementation on performance on an accelerating rotarod test

All groups were able to learn the rotarod task, showing increases in the length of time they were able to remain on the accelerating rotarod (Fig. 3), as indicated by a significant main effect of day (block of four training trials per day) ($P < 0.015$). Values are mean ± S.E.M., $n = 12$. No other significant effects were observed (P's > 0.05), suggesting that environment or treatment with UMP or DHA have no effect on rat motor activity, per se.

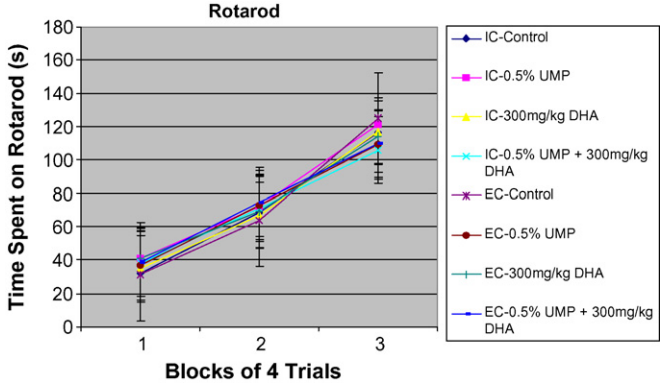

Fig. 3. The effects of a UMP-supplemented diet and/or daily administration of DHA on IC and EC rats tested on an accelerating rotarod motor activity test. Values are mean ± S.E.M., $n = 12$. The time spent on the rotarod was not affected (P's > 0.05).

3.5. Effects of a UMP-supplemented diet alone or in combination with DHA administration on brain phosphatide levels in EC and IC rats

Chronic consumption of UMP (0.5%) increased IC rats' brain PC, PE, PS, and PI levels significantly, by 23%, 28%, 46%, and 27%, respectively (Table 1). Administration of DHA (300 mg/kg) to IC rats consuming control diet also increased rats' brain PC, SM, PS, and PI levels significantly, by 26%, 49%, 71%, and 59%, respectively. Among IC rats receiving both UMP and DHA, brain PC, PE, SM, PS, and PI levels rose substantially more, i.e., by 60%, 97%, 86%, 138%, and 100%, respectively. Total phospholipid levels were also significantly increased, by 19% in IC rats receiving DHA and by 29%, in IC rats receiving both UMP and DHA (Table 1). Two-way ANOVA revealed a significant effect of dietary UMP or oral DHA on IC rats brain PC, PE, PS, and PI levels (all $P < 0.05$). Two-way ANOVA also revealed a significant effect of DHA ($P < 0.05$) or of co-administering dietary UMP and oral DHA on phospholipid levels in brains of IC rats ($P < 0.001$). Similar results were obtained when data were expressed per μg DNA (data not shown).

Administration of DHA (300 mg/kg) to EC rats consuming the control diet increased their brain levels of SM, PS, and PI significantly, by 66%, 30%, and 19%, respectively (Table 1). Among EC rats receiving both UMP and DHA, brain PC, PE, SM, PS, and PI levels all rose significantly by 18%, 46%, 100%, 107%, and 55%, respectively. Total phospholipid levels were significantly elevated in EC rats co-administered UMP and DHA, but not in EC rats receiving UMP, DHA or the combination of UMP and DHA (all $P < 0.05$ (Table 1). Two-way ANOVA revealed a significant effect of oral DHA on EC rat brain SM, PS, and PI levels (all $P < 0.05$), and a significant effect of dietary UMP and oral DHA on EC rat brain PC, PE, PS, and PI levels (all $P < 0.05$). Similar results were obtained when data were expressed per μg DNA (data not shown).

Rearing in an enriched environment increased rat brain PC, PS, and PI levels by 57%, 25%, and 41%, respectively. Total phospholipid levels were also significantly increased in EC control rats, compared with those in IC control rats, by 25%. Two-way ANOVA revealed a significant effect of rearing in the enriched environment on EC rats' brain PC, PS, and PI levels (all $P < 0.01$), and of the enriched environment on phosphatide levels in brains of EC control rats compared with those in IC control rats ($P < 0.01$).

4. Discussion

These data show that administering UMP (0.5%) or DHA (300 mg/kg) to rats reared in an impoverished environment improves their performance on the Morris water maze, a hippocampal-dependent task; moreover, co-administration of UMP and DHA further enhances this improvement, concurrently elevating brain levels of PC, PE, SM, PS, PI, and total brain phospholipids. In contrast, animals reared in an enriched environment exhibit no improvement in performance in response to UMP and/or DHA, and proportionately smaller increases in the phospholipids, primarily because control brain phospholipid levels in EC rats were higher than those in IC animals.

Among the many models of cognitive impairment available, we chose to use the environmental enrichment or impoverishment model because rats reared under IC exhibit cognitive deficits [3,4]

Table 1
Effects of giving UMP-supplemented diet (0.5%) and DHA (300 mg/kg) on phosphatide levels in whole brain samples

Treatment	Total PL (nmol/mg protein)	PC	PE	SM	PS	PI
IC-control	420 ± 9	168 ± 6	74 ± 7	49 ± 8	24 ± 4	22 ± 3
IC-0.5% UMP	442 ± 12	206 ± 8	95 ± 9*	52 ± 6	35 ± 5***	28 ± 5*
IC-300 mg/kg DHA	501 ± 7*	211 ± 11*	78 ± 6	73 ± 5***	41 ± 5***	35 ± 5***
IC-0.5% UMP + 300 mg/kg DHA	543 ± 15*	269 ± 9***	146 ± 10***	91 ± 6***	57 ± 6***	44 ± 4***
EC-control	524 ± 11[a]	223 ± 8[c]	88 ± 9	47 ± 5	30 ± 5[a]	31 ± 6[b]
EC-0.5% UMP	529 ± 9	212 ± 12	98 ± 11	49 ± 7	31 ± 4	28 ± 4
EC-300 mg/kg DHA	539 ± 14	227 ± 7	77 ± 5	78 ± 8***	39 ± 6**	37 ± 7*
EC-0.5% UMP + 300 mg/kg DHA	542 ± 12*	263 ± 11***	129 ± 8	94 ± 5	62 ± 4*	48 ± 9***

IC and EC rats were administered a UMP-containing (0.5%) diet, and received DHA (300 mg/kg) daily by gavage for 6 weeks. Values are mean ± S.E.M., $n = 12$. Brains were then obtained and their phosphatides levels determined as described in the text. Data are presented as nmol/mg protein.

[a] $P < 0.05$ EC control compared to IC control.
[b] $P < 0.01$ EC control compared to IC control.
[c] $P < 0.001$ EC control compared to IC control.
* $P < 0.05$ compared to control group.
** $P < 0.01$ compared to control group.
*** $P < 0.001$ compared to control group.

that can be corrected in part by long-term dietary supplementation with either CDP-choline or UMP [12,13]. The cellular mechanisms associated with impaired cognition in IC rats are reportedly similar to those associated with CNS damage or degeneration [39]. Thus, compounds that can improve the memory of IC rats may also benefit patients with some types of neuronal damage [40].

We propose that UMP and DHA protect against cognitive impairment in IC rats by increasing the formation of neuronal membrane, specifically synaptic membrane, [14], and dendritic spines [41], and thereby promote neurotransmission. Rats reared under IC display reduced brain phosphatide levels (Table 1), and reduced glutamatergic hippocampal transmission [3]. UMP and DHA administration increases brain phosphatide levels [14], and the density of hippocampal dendritic spines, [41] which require additional synaptic membrane. DHA administration reversed the age-related decline in GluR2 and NR2B glutamate receptor subunits, thereby improving glutamatergic transmission in the hippocampus [42]. In support of this proposed interpretation, we confirmed prior [14] findings that administration of UMP and DHA increases brain phosphatide levels (Table 1) and concurrently protects IC rats from memory impairment (Fig. 1).

UMP and DHA may protect the brains of IC reared animals by restoring neuronal function to levels normally observed in brains of control or EC rats. Rats exposed to IC conditions [43] or made DHA-deficient [44] have decreased brain weight and size, while DHA administration increases brain weight and size [44]. Brains of IC reared rats also exhibit decreased neurogenesis [45] and synaptogenesis [46], DHA has been shown to promote neurite outgrowth in hippocampal neurons [47] and uridine promotes neurite outgrowth from PC12 cells [24]. DHA supplementation increased brain-derived neurotrophic factor (BDNF) levels in rats [48] while consuming a diet deficient in DHA decreased these levels [49]; BDNF induces neurogenesis in the hippocampal dentate gyrus [50].

There are many similarities between the neural effects of rearing under EC and of chronically administering DHA, or UMP plus DHA. EC rodents exhibit improved spatial memory [8], while administration of DHA improves cognitive impairment in mouse models of mild cognitive dysfunction or in AD [51]. C57BL/6 learning-impaired mice [52] show increased survival of hippocampal cells if exposed to an EC, while DHA prolongs cell survival in retinal photoreceptors [53]. Brains of EC rodents exhibit increased NGF (nerve growth factor) expression [54]; likewise DHA administration can increase the expression of NGF [55]. Brains of EC rodents also exhibit increased expression of mRNA for BDNF [8]; BDNF modulates synapsin 1 levels during learning [56], and giving UMP plus DHA increases synapsin 1 levels in gerbil brain [14]. Brains of EC rodents have increased release of brain acetylcholine [57], while DHA supplementation increases potassium-evoked acetylcholine release [58], and UMP supplementation increases the basal and evoked release of acetylcholine [59].

In summary, the present study demonstrates that administration of UMP, DHA, or both, plus choline for 4 weeks prevents memory impairment in IC reared rats and increases brain levels of individual phosphatides and total phospholipids. The largest increase in phospholipids and greatest protection from memory impairments occur when choline, DHA, and UMP are administered in combination. EC has been implicated as a possible treatment for preventing memory impairment in such diseases as traumatic brain injury [40,60], prenatal hypoxia [61], epilepsy [62], stroke [63], Huntington's disease [64,65] and depression [66]. Co-administration of DHA and UMP may aid in their treatment as well.

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