

Omega-3 fatty acid ethyl-eicosapentaenoate, but not soybean oil, attenuates memory impairment induced by central IL-1 β administration

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Abstract Proinflammatory cytokine interleukin (IL)-1 β can cause cognitive impairment, activate the hypothalamic-pituitary-adrenal axis and impair monoaminergic neurotransmission in the rat. IL-1 β has also been shown to increase the concentration of the inflammatory mediator prostaglandin E2 (PGE2) in the blood. Omega (n)-3 fatty acids, such as eicosapentaenoic acid (EPA), which are components of fish oil, have been shown to reduce both the proinflammatory cytokines and the synthesis of PGE2. The purpose of this study was to determine whether dietary supplements of EPA would attenuate the inflammation-induced impairment of spatial memory by centrally administered IL-1 β . Rats were fed with a diet of coconut oil (contained a negligible quantity of fatty acids), soybean oil (contained mainly n-6 fatty acids), or a diet of coconut oil enriched with ethyl-EPA (E-EPA). The rats were then injected intracerebroventricularly with IL-1 β or saline. The results of this study demonstrated that the IL-1-induced deficit in spatial memory was correlated with an impairment of central noradrenergic and serotonergic (but not dopaminergic) function and an increase in the serum corticosterone concentration. IL-1 β also caused an increase in the hippocampal PGE2 concentration. These effects of IL-1 were attenuated by the chronic administration of E-EPA. By contrast, rats fed with the soybean oil diet showed no effect on the changes induced by the IL-1 administration.—Song, C., and D. Horrobin. Omega-3 fatty acid ethyl-eicosapentaenoate, but not soybean oil, attenuates memory impairment induced by central IL-1 β administration. *J. Lipid Res.* 2004. 45: 1112–1121.

Supplementary key words eicosapentaenoic acid • interleukin β • interleukin-1 β -administration • spatial memory • hippocampus • monoamine neurotransmitters • prostaglandin E2 • corticosterone

The immune system and central neurotransmitters form a complex interacting network that has been extensively studied in the last decade (1, 2). Thus, despite the widely

held view that the immune system is primarily autoregulated and is concerned with protection against infection, it is now apparent that there is “cross talk” between aspects of the immune, endocrine, and central nervous processes (1–3). This may be illustrated by the role of the proinflammatory cytokine interleukin (IL)-1 β , which is produced by activated macrophages in the periphery and by microglia, astrocytes, and neurons in the brain (4–6). Several studies have shown that central or systemic administration of IL-1 β can cause cognitive impairment (3, 7, 8), which may be related to its induced brain inflammatory response and the expression of amyloid precursor protein, IL-1 activated hypothalamus-pituitary-adrenal (HPA) axis, and changed neurotransmission (2, 9–12). IL-1 β increases the secretion of corticosterone, reduces the release of acetylcholine, and increases serotonin (5-HT) and dopamine (DA) metabolites in the hippocampus and other limbic regions of the brain (8–10). The effects of IL-1 β are mediated by IL-1 receptors that are widely distributed within the brain, the highest concentration being located within the hippocampus (13, 14). This brain region is involved in learning and memory and neuroendocrine integration (15). It has been shown that the increase in circulating glucocorticoids (GCs), and the desensitization of the central GC receptors in the hippocampus, which result in a reduction in the feedback inhibition of corticotrophin release factor (CRF), are triggered by the action of IL-1 β (10). Stress, or corticosterone injections, can

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; AA, arachidonic acid; AD, Alzheimer’s disease; COX, cyclooxygenase; CRF, corticotrophin release factor; DA, dopamine; DHA, docosahexaenoic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; E-EPA, ethyl-eicosapentaenoic acid; GC, glucocorticoids; HPA, hypothalamus pituitary adrenal; HVA, homovanillic acid; icv, intracerebroventricular; IL, interleukin; LTP, long-term potentiation; MHPG, 3-methoxy-4-hydroxyphenethyleneglycol; NA, noradrenaline; PGE2, prostaglandin E2; TRP, tryptophan.

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also induce similar neurotransmitter changes and impair learning and memory (16, 17).

Increases in IL-1 β , prostaglandin E2 (PGE2), and other proinflammatory cytokines, have been associated with cognitive impairments in patients with Alzheimer's disease (AD) and other neurodegeneration diseases (18–20). Cyclooxygenase (COX) inhibitors that reduce the production of proinflammatory cytokines and PGE2 have shown positive therapeutic effect on cognitive impairment in these diseases (21, 22).

Previous studies have demonstrated that central IL-1 β administration significantly increased PGE2 secretion in the blood (23). Therefore, drugs or nutraceuticals that have anti-inflammatory effects should be of benefit in reducing inflammation-induced memory impairment (24).

Recent studies have shown that omega (n)-3 unsaturated essential fatty acids are effective for treatment of AD and depression (25–28). Essential fatty acids synthesized from dietary precursors, such as α -linolenic (n-3) and linoleic fatty acids (n-6), are important components of membrane phospholipids in both neurons and immune cells (29). However, n-3 and n-6 fatty acids occupy different roles in terms of modulation of both the central nervous system and immune system. Ethyl-eicosapentaenoic acid (E-EPA) and other EPA derivatives (from α -linolenic fatty acids) have been used in the treatment of inflammation and autoimmune diseases, such as rheumatoid arthritis and asthma (30, 31). The therapeutic effect of EPA on these autoimmune diseases is correlated with a decrease in the production of proinflammatory cytokines and a reduction in the n-6 fatty acid arachidonic acid (AA)-derived eicosanoids. These eicosanoids have been shown to stimulate the production of proinflammatory cytokines and PGE2, as well as enhance immune cellular functions (30–32). Neurodegenerative diseases are also associated with an imbalance between n-3 and n-6 fatty acids. Decreases in the blood concentrations of docosahexaenoic acid (DHA) and its precursor EPA and an increase in the n-6/n-3 ratio have been reported in patients with AD or depression (33–35). Changes in the cholesterol and phospholipid contents of neuronal membranes can also alter membrane microviscosity (36, 37) and, consequently, can modulate the various neurotransmitter systems implicated in cognitive impairment-related AD and depression (38).

Diets enriched in n-3 fatty acids can enhance spatial learning and memory and reduce anxiety-related behavior in rats (23, 39, 40). Diets lacking in n-3 fatty acids can cause defects in learning and memory in both children and young animals (41, 42). However, the mechanism by which n-3 fatty acids modulate cognitive functions is uncertain.

The evidence presented above suggests that brain inflammatory disorders may be causally related to memory impairment. As n-3 fatty acids can modulate both CNS function and the inflammatory response, we hypothesized that dietary supplements of n-3 fatty acids might attenuate inflammation-induced impairment in spatial memory by modulation of neuroendocrine-immune axis. To test this hypothesis, rats were fed with coconut oil containing neg-

ligible amounts of essential fatty acids as a control diet, soybean oil containing mainly n-6 fatty acids, or a diet enriched with two different concentrations of E-EPA. The animals were then injected intracerebroventricularly (icv) with saline or IL-1 β . Spatial learning and memory was tested using the Morris water maze. The possible involvement of hippocampal functions and HPA axis activation in IL-1-induced memory impairment was assessed by determining the serum concentration of corticosterone, hippocampal monoamine concentrations and metabolism, and hippocampal PGE2 concentrations.

MATERIALS AND METHODS

Animals and treatment

Male Wistar rats (initially weighing 200–220 g; Charles River, Quebec, Canada) were housed two per cage and maintained in a 12-h dark-light cycle, at 21 \pm 1°C. Before feeding testing diets, the animals were fed on normal laboratory chow. After 5 days of habituation, the rats were divided into eight groups of 10 rats each and fed with one of four different diets for 8 weeks. For group 1, 5% coconut oil was fed to rats as a control diet; for group 2, they were fed with 5% soybean oil; for group 3, the diet consisted of 4.8% coconut oil mixed with 0.2% of E-EPA; and group 4 had a diet of 4% coconut oil mixed with 1% E-EPA. Each group was either treated with saline or 15 ng IL-1 β via the icv route (23). The research protocol was approved by the Animal Care Committee of the University of British Columbia, Canada.

Preparation of the diets

The basal mix (Rx 991698; Harlan Teklad Test Diet, Madison, WI), coconut oil (Harlan Teklad Test Diet), pure E-EPA (LAX-101; Laxdale Ltd., UK), and soybean oil (99.5% purification; a health food store, Vancouver) were stored at 4°C. The basal mix did not contain any fatty acids; 5% of the appropriate fatty acid mixture was added to 95% of the basal mix. The composition of diets was the same as described previously (23).

Coconut oil was added to a beaker and then melted in a water bath (<50°C). The basal diet was then mixed with the coconut oil followed by the addition of the appropriate concentration of E-EPA. Five percent coconut oil (for control diet), 5% soybean oil (for n-6 diet), 4.8% or 4% coconut oil plus 0.2% or 1% EPA (for n-3 diet) was mixed with 95% (w) of the basal mix. The food was prepared every 3–4 days and stored at 4°C until used.

Surgery

After 5 weeks on the different diets, all rats were anesthetized with 100 mg/kg ketamine and 20 mg/kg xylazine. A guide cannulae was stereotaxically implanted at a position 1 mm posterior and 1.6 mm lateral of the bregma via a 1 mm-diameter hole. The guide cannulae was cut to a 1 mm depth and secured to the skull with three screws by dental cement. A dummy cannula was then screwed into the guide cannulae (23). Tetracycline cream was used for the treatment of the wound. Animals were then allowed to recover for 14 days and were handled daily.

IL-1 β and icv injection

For the icv administration, rat recombinant IL-1 β was obtained from NIBSC (Potters Bar, UK) (biological activity: 317 IU/mg) and dissolved in sterile, pyrogen-free saline at doses of 15 ng/10 μ l/rat.

Rats were gently handled and held with a soft cotton towel every day for 3 min for 2 weeks before the start of the icv injections.

One day before the first IL-1 β injection, the rats were gently held with the towel, and the cap of the guide cannula was gently unscrewed and then screwed back. On the injection day, IL-1 β or saline in a total 10 μ l vol was taken into an internal needle (4.2 mm length), which was connected to a PE 50 polyethylene tube. After unscrewing the cap, the needle was gently inserted into the guide cannula, and IL-1 or saline was slowly infused into rat brain (in 30 s). The injection needle was allowed to remain inside the guide cannula for 1 min, then removed. Animals were returned to their cage after replacing the cap on the guide cannula.

Morris water maze and memory tests

After the rats had been fed the diets for 7 weeks, the spatial memory was tested blindly to the treatment as previously described by Morris (43), with some modifications. The water tank was 1.2 m deep, 2.0 m in diameter and divided into four quadrants of equal size and designated North (N), West (W), South (S), and East (E). The water depth was 80 cm and the platform was situated 2 cm below the water level (hidden platform learning). The water was made opaque by adding a nontoxic white paint powder (Tempera Paint, Toronto, Canada) and the temperature was held at $26 \pm 1^\circ\text{C}$. One day before receiving the injections, the rats were singly placed in a pool of water without a platform, and allowed to swim freely for 1 min with no opportunity for escape (day 0). On day 1, a platform was positioned in one of the quadrants of the maze. Each rat was put into the maze facing the wall at one of four starting directions over five trials (N, W, S, E, and N). Animals were allowed to stay in the water for 60 s and were subsequently placed into a waiting cage for 1.5 min before the next trial began. Any rat that could not find the platform within 60 s was placed on the platform by the investigator and allowed to stay there for 15 s. On day 2, animals were trained with the same procedure as on day 1. The animals then received saline or IL-1 β injection immediately after five training trials (first IL-1 injection). On day 3, rats were placed into the water maze. The training was the same as day 1 but without IL-1 β injection. On day 4, the platform was relocated to a different quadrant of the maze with the same procedure as day 2 (second IL-1 injection). On day 5, the platform was located at the same place as day 4 and rats received the saline or IL-1 injection (third IL-1 injection) immediately after training. On day 6 (probe trial), the platform was removed and the same experimental procedure was used as on day 4. On each day, the escape latency, swimming route, and speed were recorded for each rat by video camera and imaged on a monitor connected to the computer (hardware and software provided by HVS IMAGE, P.O. Box 100, Hampton TW12 2YD, UK). On day 6, the latency for each rat to reach the former platform position and the times that each rat spent in the former platform area during a 60 s period were recorded by the video-tracking computer. The aim of removing the platform on the last day was to study whether the EPA or IL-1 effect on memory was related to changes in other sensory factors, such as odor (44). To study whether the change in animal learning and memory was related to vision effects of IL-1 or EPA, a flag (5 cm \times 6 cm) was placed on the platform on day 6 after the probe trial and performance. The latency that animals found the platform was recorded for two trials.

Collection of brain and serum samples

On day 7, 50 min after saline or IL-1 β administration, all rats were sacrificed and trunk blood was collected. The blood samples were then centrifuged at 600 *g* for 15 min. Serum samples were collected for corticosterone measurement.

The brains were rapidly removed and placed on an ice block, and the locations of the cannulae and injection sites were

checked by slicing the brain coronally. Data was excluded from any animals in which the injection site failed to reach the lateral ventricle (about 10%). The hippocampus was dissected on ice and weighed.

Neurotransmitter measurement

Following sonicating in an ice-cold buffer (500 ml containing: L-ascorbic acid, 4.4 mg; 70% HClO₄, 4.66 ml; and EDTA, 50 mg), hippocampal samples were centrifuged at 7,000 *g* at 4°C for 30 min. The concentration of noradrenaline (NA), DA, 5-HT, and their metabolites, or precursor 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA), and 5-HT precursor tryptophan (TRP), were determined in the supernatants using an HPLC with electrochemical detection (464-Water; Millipore, MA) according to a procedure previously described by Seyfried, Adam, and Greve (45). The internal standard was 3,4-dihydroxybenzylamine.

Corticosterone and PGE2 assays

Serum samples from trunk blood were used for the corticosterone measurement with a commercial radioimmunoassay kit (Immucor corticosterone RIA kit for rats, catalog No. RCBK9906A; ICN Biochemical, Costa Mesa, CA). Intra- and interassay coefficients of variation were 6.8% and 5.6%, respectively.

The concentration of PGE2 in the hippocampus was measured in the supernatants of homogenized and sonicated brain samples (the same as those used for HPLC measurement) by an enzyme immunoassay (commercial kit, Assay Designs, Inc., Ann Arbor, MI) as described previously (46). Results are expressed as pg/g of fresh brain tissue.

Statistical analyses

Behavioral results were analyzed by repeated three-way ANOVA (IL-1 \times diet \times day). Neurotransmitter, corticosterone, and PGE2 data were analyzed by two-way ANOVA (IL-1 \times diet). If any statistically significant changes were found, post hoc analysis Newman-Keuls was performed for the comparison between the groups. The statistical package used for data analysis was obtained from GB-STAT, Dynamic Microsystems, Inc. Significance was set at a value of $P < 0.05$. Results were expressed as mean \pm SEM.

Neurotransmitter results were expressed as the percentage of the average value of controls (coconut oil with saline treatment). For example, the concentration in each sample, including each control sample, was divided by average value of total controls and multiplied by 100. The turnover of these monoamines was expressed as the ratios of MHPG/NA, DOPAC/DA, and 5-HIAA/5-HT based upon their respective absolute concentrations in the brain.

RESULTS

One percent EPA significantly attenuated memory impairment induced by IL-1 β

On day 0, there were no significant changes in the swimming route between the different diet groups. Compared with animals fed coconut oil or EPA, swimming speeds (cm/sec) in animals fed 5% soybean oil were significantly increased (coconut oil, 28.38 ± 2.42 ; soybean oil, 45.14 ± 6.41 ; 0.2% EPA, 24.99 ± 1.34 ; 1% EPA + saline, 28.41 ± 1.52) (ANOVA $F_{1, 64} = 5.29$, $P < 0.01$). However, for the remainder of the experiment, the swimming speeds in each group were similar (data not shown). On

day 1 and day 2 (without saline or IL-1 β administration), there were no significant changes in escape latency, swimming speed, and route between different experimental groups. Over six training and testing days, the ANOVA analysis revealed significant day interaction escape latencies, which indicated an increase in learning and memory ($F_{15,193} = 4.78$, $P < 0.001$). Newman-Keuls post hoc revealed that the escape latency in saline-treated rats or before any treatment (on day 1 and day 2) was significantly shorter when day 1 was compared to day 2, day 2 to day 3, and both day 5 and day 6 to day 4 ($P < 0.01$) (Fig. 1). The ANOVA analysis indicated that IL-1 β administration sig-

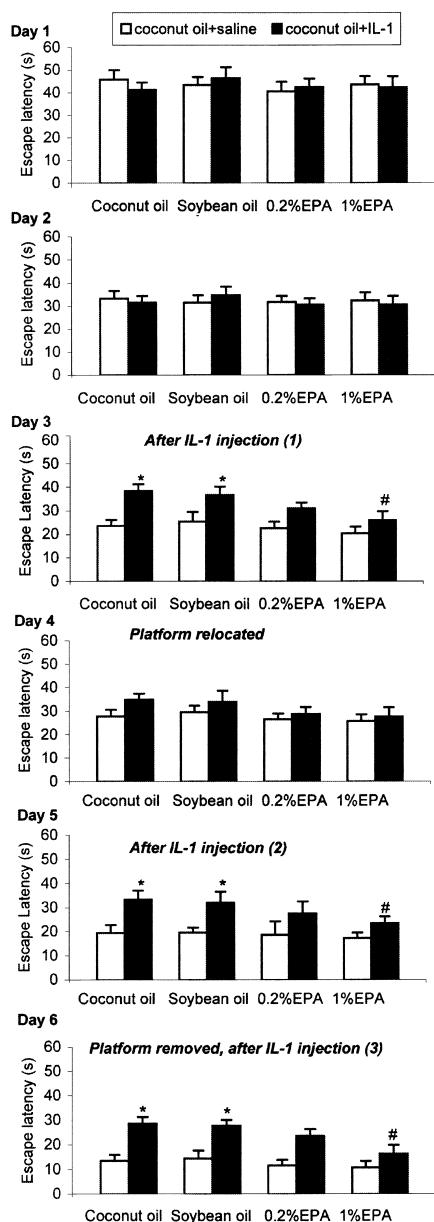


Fig. 1. Latencies (mean \pm SEM) of a located platform in a Morris water maze over six training and testing days and testing in saline- and interleukin (IL)-1-treated rats fed coconut oil, soybean oil, or eicosapentaenoic acid (EPA) diets for 7 weeks. * $P < 0.05$ versus saline-treated group with the same diet; # $P < 0.05$ versus IL-1 β -treated rats with coconut oil ($n = 8-9$).

nificantly delayed the escape latency ($F_{1,16} = 8.53$, $P < 0.001$) (Fig. 1) without changing the swimming speed ($F_{1,16} = 0.63$, $P > 0.05$; data not shown). In the IL-1 β -treated animals, the difference in the escape latency was only significant between day 4 and day 6 ($P < 0.05$). IL-1 β -treated-animals fed with soybean oil learned to locate the platform in the maze at a similar rate as IL-1-treated animals with the coconut oil diet (Fig. 1). On day 3, compared with the saline-treated rats, central IL-1 β administration (injected immediately after training on day 2) significantly increased the escape latency ($P < 0.05$). On the same day, rats with two different concentrations of EPA and those fed soybean oil did not significantly influence the escape latency after saline treatment when compared with animals fed coconut oil that had the same treatment. However, the ANOVA revealed a significant interaction between IL-1 \times EPA ($F_{3,32} = 5.72$, $P < 0.01$). Newman-Keuls post hoc revealed that the escape latency in the IL-1 β -treated rats with 1% EPA diet was significantly shorter than that in the IL-1 β -treated group fed with coconut oil ($P < 0.05$) (as shown in Fig. 1, day 3). The improvement in the time taken to locate the platform for animals fed with 0.2% EPA did not reach significance, and soybean oil did not attenuate the IL-1-induced change. This result indicates that a low concentration of EPA or soybean oil does not attenuate IL-1-induced memory impairment. On day 4, following the relocation of the platform, the escape time in each group was longer. The group treated with IL-1 β 48 h before showed a slightly longer escape time, which, however, did not reach significance. There was also no significant difference between soybean oil and coconut oil with or without IL-1 administration. After the second dose of IL-1 β on day 4, the results on day 5 were similar to those seen on day 3. There was no significant difference in the escape latency and swimming speed between saline-treated rats fed the different diets, whereas IL-1 treatment induced significantly longer escape latencies in groups fed coconut oil ($F_{1,16} = 6.31$, $P < 0.01$). Those rats fed the high concentration of EPA significantly attenuated the IL-1-induced impairment in the retrieval memory in the Morris water maze ($F_{3,32} = 4.38$, $P < 0.02$) (Fig. 1). Soybean oil and 0.2% EPA had no significant effect on IL-1-induced memory impairment. On day 6, all animals further improved their performance to locate the former platform position. The latencies to reach the former platform position are shown in Fig. 1 (day 6). The total times (s) that animals spent at the former platform area were: coconut oil plus saline, 34.21 ± 3.45 ; coconut oil plus IL-1, 17.64 ± 2.82 ; soybean oil plus saline, 32.37 ± 3.26 ; soybean oil plus IL-1, 16.58 ± 2.45 ; 0.2% EPA plus saline, 35.59 ± 3.29 ; 0.2% EPA plus IL-1, 22.94 ± 2.73 ; 1% EPA plus saline, 40.74 ± 3.29 ; and 1% EPA plus IL-1, 30.51 ± 3.64 . In IL-1-treated animals fed coconut oil, the latencies to reach the former platform position were significantly longer, and the time spent in the former platform area was significantly shorter (latencies: $F_{1,16} = 4.47$, $P < 0.02$; time spent: $F_{1,16} = 5.38$, $P < 0.01$) (Fig. 1, day 6). Similar results were also observed in the group fed soybean oil after IL-1 administration. One

percent EPA again reversed IL-1-induced changes, but soybean oil was without effect ($F_{3,32} = 4.76, P < 0.02$). Two-tenths percent of EPA seemed partially to improve memory in the IL-1 group, but this did not reach significance. When a flag was put on the platform, all animals found the platform within 6–8 s (data not shown). This result indicated that IL-1 β administration and diets did not significantly influence the visual memory.

The saline- and IL-1 β -treated rats showed different swimming routes to reach the platform. Using the software to separate the water maze into three zones (outside, middle, and central), we could see that saline-treated animals swam more in the middle and central zones, but IL-1-treated rats spent more time in the outer zone. Following the high dietary EPA treatment, the swimming route returned to the control state. **Fig. 2** shows an example of routes used by rats on day 3.

Effects of EPA on hippocampal neurotransmitter changes induced by IL-1 β

In the saline-treated animals, there was no significant difference in the concentration of NA and 5-HT neurotransmitters, their metabolites, and turnovers between groups fed diets enriched with coconut oil, soybean oil, and 0.2% EPA (**Table 1**). The ANOVA analysis only indicated that the diet factor significantly affected the dopaminergic system (DA: $F_{3,32} = 5.76, P < 0.01$; DOPAC: $F_{3,32} = 4.17, P < 0.05$; HVA: $F_{3,32} = 4.79, P < 0.01$). Post hoc comparison showed that the concentration of DA, and its metab-

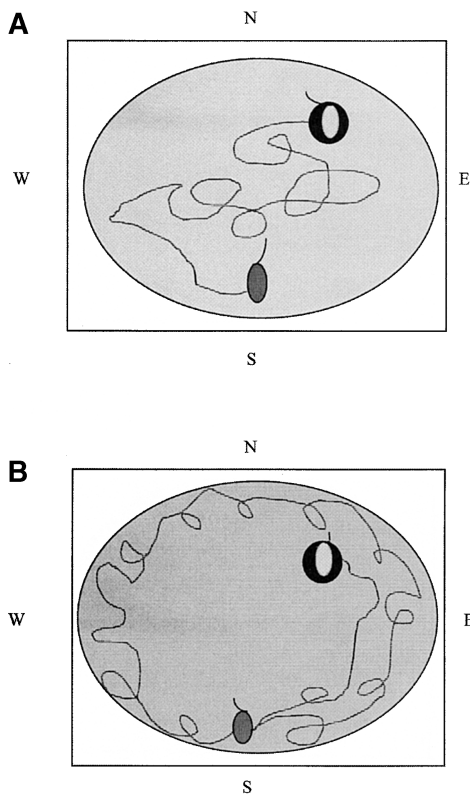


Fig. 2. Animal swimming route in a Morris water maze. A: A rat treated with saline, or an IL-1 β -treated rat fed 1% EPA. B: A rat treated with IL-1 β (15 ng) and fed coconut oil or soybean oil.

TABLE 1. Effects of supplemented diets of EPA and soybean oil on IL-1 β -induced relative changes in monoamine concentrations and metabolism in the hippocampus

Monoamines	NA%	MHPG%	MHPG/NA	DA%	DOPAC%	HVA%	DOPAC/DA	5-HT%	TRP%	5-HIAA%	5-HIAA/5-HT
Group											
5% Coconut oil + saline	98.72 ± 4.12	101.46 ± 4.73	0.102 ± 0.00061	99.69 ± 3.43	100.65 ± 4.35	99.47 ± 5.39	1.24 ± 0.066	101.05 ± 4.42	97.68 ± 7.72	100.42 ± 7.68	2.43 ± 0.17
5% Soybean oil + saline	102.49 ± 3.96	97.38 ± 4.26	0.101 ± 0.00057	104.93 ± 4.38	102.47 ± 5.27	97.34 ± 4.29	1.25 ± 0.046	96.37 ± 3.79	102.63 ± 5.82	103.75 ± 5.62	2.51 ± 0.19
4.8% Coconut oil + 0.2% EPA + saline	102.14 ± 3.46	97.68 ± 3.75	0.094 ± 0.00062	121.15 ± 6.76	112.26 ± 5.22	107.12 ± 11.92	1.17 ± 0.072	103.84 ± 15.08	104.87 ± 3.58	115.90 ± 8.90	2.27 ± 0.51
4% Coconut oil + 1% EPA + saline	106.15 ± 3.93	103.65 ± 5.36	0.098 ± 0.00046	157.06 ± 8.12**	124.26 ± 6.28*	131.82 ± 7.59*	1.14 ± 0.083	118.75 ± 12.62	86.07 ± 12.34	107.90 ± 13.29	2.62 ± 0.45
5% Coconut oil + IL-1 β (15 ng)	78.58 ± 3.39*	121.59 ± 6.32	0.15 ± 0.00083*	139.61 ± 6.53**	120.47 ± 6.32*	188.31 ± 12.52**	1.03 ± 0.049*	89.69 ± 4.32	118.61 ± 3.53*	147.48 ± 11.64**	4.07 ± 0.42**
5% Soybean oil + IL-1 β	85.83 ± 4.57*	122.82 ± 5.33 [^]	0.13 ± 0.00057*	127.75 ± 7.92*	117.38 ± 5.35	154.73 ± 19.36**	1.02 ± 0.062*	90.37 ± 5.32	120.72 ± 5.63*	143.38 ± 9.43*	3.86 ± 0.32*
4.8% Coconut oil + 0.2% EPA + IL-1	83.92 ± 3.2 [^]	107.34 ± 5.39	0.13 ± 0.00069	128.59 ± 2.96	106.26 ± 6.85	164.02 ± 12.57 ^{^^}	1.05 ± 0.067	120.09 ± 8.63	106.99 ± 9.08	126.24 ± 9.94	2.83 ± 0.31
4.0% Coconut oil + 1% EPA + IL-1	105.69 ± 5.9 ^{##}	102.53 ± 4.68 [#]	0.096 ± 0.00054 [#]	140.18 ± 5.18	112.18 ± 6.74	135.59 ± 14.95	1.04 ± 0.078	132.3 ± 11.72 [#]	84.8 ± 8.25 [#]	110.35 ± 10.32 [#]	2.53 ± 0.22 [#]

5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; EPA, eicosapentaenoic acid; HVA, homovanillic acid; IL, interleukin; MHPG, 3-methoxy-4-hydroxyphenylethylglycol; NA, noradrenaline; TRP, tryptophan. Results are expressed as mean ± SEM as a percentage change of control group (coconut oil diet with saline treatment). * $P < 0.05$; ** $P < 0.01$ versus 5% coconut oil or soybean oil + saline; # $P < 0.05$; ## $P < 0.01$ versus IL-1 group (coconut oil + IL-1); [^] $P < 0.05$; ^{^^} $P < 0.01$ versus matched same diet controls (with saline) (n = 9–11).

olites DOPAC and HVA, was significantly increased in saline-treated animals fed 1% EPA when compared with concentrations in animals fed a diet with coconut oil ($P < 0.01$, 0.05 , and 0.05 , respectively) (Table 1). There was no significant difference in the dopaminergic system between rats fed diets with soybean oil and coconut oil.

The ANOVA also indicated that IL-1 β administration significantly changed several neurotransmitters and their metabolism. In the noradrenergic system, decreased NA, increased MHPG, and increased NA turnover was observed (NA: $F_{1,16} = 5.54$, $P < 0.01$; MHPG: $F_{1,16} = 4.63$, $P < 0.05$; MHPG/NA: $F_{1,16} = 5.02$, $P < 0.01$). Newman-Keuls analysis revealed that the significant difference was between the IL-1 and saline groups fed with coconut oil and soybean oil. Two-way ANOVA further showed a significant interaction between IL-1 \times diet (NA: $F_{3,32} = 3.96$, $P < 0.01$; MHPG: $F_{3,32} = 3.27$, $P < 0.01$; MHPG/NA: $F_{3,32} = 3.58$, $P < 0.01$). The post hoc comparison revealed that 1% EPA significantly reversed the changes in NA and MHPG concentrations and NA turnover induced by central IL-1 β administration ($P < 0.05$). Soybean oil and 0.2% EPA could not attenuate noradrenergic monoamine changes induced by IL-1 β (Table 1).

In the dopaminergic system, central IL-1 β administration markedly increased hippocampal DA, DOPAC, and HVA concentrations and increased DA turnover (DA: $F_{1,16} = 4.54$, $P < 0.02$; DOPAC: $F_{1,16} = 3.94$, $P < 0.05$; HVA: $F_{1,16} = 5.07$, $P < 0.01$; DOPAC/DA: $F_{1,16} = 3.35$, $P < 0.05$). Neither the EPA nor the soybean oil diet reversed these IL-1 β -induced changes.

The ANOVA analysis also indicated a significant effect of IL-1 β on the serotonergic system. IL-1 increased the concentrations of 5-HIAA and TRP, increased 5-HT turnover, and slightly decreased 5-HT (5-HIAA: $F_{1,16} = 3.97$, $P < 0.01$; 5-HIAA/5-HT: $F_{1,16} = 3.67$, $P < 0.01$, TRP: $F_{1,16} = 3.33$, $P < 0.02$; 5-HT: $F_{1,16} = 2.35$, $P = 0.062$). There was a significant IL-1 \times EPA interaction (5-HIAA: $F_{3,32} = 3.19$, $P < 0.02$; TRP: $F_{3,32} = 3.23$, $P < 0.02$, 5-HIAA/5-HT: $F_{3,32} = 2.94$, $P < 0.05$). The Newman-Keuls comparison between groups showed IL-1-induced changes occurring in groups fed coconut and soybean oil ($P < 0.05$ or $P < 0.01$), whereas 1% EPA, but not 0.2% EPA, significantly increased 5-HT and decreased 5-HIAA, TRP, and 5-HT turnover ($P < 0.05$) (Table 1).

Effects of different diets on IL-1-induced secretion of corticosterone and PGE2

The ANOVA analysis did not show significant dietary effects on serum corticosterone concentrations, but there was a significant effect of IL-1 β on corticosterone secretion ($F_{1,16} = 9.83$, $P < 0.0001$). The comparison between groups showed that soybean oil induced a small, but significant, increase in the serum concentration of corticosterone compared with saline-treated rats fed coconut oil ($P < 0.05$). IL-1 β elevated the corticosterone concentration in animals fed coconut oil and soybean oil ($P < 0.01$). This hormone concentration was also significantly increased in the group fed with 0.2% EPA ($P < 0.05$), even though this diet partially reduced IL-1-induced corti-

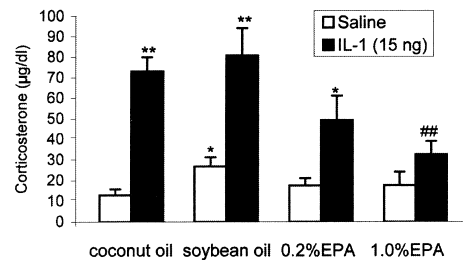


Fig. 3. The effect of a diet enriched with coconut oil, soybean oil, or EPA on a central IL-1 β -induced increase in serum concentrations of corticosterone. The results are expressed as mean \pm SEM. * $P < 0.05$; ** $P < 0.01$ versus coconut oil or soybean oil + saline; ## $P < 0.01$ versus coconut oil + IL-1 groups ($n = 8-9$).

sterone elevation. Two-way ANOVA analysis revealed significant interaction between EPA and IL-1 β ($F_{3,32} = 7.46$, $P < 0.0001$). When compared with rats fed coconut oil diet and challenged with IL-1, the 1% EPA diet significantly reduced serum concentration of corticosterone following IL-1 β administration ($P < 0.01$) (Fig. 3).

There was no significant difference in hippocampal PGE2 concentrations between four groups on the different diets following saline icv administration. However, following IL-1 β administration, PGE2 was largely increased in the group fed with coconut oil or soybean oil ($F_{1,16} = 4.87$, $P < 0.02$). The interaction between IL-1 and EPA was also significant ($F_{3,32} = 4.05$, $P < 0.05$). The group comparison showed that only 1% of EPA significantly attenuated the IL-1-induced increase in PGE2 ($P < 0.05$) (Fig. 4).

DISCUSSION

Previous studies have shown that central administration of murine recombinant IL-1 β to rats at a high dose (100 ng) or peripheral injection into mice 1 h before training impaired animal memory in the Morris water maze (7, 8). However, Yirmiya, Winocur, and Goshen (47) recently reported that human IL-1 β failed to change the spatial memory of rats in the Morris water maze. The present study has shown that rat recombinant IL-1 β injection significantly impaired rat spatial memory in the water maze.

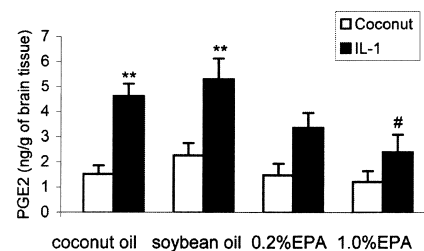


Fig. 4. The effect of a diet enriched with coconut oil, soybean oil, or EPA on IL-1 β -induced increase in hippocampal prostaglandin E2 (PGE2) concentrations. The results are expressed as mean \pm SEM. ** $P < 0.01$ versus coconut oil or soybean oil + saline; # $P < 0.05$ versus coconut oil + IL-1 groups ($n = 8-9$).

These results are similar to those reported by Oitzl et al. (7), who found that IL-1 impaired memory retrieval without affecting acquisition. The present study, by injecting IL-1 β immediately after training, demonstrated that IL-1 β significantly impaired memory retrieval on the second day of testing.

Several previous studies have reported that n-3 fatty acid deficiency can impair spatial learning and memory, which can be reversed by a diet enriched with n-3 fatty acids (39, 48). Some studies have also shown that DHA or fish oil (containing both DHA and EPA) enhanced spatial memory or other types of memory in normal and aged animals (40, 42, 49). However, the durations of exposure to these diets were over two or four generations, or, alternatively, following high n-3 fatty acid concentrations (5%). In the present study, the effect of pure EPA on the spatial memory of IL-1-treated animals has been reported for the first time. EPA, as a precursor of DHA, did not significantly enhance memory in the control rats. These results were similar to the findings of others who reported that feeding an n-3 fatty acid-enriched diet for two generations, or for two months, did not enhance memory in control animals (50, 51). However, the present study demonstrated that 1% EPA significantly attenuated memory impairment induced by IL-1 β . It was also demonstrated that IL-1-induced memory impairment and EPA effect were not related to changes in swimming speed or vision. Changes in the concentration of neurotransmitters, corticosterone, and PGE2 may be involved in the mechanism, thereby IL-1 affected spatial learning and memory because EPA improved learning, and memory was associated with the attenuation of these changes. In the hippocampus, IL-1 β significantly reduced the NA concentration. Spatial memory in the Morris water maze is hippocampus-dependent learning (52, 53). The central functions of NA have been shown to regulate alertness, the wakefulness-sleep cycle, attention, learning, and memory. A reduction of NA concentrations in the hippocampus has been associated with impairment in spatial learning and memory (53). A previous study has also demonstrated that depletion of NA, but not 5-HT, reduced long-term potentiation in the hippocampus, which is assumed to be a major cause of memory impairment (54). *In vitro* studies have found that IL-1 β can significantly inhibit LTP in the hippocampal slice (55). In addition, changes in brain NA is also associated with an anti-inflammatory response in the brain. Recently, Heneka et al. (56) reported that NA depletion in the locus ceruleus enhanced aggregated amyloid β -induced brain inflammation and IL-1 β expression through the inhabitation of I κ B and heat shock proteins. Thus, the present study suggests that decreased NA concentration plays an important role in IL-1-induced spatial memory impairment.

In contrast to the effect of decreased NA concentrations on memory, increased 5-HT and its metabolite, 5-HIAA, have been associated with impaired spatial memory in animal models of sleep disorder and aging (57, 58). Stress exposure, exogenous corticosterone, or IL-1 administration can all increase 5-HT metabolism and 5-HT re-

ceptor functions in the limbic system of the brain, which is accompanied by impairment of spatial memory (59, 60, 61). It has been found that the neurotoxin, 5,7-dihydroxytryptamine, causes serotonergic deafferentation in the hippocampus and enhances spatial discrimination learning in rats (62). In the present study, IL-1 β significantly increased blood corticosterone concentrations, hippocampal 5-HIAA and TRP concentrations, and 5-HT turnover, which may contribute to the effect of IL-1 on spatial discrimination.

In the present study, an increase in DA and its metabolite HVA in the hippocampus were found after central IL-1 β administration. Even though some studies have shown that the D1 receptor is involved in location of both hidden and visible platforms in water maze learning, the relationship between the DA, its metabolites, and spatial memory is unclear. Changes in the function of the dopaminergic system may be related to the emotional state, stress response, and anhedonic behavior (63, 64), which may indirectly influence memory. In this study, EPA dose dependently attenuated an IL-1-induced decrease in NA and an increase in MHPG concentrations, and 1% EPA also reversed an IL-1-induced increase in the turnover of NA. In the serotonergic system, EPA dose dependently reduced IL-1-induced increases in 5-HIAA, TRP, and the 5-HT turnover. However, neither concentration of EPA significantly changed NA or 5-HT, or their metabolites, in the rats treated with saline. By contrast, in the dopaminergic system, the EPA dose dependently increased hippocampal DA, DOPAC, and HVA concentrations in the saline-treated group. IL-1 β administration induced similar changes in DA, DOPAC, and HVA as EPA and also decreased DA turnover, which was not significantly attenuated by either the 0.2% or 1% concentrations of EPA. These results suggest that EPA attenuated the IL-1 effect on memory may be related to a modulation of noradrenergic and serotonergic systems but not the dopaminergic system. How EPA enters the brain is still unclear. The vascular system of the brain is lined with endothelial cells that are a rich source of EPA. Several papers have reported that unsaturated fatty acids (which may include EPA) can pass through the blood-brain barrier or can be taken up by the brain (65, 66).

IL-1 β significantly increased the serum corticosterone secretion and hippocampal PGE2 concentration, changes that were consistent with previous findings (23). A hypersecretion of corticosterone not only suppressed LTP and increased 5-HIAA but also reduced the neuronal number and hippocampal volume (67, 68). In aging rats, an anti-inflammatory COX2 inhibitor, celecoxib, has been shown to significantly reverse these changes (69). The present study showed that n-3 fatty acid EPA has similar effects on a COX2 inhibitor, which significantly reduced IL-1-induced increases in hippocampal PGE2 secretion and serum corticosterone concentrations. Unsaturated fatty acids can modulate GC receptor function and intracellular signaling pathways (70, 71). The inhibitory effects of these fatty acids on GC receptors are ranked in the order n-3 > n-6 > n-9. However, Gottlicher et al. (72) reported that linoleic

acid and AA (both n-6 fatty acids) have similar effects to dexamethasone and activated a chimera of GC receptors. The present study demonstrated that soybean oil (ratio of n-6/n-3, approximately equal to 7) induced a small, but significant, increase in serum corticosterone concentration without significantly exacerbating IL-1 effects. Similar results were found in our previous study in which a diet enriched with 0.5% AA significantly increased the corticosterone concentration in saline-treated animals (23), although the increase in hippocampal PGE2 concentration did not reach statistical significance after feeding with soybean oil. The lack of effects of soybean oil on the concentration of PGE2 in the brain may be partly attributed to the presence of n-3 fatty acids as a minor component of the oil, while the slight rise in the basal corticosterone concentration following the soybean oil diet could be attributed to an increase in adrenal steroidogenesis caused by the fatty acids (73).

In double-blind clinical trials, EPA has been shown to reduce plasma concentration of IL-1 β by approximately 54% (74). The modulation of corticosterone secretion by E-EPA may be related to its anti-inflammatory effect on PGE2, as both EPA and PGE2 can pass through the blood-brain barrier (75, 65, 66). The IL-1-induced changes in body temperature, inflammatory responses, and corticosterone secretion have been shown to be mediated by activation of PGE2 and its receptors (75, 76). IL-1 β activates phospholipase A2 to release AA (n-6) that is converted to PGE2 (30, 32). The PLA2 inhibitor mepacrine has been shown to dramatically inhibit PGE2 synthesis and ACTH secretion (77). PGE2 also plays an important role in IL-1 β -induced neuronal activation and upregulation of CRF mRNA in the paraventricular nucleus of the hypothalamus, which, via ACTH, induces corticosterone secretion (75). Systemic administration of the COX inhibitor indomethacin can attenuate IL-1 effects in the hypothalamus (78). Therefore, the attenuated memory deficit and decreased corticosterone concentration after feeding E-EPA for 8 weeks may result, in part, from the suppressive action of EPA on the release of AA and the formation of PGE2.

In summary, the IL-1 β -induced spatial memory deficit observed in the present study appears to be related to changes in the noradrenergic and serotonergic system and an increase in corticosterone concentration. Furthermore, the mechanism whereby E-EPA attenuated the impairment of spatial memory by IL-1 β may be due in part to a reduction of PGE2, as EPA can inhibit thromboxane A2, leukotriene B4, and eicosanoid production from AA (30, 32). Soybean oil, containing mostly n-6 fatty acids, had no effect on IL-1-induced changes. ■

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