

ORIGINAL RESEARCH ARTICLE

Ethyl-eicosapentaenoic acid ingestion prevents corticosterone-mediated memory impairment induced by central administration of interleukin-1 β in rats

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In memory of Dr David Horrobin

Central or peripheral administration of the proinflammatory cytokine interleukin (IL)-1 β can impair performance on spatial memory tasks and also elevate circulating concentration of corticosterone. The present experiment provides independent confirmation that intracerebroventricular administration of 10 ng IL-1 β in the rat can have a selective effect on the retrieval of trial unique information about the location of food on an eight-arm radial maze. The probable involvement of corticosterone in IL-1 β -induced memory impairment was indicated by elevated corticosterone levels after IL-1 β administration. Further evidence comes from the blockade of the associated impairment in working memory by coadministration of the glucocorticoid receptor antagonist RU486. Ingestion of diet containing omega-3 fatty acid eicosapentaenoic acid (EPA) is known to antagonize the synthesis of prostaglandin (PG) E2 from arachidonic acid, and the present study confirmed that ethyl EPA (1%) reduced IL-1 β -elevated concentrations of PGE2 and corticosterone. Furthermore, rats given the ethyl-EPA diet for 8 weeks were unaffected by the disruptive effects of IL-1 β on working memory. IL-1 β -induced suppression of mitogen-stimulated release of the anti-inflammatory cytokine IL-10 was also blocked by treatment with ethyl-EPA. Collectively, these data demonstrate that IL-1 β can impair memory function by elevating the concentration of corticosterone and that prior consumption of 1% ethyl-EPA can block both the neuroendocrine and cognitive effects of IL-1 β . These findings in turn may indicate beneficial effects of ethyl-EPA in the treatment of cognitive and affective disorders in which inflammation and stress play a critical role.

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Introduction

A growing body of evidence indicates that inflammatory disorders in the periphery or in the brain may be related to cognitive impairment. Intracerebroventricular (i.c.v.) injection of 100 ng murine interleukin (IL)-1 β to rats impairs spatial memory on the first trial of a retention test.¹ Spatial learning is also impaired in mice given IL-1 β (100 ng) daily prior to training,¹ and peripheral IL-1 β or bacterial endotoxin administration to rats also disrupts performance in the Morris water task.¹ Furthermore, IL-1 β concentrations are increased during isolation stress, and an IL-1 receptor antagonist given after conditioning can prevent impairment of contextual fear conditioning following isolation.²

During an inflammatory response, activated T and B lymphocytes, macrophages and proinflammatory cytokines may cross the brain–blood barrier to activate brain microglia cells,^{3–5} which in turn increase the synthesis of IL-1 β and the proinflammatory prostaglandin (PG) E2 in the brain.^{6,7} Central or peripheral administration of IL-1 β increases the release of corticotropin-releasing factor (CRF) that triggers sequentially adrenocorticotrophic hormone (ACTH) and glucocorticoid (GC) secretion.^{8,9} Recent data indicate that IL-1 β may elevate corticosterone levels indirectly by the induction of cyclo-oxygenase-2 mRNA expression that results in PGE2 synthesis since COX inhibitors can block the increase in corticosterone induced by IL-1 β .^{7,10} Both IL-1 and GC receptors are located in the hippocampus,¹¹ a brain region that is essential for many aspects of declarative and contextual forms of memory.^{12,13} Acute GC treatment can suppress long-term potentiation (LTP) and synaptic transmission in the hippocampus,¹⁴ and excessive activation of GC receptors

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can impair memory.^{15,16} The GC receptor antagonist RU486 can block GC-impaired memory.^{14,17} Therefore, we hypothesize that memory impairment induced by IL-1 β may be a consequence of elevated corticosterone via its direct effects on the hypothalamic–pituitary–adrenal (HPA) axis, or indirect effects on the HPA axis via activation of PGE2.

The present study first established a selective effect of IL-1 β on a form of spatial working memory that is dependent on a neural circuit linking the hippocampus with the medial prefrontal cortex (mPFC) in the rat.¹⁸ The possible involvement of the HPA axis in IL-1 β -induced memory impairment was assessed by determining the serum concentrations of corticosterone after i.c.v. administration of IL-1 β and by coadministration of the GC receptor antagonist RU486. Omega (n)-6 unsaturated fatty acid arachidonic acid (AA) is the major substrate for PGE2, and recently it was reported that treatment with n-3 fatty acid eicosapentaenoic acid (EPA) can compete with AA, thereby reducing PGE2 synthesis.¹⁹ A previous study has shown that diet enriched with AA increase GC and PGE2 secretion.²⁰ Furthermore, EPA can suppress the proinflammatory effects of AA.¹⁹ In a separate experiment, a dietary supplement of ethyl-EPA was employed as a means of attenuating IL-1 β -induced activation of PGE2. This strategy was selected because EPA, a compound derived from natural food sources, has minimal side effects as compared to COX inhibitors, and therefore may ultimately have greater benefit in the treatment of memory deficits related to elevated GCs induced by IL-1 β . Serum concentrations of proinflammatory PGE2 and the release of the anti-inflammatory cytokine IL-10 were also measured to determine whether ethyl-EPA can restore a balance between these pro- and anti-inflammatory cytokines.

Materials and methods

Experimental design

In experiment 1, the effects of different doses of IL-1 β administration on the acquisition and retrieval of spatial memory in an eight-arm radial maze were studied. Animals were divided into eight groups ($n=6-7$). Four groups of rats received saline (10 μ l), IL-1 β (10 ng), IL-1 β (50 ng) or RU486 (100 ng), respectively, 45 min before training. The other four groups received these treatments 45 min before a memory test.

Drug treatment continued for 3 days, and the long-term consequences of the drugs were assessed over three additional daily tests without drug treatment.

The second experiment examined whether central administration of the GC receptor antagonist RU486 could block IL-1 β -induced impairment in spatial working memory. The experimental procedure and drug doses were the same as experiment 1. However, i.c.v. injections were only given during the delay interval, 45 min before the memory test. Rats were divided into six groups ($n=6-8$): saline or IL-1 β (10 or 50 ng), RU486 alone and RU486 coadministration with both doses of IL-1 β . RU486 was injected 5 min before IL-1 β administration.

In the third experiment, the effects of an ethyl-EPA-enriched diet on IL-1 β -induced impairment in memory and elevated corticosterone were evaluated. Animals were divided into six groups ($n=8$). Two groups of rats were fed with 5% coconut oil, two groups with 4.8% coconut oil mixed with 0.2% ethyl-EPA and two groups with 4% coconut oil mixed with 1% ethyl-EPA. Rats were subsequently injected with saline or 10 ng IL-1 β i.c.v. 45 min before the memory test on three successive days. A dose of 50 ng IL-1 β was not included because of the nonspecific effects observed in experiments 1 and 2. One day after the end of behavioral testing, rats received a fourth dose of IL-1 β and were killed 45 min later under halothane anesthesia. Blood samples for culture were taken by cardiac puncture, while trunk blood was collected for corticosterone and PGE2 assay. The experimental time line was shown in Table 1.

Animals and diets

Male Long–Evans rats (280–300 g for experiments 1 and 2, 200–220 g for experiment 3 at the start of the experiment) were purchased from Charles River, Quebec, Canada. Rats were allowed free access to food and water except for food deprivation before and during training and testing on the maze. The colony was maintained at $21 \pm 1^\circ\text{C}$ with a 12 h light–dark cycle (at 0730 to 1930). Animals were handled daily. The research protocol was approved by the Animal Care Committee of the University of British Columbia, Canada, and conformed to the guidelines of the Canadian Council for Animal Care.

In experiments 1 and 2, rats were fed a diet of normal laboratory chow. In experiment 3, rats were fed a diet with or without ethyl-EPA. Coconut oil that

Table 1 Experimental Time Line

Exps. 1 and 2.	Habituation	Surgery	Recover	Weight reduction	Training	Saline or IL-1 β injection	Testing	Histology
	7 days	1 day	14 days	10–14 days	10–14 days	45 min before training or test	6 days	1 day
Exp. 3.	Habituation		Same procedure as Exps. 1 and 2					
	7 days		Diet + EPA (0.2; 1.0%) or coconut oil (5%) for 49 days					

Table 2 Nutrition composition in basal mix diet

Names	g/kg
Casein, 'vitamin-free' test	202.11
DL-methionine	3.16
Sucrose	688.39
Cellulose	52.63
Mineral mix, AIN-93-MX (TD 94046)	36.85
Calcium phosphate, dibasic CaHPO ₄	3.69
Vitamine mix, AIN-93-VX (TD 94047)	10.53
Choline bitartrate	2.64

contains negligible amount of n-3 and n-6 fatty acids was added to the control diet to ensure comparable texture and caloric value. Basal Mix (Harlan Teklad Test Diet, USA), coconut oil (Harlan Teklad Test Diet, USA) and ethyl-EPA (Laxdale Ltd, UK) were stored in a 4°C refrigerator. Basal mix without fat was designed for use at 95% in preparing diets with the addition of 5% (50g/kg) of a selected fat/oil. The composition of the diets is shown in Table 2. In all, 5% coconut oil, 4.8 or 4% coconut oil + 0.2 or 1% ethyl-EPA (for EPA diet) was mixed with 95% Basal Mix and fed to rats for 8 weeks.

Food deprivation

Before animals began training on the maze, they were food deprived gradually to 90% of their free feeding weight. Throughout the experiments all animals were weighted daily and were maintained at the 90% weight level by adjusting the quantity of the assigned diet provided each day between noon and 1300.

Surgery

All rats were anesthetized with 100 mg/kg ketamine and 10 mg/kg xylazine. Tetracycline was used to treat incisions. 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 1 mm depth and secured to the skull with three screws by dental cement. A dummy cannula is then screwed into the guide cannulae.²¹ Animals were allowed to recover for 10–14 days and handled daily.

Reagents and i.c.v. injection procedure

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 10 and 50 ng/10 μ l/rat and prepared for i.c.v. administration. The GC receptor antagonist RU486 was obtained from Sigma, Canada, and dissolved with 99% ethanol and then diluted with sterile saline to 100 ng/10 μ l/rat. The final concentration of ethanol was less than 1%.²²

Rats were gently held in a soft cotton towel every day for 2 weeks before the injection. On the injection day, IL-1 β , RU486 or saline were taken into an injection needle (4.2 mm length) that was connected to a PE 50 polyethylene tube. After removing the

dummy cannulae, the needle was gently inserted into the guide cannula and IL-1 β or saline was slowly infused into the brain over a 30 s interval. The injection needle remained in place for 1 min.

Apparatus

The eight-arm radial maze had an octagonal center platform 40 cm in diameter connected to eight equally spaced arms, 50 cm \times 9 cm, each with a food cup at the end. Removable plastic barriers (9 \times 13) were used to block arms of the maze. The maze was elevated 40 cm from the floor and was surrounded by numerous extra maze cues.

Memory task

A delayed spatial win-shift version of an eight-arm radial maze task was adapted from previous studies.^{23,24} Rats were habituated to the maze for 2 days, then memory tasks were given once daily. Each trial consisted of a training phase and a memory test phase, separated by a delay (initial set at 5 min and increased to 50 min). Before the training phase, four arms were randomly baited with food pellets (Bioserv, French, NJ, USA). The other arms were blocked. In the training phase, each rat was given 5 min to retrieve the food from the four open arms. During the memory test phase, all arms were opened and rats explored the maze until they had retrieved food located in the four arms that were blocked during training or 5 min had elapsed. An arm entry was defined as movement along the arm to the food cup. Criterion performance during the memory tests was defined as five or fewer arm entries to locate four food pellets.

Assays for IL-10 and PGE2

Heparinized blood, incubated with and without mitogens (phytohemagglutinin, 5 μ g/ml, and lipopolysaccharide, 20 μ g/ml), was used to study the release of IL-10 as described previously.²⁵ Briefly, samples (200 μ l) were pipetted into 24-well plates filled with medium (1800 μ l) and incubated for 72 h in a humidified atmosphere at 37°C, 5% CO₂. After incubation, the plates were centrifuged at 1500 r.p.m. for 15 min. The supernatant was removed under sterile conditions and frozen immediately at -70°C. The release of IL-10, and serum PGE2 concentrations, were measured by a quantitative enzyme-linked immunosorbent assay (Biosource International, CA, USA) and by enzyme immunoassay (Assay Designs, Inc., Ann Arbor, MI, USA) respectively.

Corticosterone assay

Serum samples from trunk blood were used for corticosterone measurement with a commercial radioimmunoassay kit (Immucor corticosterone RIA kit for rats; catalogue no. RCBK9906A; ICN Biochemical, Costa Mesa, CA, USA). Intra- and interassay coefficients of variation were 6.8 and 5.6%, respectively.²⁶

Histology

After decapitation, brains were rapidly removed, placed on ice block and the locations of the cannula and injection site were quickly checked by sectioning the brain coronally. The data were excluded from any animals with injection sites outside the lateral ventricle.

Data analysis

The behavior results were analyzed by two-way ANOVA with repeated measurement. Results for corticosterone, cytokines and PGE2 were analyzed by two-way ANOVA. The difference between groups was assessed by Scheffe's *post hoc* tests (the package was from GB-STAT, Dynamic Microsystems, Inc., USA). Significance was set at $P < 0.05$. Results are expressed as mean \pm SEM.

Results

Impairment of working memory by IL-1 β

Rats that received saline i.c.v. administration prior to either the training or memory test phase of the delayed working memory task were able to acquire trial unique information about the location of food pellets in specific arms on the maze. These rats were also able to utilize this information after a 50 min delay to select and enter a subset of four arms that now contained food (Figure 1a and b). Central administration of either dose of IL-1 β prior to training had no effect on the time taken or the number of arm choices to find food reward during the training period. However, the ANOVA confirmed that IL-1 β administration significantly increased errors during the memory test (day 1, $F_{3,22} = 33.96$, $P < 0.0001$; day 2, $F_{3,22} = 14.16$, $P < 0.001$; day 3, $F_{3,22} = 22.34$, $P < 0.0001$). The high dose IL-1 β (50 ng) given prior to training or testing phase disrupted memory-based food searching behavior during all three test days ($P < 0.01$) (Figure 1e and f). Treatment with a 10 ng dose of IL-1 β prior to the training had no effect on memory for food location during the first two days, but a slight increase in errors was observed on treatment day 3 (Scheffe's *post hoc* tests, $P = 0.07$) (Figure 1c). The administration of 10 ng IL-1 β prior to the memory test significantly increased errors on all 3 days (Figure 1d). A two-way ANOVA indicated that the interaction between the time of injection and IL-1 β -induced memory impairment was significant (day 1, $F_{3,51} = 4.19$, $P < 0.05$; day 2, $F_{3,51} = 9.78$, $P < 0.001$ ANOVA). On day 4 (post-treatment), a significant increase in errors was still observed in the groups that received 50 ng IL-1 β infusion prior to both training and testing phases ($F_{3,54} = 4.28$, $P < 0.05$) (Figure 1e and f). On days 5 and 6 (post-treatment), the memory-based choice of correct arms recovered to control levels (Figure 1e and f).

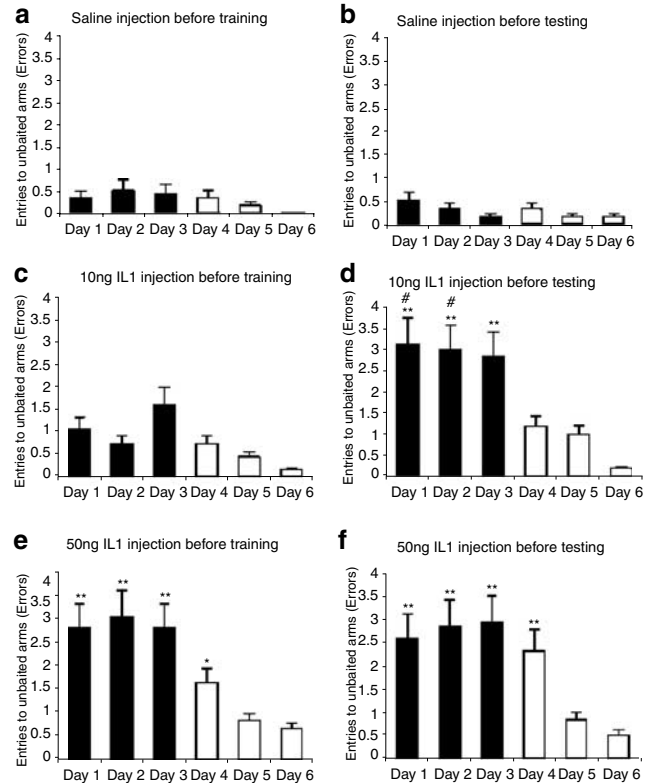


Figure 1 Memory impairment following i.c.v. administration of IL-1 β (10 or 50 ng) prior to training or memory-based retrieval of food on an eight-arm maze. Black columns represent 3 treatment days, and white columns represent 3 post-treatment days. (a) Saline treatment before training; (b) saline treatment before testing; (c) 10 ng IL-1 β treatment before training; (d) 10 ng IL-1 β before testing; (e) 50 ng IL-1 β treatment before training; and (f) 50 ng IL-1 β before testing. * $P < 0.05$; ** $P < 0.01$ vs saline group, # $P < 0.01$ vs IL-1 (10 ng) injection before training.

Central RU486 administration prevents IL-1 β -induced memory impairment

I.c.v. administration of RU486 alone prior to or after training had no effect on acquisition or retrieval of memory for the correct location of food in the radial maze (result from RU486 administration alone before training not shown). In the second experiment, administration of 10 or 50 ng IL-1 β before testing induced similar working memory deficits as described in experiment 1 (day 1, $F_{2,22} = 9.68$, $P < 0.001$; day 2, = 14.31, $P < 0.001$; day 3, = 6.21, $P < 0.01$) (Figure 2a). Central RU486 infusions significantly attenuated the memory deficit induced by IL-1 β (day 1, $F_{1,41} = 19.71$, $P < 0.001$; day 2, = 16.73, $P < 0.01$; day 3, = 7.31, $P < 0.01$) (Figure 2a). On the first post-treatment day, errors in the groups that received 10 or 50 ng IL-1 β alone prior to testing were still significantly greater than in the saline-treated groups ($F_{2,24} = 4.21$, $P < 0.05$) (Figure 2b). In the last two post-treatment days, there were no significant group differences (Figure 2b).

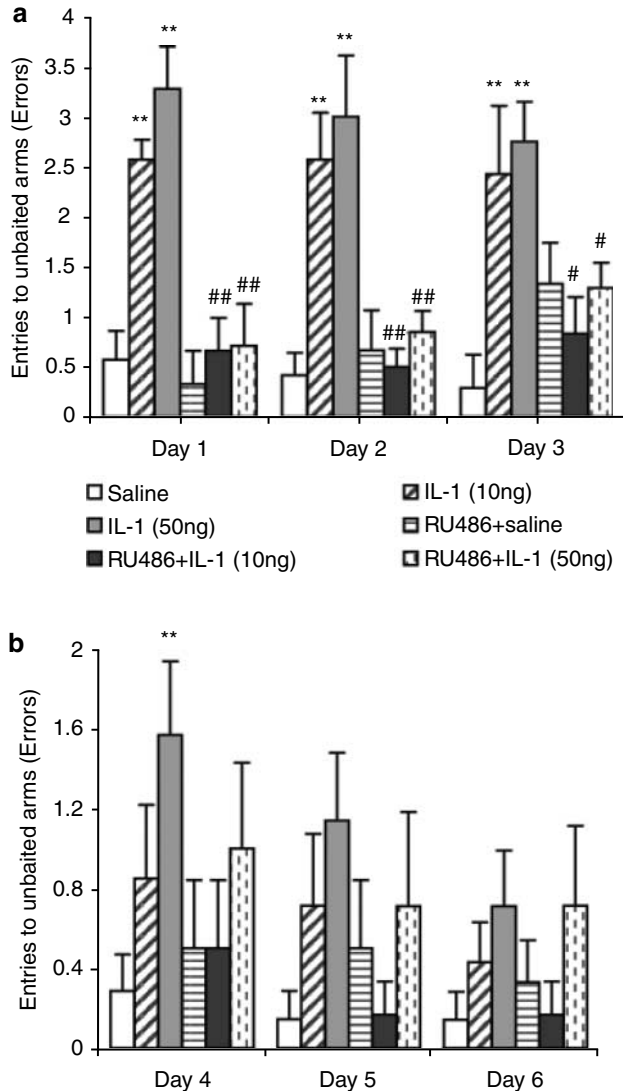


Figure 2 I.c.v. RU486 administration blocks IL-1 β -induced impairment of memory-based retrieval of food on an eight-arm maze. (a) Saline or IL-1 β treatment for 3 days and (b) 3 days of post-treatment. ** P <0.01 vs saline group; # P <0.05; ## P <0.01 vs IL-1 (10 ng) or IL-1 (50 ng) group.

Dietary supplement of ethyl-EPA prevents IL-1 β -induced memory impairment

Memory errors defined as entries into unbaited arms were increased significantly relative to controls, following i.c.v. administration of 10 ng IL-1 β to rats fed with the coconut diet (day 1, $F_{1,13} = 39.38$; P <0.0001; day 2, =42.25, P <0.0001; day 3, =31.36, P <0.0001) (Figure 3). In contrast, rats fed with ethyl-EPA diets failed to display any working memory impairment despite prior treatment with IL-1 β (day 1, $F_{2,24} = 7.26$, P <0.01; day 2, $F_{2,24} = 8.76$, P <0.01; day 3, $F_{2,24} = 6.51$, P <0.01) (Figure 3). Scheffe's *post hoc* test revealed that a 0.2% EPA diet only partially attenuated IL-1 β -induced disruption in memory (P <0.05 on day 3), whereas 1% EPA completely blocked IL-1 β effects (P <0.01 for 3 days).

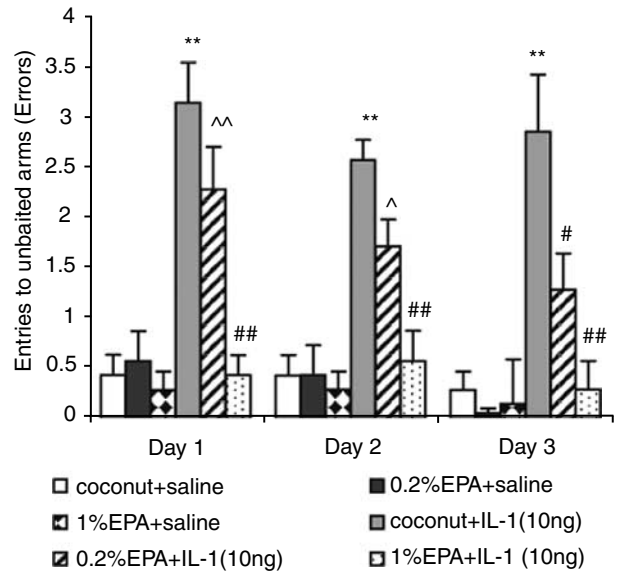


Figure 3 Effects of diet enriched with 0.2 or 1% ethyl-EPA on IL-1 β -induced impairment of memory-based retrieval of food on an eight-arm maze. ** P <0.01 vs coconut oil + saline; ^ P <0.05; ^^ P <0.01 vs 0.2% ethyl-EPA + saline; # P <0.05, ## P <0.01 vs coconut oil + IL-1.

Saline-treated rats fed with 0.2 or 1% ethyl-EPA displayed few errors and did not differ significantly from saline-treated rats fed with the coconut oil diet. After saline treatment, rats fed with diet containing 5% coconut oil, displayed normal learning and memory in the maze when compared to the rats fed with rat chow after saline treatment (Figure 3).

Ethyl-EPA blocked IL-1 β -induced increases in PGE2 and corticosterone concentrations and suppression of IL-10 release

Central administration of IL-1 β significantly elevated serum concentrations of corticosterone in rats fed with a diet containing 5% coconut oil ($F_{2,24} = 22.83$, P <0.0001). The effect of ethyl-EPA diet on corticosterone levels showed a similar pattern to that of ethyl-EPA on memory. EPA (0.2%) partially attenuated, while 1% EPA significantly blocked IL-1 β -induced elevation in this stress hormone ($F_{2,24} = 5.57$, P <0.01) (Figure 4). IL-1 β administration also significantly increased serum PGE2 concentrations in the coconut oil group ($F_{2,24} = 13.53$, P <0.0001), but suppressed IL-10 release from the blood culture following mitogen stimulations ($F_{2,24} = 10.07$, P <0.001) (Figure 5a, b). The release of IL-10 in the blood without mitogen stimulation was undetectable. Following i.c.v. administration of IL-1 β rats fed with diets enriched with either concentration of ethyl-EPA showed significantly decreased PGE2 concentrations and increased IL-10 release relative to those maintained on a diet containing coconut oil (PGE2: $F_{4,70} = 5.57$, P <0.01; IL-10: $F_{4,70} = 7.36$, P <0.01) (Figure 5a, b).

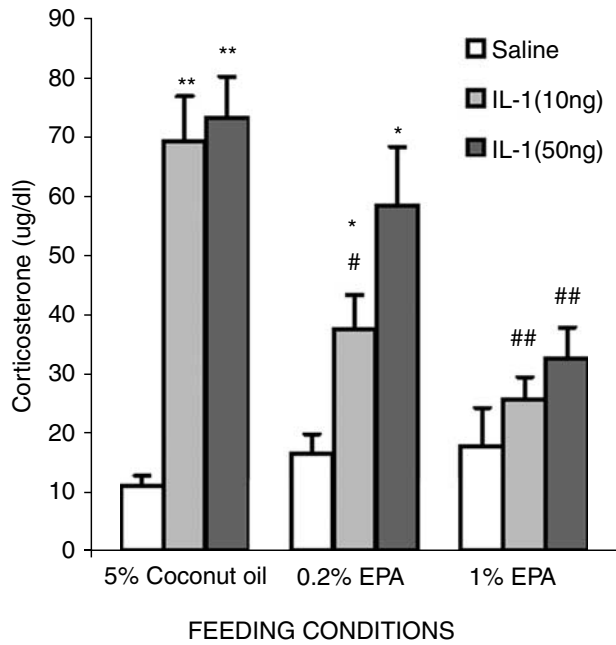


Figure 4 Effects of diet enriched with ethyl-EPA on IL-1 β -induced increase in serum corticosterone concentrations. * $P < 0.05$ vs 0.2% ethyl-EPA + saline; ** $P < 0.01$ vs coconut oil + saline, * $P < 0.05$ vs coconut oil + IL-1 (10 ng); ## $P < 0.01$ vs coconut oil + IL-1 (both doses) groups.

Discussion

The present study provides clear evidence that i.c.v. administration of IL-1 β can produce a significant deficit in spatial working memory. Rats treated with IL-1 β could not utilize information acquired 50 min earlier to locate food pellets in a complex spatial environment. This form of working memory is mediated by a neural connection between the hippocampus and mPFC.¹⁸ Importantly, rats treated with either dose of IL-1 β did not differ from saline controls with respect to the number of food pellets eaten and latency to find the food on the maze in the training phase. This suggests that IL-1 β did not change the motivation to search for the food reward. Specifically, administration of 10 ng IL-1 β prior to training did not impair memory retrieval in the radial maze in the first two days, whereas infusion of the same dose of IL-1 β prior to memory test significantly increased errors defined as entries into unbaited arms. This finding suggests that IL-1 β selectively disrupts memory retrieval but not encoding. The administration of 50 ng IL-1 β either before training or testing increased memory errors during the testing phase, and this may be due to an extended duration of action. Other investigators have reported that administration of murine IL-1 β to rats or to mice can impair spatial learning in a Morris water task.¹

High concentration of corticosterone can decrease LTP in the CA1 region of the hippocampus, which can be blocked by the GC receptor antagonist RU486.^{14,17} In the present study, i.c.v. administration of RU486 alone did not affect spatial memory, whereas its

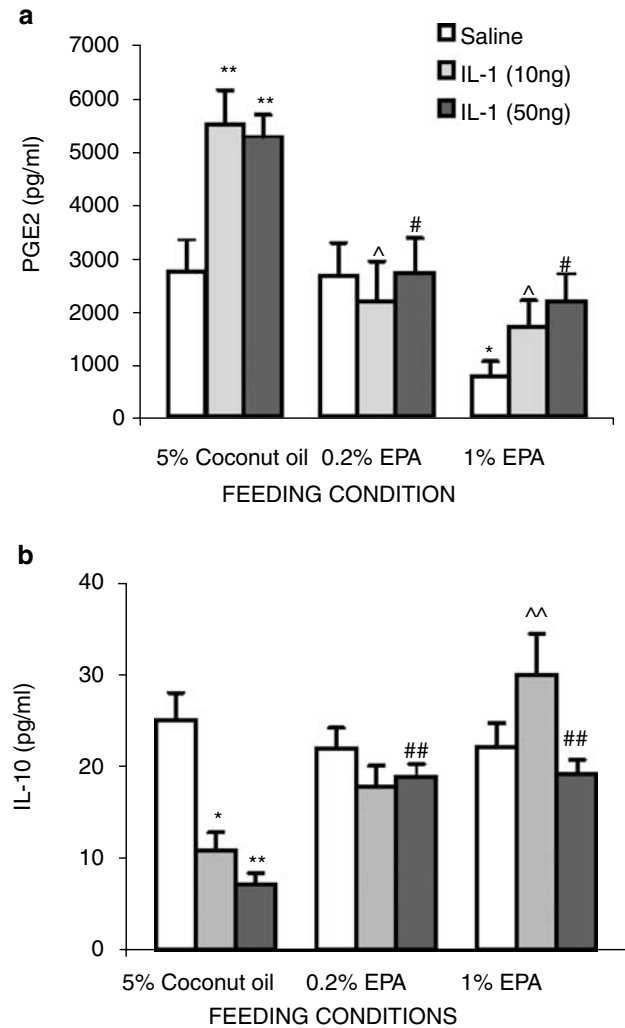


Figure 5 Effects of diet enriched with ethyl-EPA on IL-1 β -induced changes in serum PGE2 concentrations (a) and IL-10 release from blood culture (b). * $P < 0.05$, ** $P < 0.01$ vs coconut oil + saline; ^ $P < 0.05$, ^^ $P < 0.01$ vs coconut oil + IL-1(10 ng); # $P < 0.05$, ## $P < 0.01$ vs coconut oil + IL-1(50 ng).

administration 5 min before IL-1 β infusion did block the disruption of IL-1 β on working memory. In contrast with our result, other investigators have reported that acute i.c.v. but not chronic administration of RU486 impaired spatial memory.^{27–29} Our results are in agreement with Douma *et al*,³⁰ who failed to impair spatial learning by acute or chronic subcutaneous administration of RU486 (2.5 mg/100 g). When RU486 is given to rats peripherally, 28% can cross the blood–brain barrier.³¹

The present study provides the first demonstration that the n-3 fatty acid ethyl-EPA can significantly attenuate memory impairment in an eight-arm radial maze produced by the proinflammatory cytokine IL-1 β . Previous studies have reported that n-3 fatty acids may modulate cognitive behavior. Rats or mice fed an n-3-deficient diet for two generations exhibit

spatial memory impairment accompanied with increased n6/n3 ratio in the brain,³² which can be prevented by feeding an n-3 diet.³³ There is a similar report in the senescence-accelerated mouse, a model of age-related deterioration of memory and learning ability.³⁴ Results from the present study also indicate that the possible mechanism by which ethyl-EPA blocked the effect of IL-1 β may be related to the modulation of GC secretion and receptor activation, as well as the inflammatory response.

In the present study, we also provide the first report that ethyl-EPA can significantly modulate IL-1 β -induced corticosterone secretion, which in turn may influence learning and memory function. Previous studies have reported that unsaturated fatty acids can modulate GC receptor function and intracellular signaling pathway.^{35,36} The inhibitory effects of these fatty acids on GC receptors were ranked as n-3 > n-6 > n-9. Whereas, Gottlicher *et al*³⁷ reported that linoleic acid (n-6 fatty acid) and AA, similar to dexamethasone, activated chimera of GC receptors. It has also been demonstrated that RU486 and n-3 fatty acids bind to different sites on GC receptors to inhibit GC functions.³⁸ In a previous study, we observed that RU486 significantly reduced corticosterone concentrations that were elevated by IL-1 β administration.³⁹ This finding is similar to the present demonstration that EPA can suppress corticosterone concentrations.

The modulation of corticosterone secretion by ethyl-EPA may be related to its anti-inflammatory effect on PGE2 that can pass the blood-brain barrier.⁷ IL-1 β -induced changes in body temperature, immune inflammatory response and corticosterone secretion have been shown to be mediated by activation of PGE2 and its receptors.^{7,40} IL-1 β activates phospholipase (PL) A2 to release AA (n-6) that is converted to PGE2.^{19,41} The PLA2 inhibitor mepacrine has been shown to inhibit PGE2 synthesis and ACTH secretion dramatically.⁴² PGE2 also plays an important role in the IL-1 β -induced neuronal activation and upregulation of CRF mRNA in the paraventricular nucleus of the hypothalamus, which via ACTH induces corticosterone secretion.⁷ Systemic administration of the cyclo-oxygenase inhibitor indomethacin can attenuate IL-1 β effects in the hypothalamus.⁴³ Therefore, the attenuated memory deficit and decreased corticosterone concentration after ethyl-EPA feeding for 8 weeks in the present study may result in part from the suppressive action of EPA on the release of AA and formation of PGE2.

It has been reported that ethyl-EPA and other EPA derivatives, acting as anti-inflammatory modulators, can inhibit the production of proinflammatory cytokines and suppress macrophage and other immune cellular functions by inhibiting thromboxane (TX)A2, leukotriene B4 and eicosanoid production.^{19,41} Kozak *et al*⁴⁴ found that a fish oil diet (with high concentration of EPA) prevented weight loss and also reduced the production of PGE2 and proinflammatory cytokines induced by the systemic injection of bacterial

endotoxin. In the present study, IL-1 β -induced suppression of IL-10 was blocked by ethyl-EPA. IL-10 produced by T-helper 2 (Th2) cells, is an anti-inflammatory cytokine that can antagonize the effect of proinflammatory cytokines (such as IL-1 β) produced by Th1 and macrophages.^{45,46} In patients with depression or Alzheimer's disease, IL-10 concentrations were decreased, whereas the production of proinflammatory cytokines and PLA2 were elevated.⁴⁷⁻⁴⁹ Decreased n-3 fatty acids DHA and EPA have also been reported in these patients.^{50,51} EPA was very effective in the treatment of depression and cognitive impairment.⁵²⁻⁵⁵ It is particularly interesting that in the present study ethyl-EPA blocked IL-1 β -induced inflammatory response in two ways: it increased the production of anti-inflammatory cytokine IL-10 and decreased PGE2 synthesis.

In summary, the IL-1 β -induced spatial working memory deficit observed in the present study appears to be related to an increase in corticosterone concentration because central RU486 administration blocked the effect of IL-1 β . Furthermore, the mechanism whereby ethyl-EPA attenuated the impairment of working memory by IL-1 β may be due in part to a reduction of hypersecretion of corticosterone. Immune modulation effects of ethyl-EPA including the reduction in PGE2 and an increase in the release of IL-10 may also be related to the protective effect of ethyl-EPA against the disruption of spatial working memory induced by IL-1 β . Furthermore, these mechanisms may explain the possible therapeutic effects of ethyl-EPA treatment of unipolar depressive disorder and memory impairment.⁵²⁻⁵⁵

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