Reductions of acetylcholine release and nerve growth factor expression are correlated with memory impairment induced by interleukin-1β administrations: effects of omega-3 fatty acid EPA treatment

Pornmarin Taepavaranuprak*† and Cai Song*

*Department of Biomedical Sciences, AVC, University of Prince Edward Island, Charlottetown, PE C1A 4P3, Canada
†Department of Physiology, Faculty of Medical Science, Naresuan University, Phitsanulok, 65000, Thailand

Abstract
Interleukin (IL)-1β may play an important role in Alzheimer’s disease. However, the relationships between glucocorticoids and acetylcholine (ACh), and between neurotrophins and ACh in IL-1-induced memory deficits are unknown. While ethyl-eicosapentaenoate (E-EPA) has recently been reported to reduce inflammation and improve memory, cholinergic and neurotrophic mechanisms by which E-EPA improves memory is unclear. This study evaluated: (i) the correlation between ACh release and memory impairment; (ii) the effect of glucocorticoids on ACh release; (iii) the relationship between nerve growth factor (NGF) and inflammation; and (iv) the effects of E-EPA treatment on IL-1β-induced changes. Intracerebroventricular IL-1β administrations produced a significant reduction in hippocampal ACh release in rats fed control diet, which was partially attenuated by mifepristone (RU 486) and completely blocked by IL-1 receptor antagonist. In eight-arm radial maze, significantly less ACh release was correlated with the memory deficits after IL-1β administrations. mRNA expression of hippocampal NGF was lower, whereas IL-1β was higher when compared with controls. E-EPA treatment significantly improved the memory, which was correlated with normalizing ACh release, and expressions of NGF and IL-1β. This study revealed important mechanisms by which IL-1β impairs, while E-EPA improves memory through IL-1-glucocorticoid-ACh release and IL-1-NGF-ACh release pathways.

Keywords: acetylcholine, eicosapentaenoate, interleukin-1beta, memory impairment, microdialysis, nerve growth factor.

an increase in IL-1β concentration was found to relate to impaired long-term potentiation (LTP) in aged and stressed rats (Murray and Lynch 1998).

On the other hand, inflammatory responses in the brain may change astrocyte function, and thereby cause the dysfunction of neurotrophin systems (Takuma et al. 2004). Indeed, increased and decreased neurotrophin concentrations and receptor functions have been reported in AD patients (Counts and Mufson 2005; Peng et al. 2005). Furthermore, neurotrophins, such as nerve growth factor (NGF) and brain-derived neurotrophic factor have been used to treat AD (Cattaneo et al. 2004; Nagahara et al. 2009). In experimental studies, NGF was reported to attenuate cholinergic deficits following traumatic brain injury in rats (Dixon et al. 1997), and NGF gene transfer into aged animals can increase the level of depolarization-induced ACh release from hippocampal synaptic terminals (Wu et al. 2004).

However, in the last 20 years, most studies have only focused on the effects of IL-1β on behavior, the endocrine system, and catecholamine functions in IL-1-induced memory impairment is still unknown. The possible pathways of both IL-1-glucocorticoid-ACh release and IL-1-NGF-ACh release are presented by Fig. 1. Thus, the first aim of this study was to demonstrate these pathways. Our hypotheses were that (i) the reduction of ACh release is correlated with memory deficits after IL-1 administration; (ii) a glucocorticoid RA or IL-1 RA can block ACh reduction; and (iii) IL-1 would decrease NGF expression but increase brain inflammation, which is correlated with the reduction of ACh release. To demonstrate these hypotheses, an in vivo microdialysis technique was employed to measure ACh release from the dentate gyrus (DG) of the hippocampus, following IL-1β and saline injections during animal training and testing in an eight-arm radial maze, an apparatus for testing working memory. The reasons to choose the DG for this study are based on that the DG is innervated by basal forebrain cholinergic neurons and the loss of neurogenesis and cholinergic innervations in the DG play an important role in AD (Tatebayashi et al. 2003; deToledo-Morrell et al. 2007). Several studies have demonstrated that lesion of the DG significantly impairs hippocampus-dependent memory (Mohapel et al. 2005). In addition, the DG plays an important role in suppressive control of the HPA axis in AD. Lesion of the cholinergic projection in the basal forebrain is correlated with a significant increase in glucocorticoid receptor expression in the DG (Yau et al. 1992).

IL-1RA and RU 486 were administered before IL-1β administrations to demonstrate IL-1β and glucocorticoid effects on ACh release. mRNA expressions of NGF and IL-1β were measured by quantitative PCR in the hippocampus after IL-1 and IL-1RA administrations. Furthermore, this study also compared the effect of acute (1 day) and subchronic (7 days) IL-1β administrations on ACh release and memory performance in the rat because an increase in IL-1β release in acute brain conditions has been reported to protect neurons (Kuhlow et al. 2003; Dietrich et al. 2004), while in chronic neurodegeneration, excessive produced IL-1β has been considered to contribute to neuron death (Cunningham et al. 2005; Ferrari et al. 2006).

As introduced above, if inflammation can significantly reduce ACh release and neurotrophin function, any drug or natural product that reduces inflammation and enhance neurotrophin function could be an effective treatment for AD. Recently omega (n)-3 fatty acids have been suggested to treat AD because a higher n-3 fatty acid intake was associated with a lower risk of AD onset (Otsuka 2000; Morris et al. 2003). In a pilot clinical trial, a treatment with eicosapentaenoic acid (EPA) and docosahexaenoic acid significantly improve cognitive function in patients with mild cognitive impairment (Chiu et al. 2008). In animal models of AD or aging, significantly reversed learning deficits and normalized the expression of proteins involved in neuronal plasticity (Hashimoto et al. 2008). The mechanism by which n-3 fatty acids protect neurons may be though anti-inflammatory (inhibit nuclear factor-kappaB and reduce n-6 fatty acids to produce inflammatory mediators), anti-stress (reduce glucocorticoids), and modulation of apoptosis genes (Song et al. 2003; Lynch et al. 2007; Singer et al. 2008). Furthermore, we have reported that EPA can up-regulate the brain-derived neurotrophic factor receptor tropomyosin-related protein in fully differentiated SH-SY5Y cells (Kou et al. 2008). However, the ACh and neurotrophin mechanisms by which EPA improves memory are still unknown. The possible target

![Fig. 1](image-url) The hypothesized pathway from IL-1 administration to memory impairment, and EPA effects.

© 2009 The Authors
points of EPA are also presented in the Fig. 1. Our hypothesis was that EPA might improve memory by blocking IL-1β effects on ACh and NGF. To demonstrate this hypothesis, as the second aim of this study, EPA effects on ACh release, memory and mRNA expressions of NGF and IL-1 were evaluated after saline or IL-1β administrations.

In this study, EPA in ethyl ester form was used to feed animals. The main advantages of the ethyl ester appears to be that it can be concentrated to a greater degree and has greater bioavailability than when provided as triglycerides (Brunton and Collins 2007). It also has the potential to be FDA approved, which requires it to be manufactured by good manufacturing practice facilities requiring consistency, purity, and minimal levels of toxins as has been done for prescription omega-3-acid ester. However, it remains unclear whether the advantages gained by esterification are lost by the fact that people absorbed ethyl-EPA less than the unesterified EPA (Lawson and Hughes 1988; Beckermann et al. 2003). The total feeding period was 7 weeks.

The food was prepared every 3–4 days and stored at 4°C dark cycle (7:00–19:00 hours).

Animals and diets
Male Long-Evans rats (280–320 g, Charles River; Canada) were housed two per cage in a colony room (22 ± 1°C) with a 12 h light–dark cycle (7:00–19:00 hours).

For a control diet, 0.8% (v/w) palm oil (Harlan Teklad Test Diet; Sigma, Oakville, ON, Canada), which contained negligible amount of n-3 fatty acids and low n-6 fatty acids, was chosen to ensure comparable texture and calorific value as 0.8% pure ethyl-EPA (Amarin Neuroscience Ltd., Oxford, UK). Food was either prepared as palm oil melted in a water bath (<50°C) (Amarin Neuroscience Ltd.), or EPA added to the rat-chow powder and mixed properly. The food was prepared every 3–4 days and stored at −20°C (Song et al. 2003). The total feeding period was 7 weeks.

There were three experiments. The first experiment was to evaluate acute and subchronic effects of IL-1β and saline administrations on ACh release from the hippocampus, and the correlation between ACh release and animal learning and memory performance in the maze. Fourteen rats were fed the control diet and divided into two groups as: (i) saline injection (n = 7), and (ii) IL-1β injection (n = 7). On day 1 and day 7, 50 min following administrations of acute (one injection) and subchronic (seven injections) saline or IL-1β, ACh release was measured in the hippocampus, and animal learning (in training phase) and memory (in testing phase) were tested in eight-arm radial maze.

The second experiment was to study whether IL-1RA or RU 486 can modulate ACh release in the rats that received subchronic IL-1β-administration. Twenty-one rats were divided into three groups: (i) subchronic saline (for 7 days, n = 7); (ii) RU 486 (once on day 7, n = 7); and (iii) IL-1RA administrations (once on day 7, n = 7). Because previously we reported that acute (once) RU 486 or IL-1RA administration did not induce any changes in the maze learning and memory (Song et al. 2004), we did not use their control groups in this study. The third experiment was for evaluating ethyl-eicosapentaenoate (E-EPA) effects on ACh release and memory performance after acute and subchronic saline or IL-1β administrations. Twenty-eight rats were fed E-EPA for 7 weeks (Song et al. 2004) and divided into four groups as: (i) acute saline group fed E-EPA (n = 6); (ii) subchronic saline group fed EPA (n = 6); (iii) acute IL-1β group fed E-EPA (n = 6); and (iv) subchronic IL-1 group fed E-EPA (n = 8). After collecting dialysates, animals were decapitated and the hippocampus was dissected and stored in −80°C for the measurement of NGF and IL-1 mRNA expressions. The number of animals in each group was the final number. About 10% of animals were excluded because of the loss of cap and the wrong location of cannulae or probes.

Animal body weights were measured weekly before and 7 days after the surgery, and measured daily after surgery and each IL-1 administration. IL-1 induced sickness behavior was also checked a couple of times per day. The research protocol was approved by the Animal Committee, University of Prince Edward Island and conformed to the guidelines of the Canadian Council for Animal Care.

Surgery
Each rat was placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA) after being anesthetized with ketamine [100 mg/kg, intraperitoneal (i.p.)] and xylazine (10 mg/kg, i.p.). The coordinates for the cannulae of microdialysis probes (19 G, 15 mm) aimed at the DG of the hippocampus was AP = −3.8 mm from bregma, ML = 2.0 mm, and −4 mm depth. The coordinates for guide cannulae (22 G, 10 mm) for intracerebroventricular (i.c.v.) administrations of IL-1β or saline were AP = −0.8 mm, ML = 1.4 mm, −1 mm depth. Cannulae were secured to the skull with screws and dental acrylic. Rats were allowed to recover for 14 days, and were handled daily.

Central administrations of IL-1β and other drugs
Rat recombinant IL-1β (NIBSC, Potters Bar, UK) was dissolved in sterile, pyrogen-free saline at doses of 15 ng/5 μL/rat/day. IL-1β or the saline (5 μL) was slowly infused into the lateral ventricle over the period of 60 s daily for 7 days (Song et al. 2004). IL-1RA (R&D System, Minneapolis, MN, USA, 100 ng/5 μL/rat) or RU 486 (Sigma, Canada; 1 μg/5 μL/rat) was applied i.c.v. 10 min prior to IL-1β injection on day 7 with the same method as IL-1β administration. The reason for the administration of RU 486 and IL-1RA only once before the last injection of IL-1β is because of administrations of RU 486 for 3 days could impair working memory in the radial maze (Song et al. 2004), and the impairment of spatial memory was observed in IL-1 receptor knock-out mice (Avital et al. 2003).

Microdialysis procedures
The probe was secured in a metal collar and its inlet and outlet tubes were inserted through a stainless steel coil tether and connected to a swivel (Instech, Inc., Plymouth Meeting, PA, USA) located at the end of the post. The perfusate solution consisted of 147 mM NaCl, 3.0 mM KCl, 1.3 mM CaCl2·H2O, 1.0 mM MgCl2·6H2O, 0.01 M sodium phosphate buffer; pH 7.4 ± 0.2. Neostigmine bromide (10 μM, Sigma Chemical Co., St Louis, MO, USA) was added to the perfusate solution in order to retard ACh metabolism (Rada et al. 1991).

Dialysis experiments were conducted on day 1 and day 7, 50 min after IL-1 administration (Song et al. 2004). On the memory test day, the probe was inserted into the guide cannula and four baseline
dialysate samples were collected after the release of ACh was stable for 2–3 h, as checked by HPLC. Saline or IL-1β was administered i.c.v. and dialysate samples were continuously obtained at 20 min intervals for 180 min. Meantime, rat learning and memory were tested in the radial maze as described below.

In the second experiment, IL-1RA (100 ng/5 μL/rat), RU 486 (1 μg/5 μL/rat), or saline (5 μL) was i.c.v. injected on day 7, 10 min prior to IL-1β injections (15 ng/5 μL/rat), and dialysate samples were also continuously obtained at 20 min intervals for 180 min. The dialysis procedure in the third experiment was the same as the first experiment.

**Behavioral testing and dialysate collection**

The eight-arm radial maze consisted of an octagonal center platform (85 cm in diameter, arm-to-arm) connected to eight equally spaced arms (LxWxH, 42.5 × 14.5 × 22.5 cm), with a food cup at the end of each arm. A metallic post (100 cm in length) was positioned between two of the arms. The liquid swivel (Instech 375 s) located at the top end of the post was connected directly to the tubing, permitting rats unimpeded movement (Phillips et al. 2004).

Seven days after the surgery, rats were food deprived to ≈90% of their free-feeding weight and the delayed spatial win-shift version of radial maze task was adapted from Song et al. (2004). After habituating to the maze, animals were tethered to the dialysis assembly during training trials once daily. Each trial consisted of a training phase and a memory test phase, separated by a delay (from 5 min 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 until 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. On the behavioral testing day, after implanting the probe, the rats remained within a box with an open top in the center of the maze ≈3 h prior to training phase (they had been previously habituated to stay the box for 3–4 h/day). The dialysate samples were collected continuously at 5 min intervals at flow rate of 2 μL/min while the rat was confined in the center of the maze and the samples were analyzed immediately for ACh with a HPLC system until the release of ACh was stable. After collecting four baseline samples, the box was removed, rats were allowed to explore the four open arms on the maze that contained food. Animals were then immediately injected with saline or IL-1 (for the first and third experiment), or injected with RU 486 or IL-1RA and then IL-1 administration (for the second experiment). Following the delay, the box was removed again, and rats were allowed to explore all eight-armed open arms (test phase). Dialysis samples were obtained during the training phase, the delay, the recalling test phase, and after completing the test phase.

**Analysis of dialysates**

Immediately after a sample collection, ACh concentrations in the dialysates were measured by HPLC and an immobilized-enzyme reactor kit (BASi, West Lafayette, IN, USA). An isocratic pump (PM-92e; BAS) delivered the mobile phase (NaH₂PO₄ 6.9 g/L, Na₂EDTA 0.18 g/L, and Proclin 5 mL/L, pH 8.50 ± 0.05) at a flow rate of 0.1 mL/min. ACh was separated on an ion exchange ACh/Ch microbore analytical column and converted to H₂O₂ on a post-column immobilized-enzyme reactor in which the choline oxidase/acyetylcholinesterase was immobilized. The H₂O₂ was detected by a glass carbon-working electrode modified with horseradish peroxidase polymer. The applied potential was +100 mV (vs. Ag/AgCl) and the reduction current was measured by Epsilon and BAS ChromGraph software.

**Histology**

After finishing the behavioral test, rats were decapitated and brains were rapidly removed and placed on ice. One hemisphere of the hippocampus was used for checking the location of the probes after slicing (50 μm) and staining with crystal violet, and the other hemisphere was used for measuring NGF and IL-1 mRNA expressions. Only data from rats whose probe tips were located within the DG/CA1 were included and analyzed.

**Quantitative reverse transcription-PCR**

NGF and IL-1 mRNA expressions were studied in the hippocampus by quantitative RT-PCR. The total RNA was purified using RNeasy Mini Kit (Qiagen, Valencia, CA, USA). cDNA was synthesized using Ominiscript RT kit (Qiagen). Primer sequences for NGF (5'-AAACGGAGACTCCG- TTCACC-3') and IL-1 (5'-GGATGATGACGA-CCTGCT-AGTG and ACATGGTGACGACGCA) were purchased from Invitrogen (Carlsbad, CA, USA). The real-time PCR was performed with QuantiTect SYBRGreen PCR Kit (Qiagen) at conditions of the initial activation at 95°C for 15 min, denaturation at 94°C for 15 s, annealing at 55°C for 20 s, and extension at 72°C for 15 s with a single fluorescence measurement and up to 45 cycles. Then a melting step with the temperature ramps was set from 40 to 95°C. The values were normalized against the endogenous control, β-actin.

**Data analysis**

All results were expressed as mean ± SEM. The body weights were analyzed by one-way repeated ANOVA before IL-1 administrations and two-way (diet × injection) repeated ANOVA after IL-1 administrations. Behavioral data were analyzed by two-way (diet × injection) repeated ANOVA in train and test phases. Data points for the ACh release from each rat were expressed as the percentage of the average baseline values, and analyzed by three-way (sampling point × injection × duration) repeated ANOVA in the first and third experiments. For the measurement of ACh release in the second experiment with subchronic IL-1 administrations, data were measured by two-way (sampling point × drug treatment) ANOVA. For analyzing the expressions of NGF and IL-1 mRNAs, two-way (diet × injection or drug treatment × IL-1 injection) ANOVA was used. A post hoc Newman-Keuls test was used to compare any difference between groups (GB-STAT; Dynamic Microsystems, Inc. USA). The correlation between ACh release and maze performance (error entries), and between the NGF expression and ACh release were also measured by the same software. Significance was set at p < 0.05 for all parameters.
Results

Before IL-1β administration, rats fed palm oil gained a similar amount of weight as rats fed E-EPA enriched diet. Three days after IL-1β administration, body weight increase in the palm oil group was significantly lower than that with saline administration or fed E-EPA enriched diet (Palm-saline: 2.1 g ± 0.33; Palm-IL-1: −3.6 g ± 0.042; E-EPA-saline: 2.53 g ± 0.36; E-EPA-IL-1: 2.06 g ± 0.28. \( F_{1,27} = 10.72, p < 0.001 \)). The one-time IL-1RA or RU 486 injection on day 7 did not significantly change animal weights (similar results were previously published by Song et al. 2003, 2004, results not shown here).

**Acute and subchronic effects of IL-1β on ACh release in rats fed control or E-EPA diet**

Basal concentrations of ACh in dialysates (10 μL) collected from the DG of the hippocampus was 0.51 ± 0.07 μM. The three-way ANOVA analysis indicated a significant interaction between sampling points and injections in ACh release (\( F_{12,363} = 19.11, p < 0.0001 \)). The Newman-Keuls post hoc showed that both acute and chronic administrations of either IL-1β or saline significantly increased the release of ACh, which peaked at ≈40–60% above baseline values in the first 20 min (\( p < 0.001 \)), and then gradually returned to baseline in rats fed control diet (Fig. 2a). Following the transient increase, the both acute and chronic IL-1β, but not saline administrations, induced a significant decrease in ACh release for over 140 min in rats fed control diet when compared with baseline value (\( p < 0.01 \)). The ANOVA further showed that the duration of IL-1 administration significantly affected ACh release (\( F_{12,363} = 6.23, p < 0.05 \)). The post hoc indicated that subchronic IL-1β administration-induced reduction of ACh release was more pronounced than the effect of acute IL-1β administration (\( p < 0.05 \)).

In the rats fed the E-EPA enriched diet, the ANOVA also showed a significant interaction between sampling points and injection in the ACh release (\( F_{12,363} = 7.95, p < 0.01 \)). A similar short increase in the ACh release was found after both the saline and IL-1β injections (\( p < 0.01 \)). After the transitory increase, the post hoc demonstrated that there was no significant decrease in ACh release following IL-1β administration on day 1 or day 7 (Fig. 2b). Saline administrations on day 1 or day 7 did not yield any significant decrease in ACh release in rats fed control or E-EPA diet either (Fig. 2b).

**IL-1RA and RU 486 attenuated the reduction of ACh release induced by subchronic IL-1β injections**

Two-way ANOVA analysis revealed a significant interaction between sampling point and drug treatment (\( F_{12,272} = 7.23, p < 0.01 \)) in the second experiment. Saline and IL-1β injections induced a similar increase in ACh release (79.8 ± 8.98%) above baseline values. Then a similar decrease in ACh release started at 60 min time points and lasted 120 min was found, but only in the group with IL-1 administration alone. The application of RU 486 prior to IL-1β administration produced less increase (45.2 ± 7.64% above baseline) in ACh release in the first 20 min when compared to saline effect (\( p < 0.05 \)) (Fig. 2c). RU 486 administration also partially but significantly attenuated IL-1β-induced ACh decrease at 120, 140, 180 min time points (\( p < 0.01 \)) (Fig. 2c). In a separated group of rats, IL-1RA i.c.v. applied 10 min before IL-1β administration also
rats that received IL-1 confirmed these changes. The expression of NGF mRNA in saline group fed control diet. In saline-treated groups, NGF significantly lower (p < 0.01 versus IL-1 group with saline injection; *p < 0.05 versus IL-1 group with saline injection) 

**Effects of IL-1 administration on behavioral performance in the testing phase**

The correlation between ACh release and memory acquisition (training) and retrieval (testing) following injections of saline and IL-1β caused more errors after the third injection (p < 0.05) (Fig. 4a). The ANOVA also suggested that the interaction between IL-1β and E-EPA treatment was significant (F1,13 = 7.42, p < 0.01). The post hoc indicated that E-EPA treatment significantly blocked IL-1-induced memory errors in both the training (only on day 3) and testing phases (day 3–7) (p < 0.05) (Fig. 4b).

**The correlation between the reduction of ACh release and error entries into the arms of the maze and between ACh release and NGF mRNA expressions**

The correlation between ACh release and memory acquisition (training) and retrieval (testing) following injections of saline and IL-1β were measured by a ‘simple correlation’ (GB-STAT) (Fig. 5a and b), respectively. In rats fed control diet, significant increases in ACh release were observed following saline injection at the start of the training session (214.66 ± 21.60%) and during the testing sessions (225.30 ± 37.09%, p < 0.05 when compared with baseline) (Fig. 5a). Similar results were produced a significant increase in ACh release (70.33 ± 7.61% above baseline) for 30 min when compared to baseline values, and then completely blocked IL-1β-induced ACh decrease (p < 0.01), as indicated by the post hoc (Fig. 2c).

**E-EPA- and IL-1RA-attenuated reduction in NGF and increased IL-1 mRNA expressions after IL-1β administrations**

In the third experiment, two-way ANOVA indicated that the effects of IL-1 administration and E-EPA feeding are both significant (IL-1: F1,27 = 13.41, p < 0.01; E-EPA: F1,27 = 34.67, p < 0.0001) for NGF expression. The post hoc further confirmed these changes. The expression of NGF mRNA in rats that received IL-1β administration for 7 days was significantly lower (p < 0.01) when compared with the saline group fed control diet. In saline-treated groups, NGF expression was significantly up-regulated in the hippocampus of rats fed E-EPA when compared with the group fed control diet (p < 0.01) (Fig. 3a).

**Effects of E-EPA treatment on IL-1β-induced changes in memory performance in training and testing phases**

Two-way repeated ANOVA showed a significant effect of IL-1 administration on behavioral performance in the testing phase (F1,13 = 9.54, p < 0.01) but not in the training phase. In correlation with this statistical result, the Newman–Keuls test revealed that subchronic administrations of IL-1β caused more error entries into the arms of the maze in rats fed control diet during the testing (retrieval and post-testing) phase (p < 0.05) on day 3–7 (Fig. 4b). In the training phase, following a series of IL-1β injections, the number of arm entries generally showed no significant difference between saline and IL-1 groups except that rats fed control diet made more errors after the third injection (p < 0.05) (Fig. 4a). The ANOVA also suggested that the interaction between IL-1β and E-EPA treatment was significant (F1,13 = 7.42, p < 0.01). The post hoc indicated that E-EPA treatment significantly blocked IL-1-induced memory errors in both the training (only on day 3) and testing phases (day 3–7) (p < 0.05) (Fig. 4b).
observed in rats fed E-EPA, in which significant increases in ACh release (229.44 ± 30.08% and 231.59 ± 38.26%) were observed for 20–30 min during the training and the testing sessions (Fig. 5a). There was no significant difference in the number of arm entries between these two groups of rats. In contrast, IL-1β injection on day 7 evoked lower increases in ACh release during both training and testing sessions in rats fed control diet (201.82 ± 33.37% and 185.30 ± 41.30%), but not in rats fed E-EPA diet (278.30 ± 36.17% and 259.43 ± 27.96%). After IL-1β administrations, a significant reduction in the magnitude of the evoked ACh release was observed during post-testing phase (p < 0.05) (Fig. 5b). The correlation between the reduction of ACh release and the higher number of error entries in group treated with IL-1β was significant (r = −0.83, p < 0.01).

In Figs 2 and 3, a decreased ACh release was associated with a down-regulation of NGF mRNA expression in rats with subchronic IL-1β administrations and fed control diet. In rats fed E-EPA enriched diet, IL-1 effect on ACh release was blocked, which was also associated with an increase in NGF expression. The statistics also showed a significant and positive correlation between ACh and NGF expression (r = 0.79, p < 0.05). However, RU 486 partially reversed IL-1-induced decrease in ACh release but did not significantly reverse NGF expression.

**Discussion**

This study, for the first time, reported four important findings in the IL-1-induced model of memory deficits: (i) a clear correlation between the decrease in ACh release and memory deficit was found during rat memory retrieval phase; (ii) glucocorticoids are involved in the reduction of ACh release; (iii) a down-regulated NGF expression in the hippocampus is correlated with the reduction of ACh release and associated with memory impairment; and (iv) E-EPA improves memory by attenuating the reduction of ACh release and NGF expression. Thus, the results have demonstrated our hypoth-
thesis that inflammation may increase glucocorticoids but suppress NGF to reduce ACh release, which is responsible to the memory deficit, and EPA treatment significantly reversed IL-1-induced changes by targeting inflammation and gene expression.

Using a unique technique combining behavioral test with microdialysis, this study observed a dynamic correlation between hippocampal ACh release and memory performance after each IL-1β administration. The results demonstrated that the reduction of ACh release was directly related to the memory deficit during memory retrieval phase, and E-EPA blocked the ACh reduction after IL-1β administration, and improved the memory. Furthermore, we have previously reported that IL-1RA can reverse IL-1-induced memory impairment, and this study further demonstrated that the effect of IL-1RA on memory is through reversing IL-1-induced decreases in ACh release and NGF expressions. The section below will discuss each step of the two pathways (shown by Fig. 1), by which IL-1 may impair memory and E-EPA improves memory.

IL-1β is a well-known stimulator for the HPA axis (Dunn 2000), and administration of RU 486 can significantly reduce corticosterone elevation and attenuate the memory impairments induced by i.c.v. IL-1 β administration (Song et al. 2004). This study revealed the mechanism by which glucocorticoids may affect ACh function in this model. It has been reported that stress can increase or decrease ACh release from the hippocampus, which is causally related to an increase in glucocorticoid secretion (Mizuno and Kimura 1997; Mitsushina et al. 2003). In this study, saline or IL-1 injections, as a mild stressor, significantly increased ACh release (for 20 min). After RU 486 treatment, the short increase in ACh release was significantly lower than that observed in the saline group, suggesting that the stress-induced ACh release may be partially related to glucocorticoid effects. On the other hand, a deficit in working memory and decreased acetylcholinesterase activity or ACh release were reported in the hippocampus of rats that received chronic stress or chronic corticosterone administrations (Masuda et al. 2005; Srikumar et al. 2006). In this study, we showed that IL-1β administrations induced a significant and longer reduction in ACh release, which is different from the effect of the injection. Since both changes in ACh release could be partially but significantly attenuated by RU 486, it is possible that amount and duration of glucocorticoid secretion in response to different stressors may exert different effects on ACh release. The result from this study demonstrated that the fact that RU 486 improved memory may be, at least partially, related to the glucocorticoid effect on hippocampal ACh release. Since RU 486, an antagonist of glucocorticoid II receptor, did not completely reversed injections- and IL-1-reduced ACh release, there could be other factors, such as other glucocorticoid receptors, involved in ACh increase.

NGF also plays an important role in learning and memory as result of its enhancement of ACh and LTP functions (Isacson et al. 2002). This study is the first to demonstrate that subchronic IL-1β administration significantly reduced hippocampal NGF expression, which is associated with reduced ACh release and the memory impairment. IL-1β may reduce NGF in two ways. First, glucocorticoids may up-or down-regulate NGF mRNA expression, depending on its amount and acting duration (Sapolsky 2000; Nichols et al. 2005). Excessive glucocorticoid secretion may suppress NGF functions (McLay et al. 1997). Down-regulated NGF functions were reported in animals exposed to psychological stress (Colangelo et al. 2004), and in an AD model (Salehi et al. 2006). However, once RU 486 administration in this study did not significantly attenuate the down-regulation of NGF mRNA expressions. This result may suggest that glucocorticoids were not involved in the down-regulation of NGF and mRNA expressions or longer treatment with RU 486 may be needed.

The other possible mechanism could be that IL-1β directly affects astrocyte functions, which changes NGF synthesis. In an AD model induced by aluminum, increased proinflammatory cytokines were associated with a decrease in NGF expression (Johnson and Sharma 2003). The result from this study supports that subchronic IL-1-induced inflammation down-regulates NGF expressions since IL-1RA significantly reversed the reduction of NGF and increased hippocampal IL-1 expression. In addition, we have previously reported that a treatment with anti-inflammation drug celecoxib inhibits glucocorticoid secretion, reverses NGF reduction, and improved memory in a rodent model of depression (Song et al. 2009). Therefore, both glucocorticoids and NGF may contribute to the reduction of ACh release and memory impairment in this model.

It should be emphasized that many studies have reported that inflammation can increase NGF expression and IL-1 has neuroprotective effects (Shaftel et al. 2007). We have recently found that acute IL-1β administration up-regulates, while subchronic IL-1 β administration down-regulates NGF mRNA expressions (Song et al. 2008). It is possible that during acute neuroinflammation and brain injury, the function of NGF and other neurotrophins may be enhanced to protect the brain. However, subchronic and chronic inflammation may cause astrocyte apoptosis, which reduces neurotrophin synthases (Takuma et al. 2004).

Whether IL-1 is degenerative or protective in neurodegeneration and brain injuries also depend on its concentrations (Pintaux et al. 2009). Low concentrations of IL-1, such 1–15 ng, were found to favor late microglia-initiate neuroinflammation, which causes cell death, while high concentration of IL-1 (500 ng) exerts neuroprotective effects (Pintaux et al. 2009). In a model of IL-1β over-expression, increased IL-1 expressions (8–130 folds) was associated with up-regulation of astrocyte marker glial fibrillary acidic
IL-1 administration (Song 2009). Previously, we have also reported that E-EPA can significantly improve memory by the modulation of ACh and neurotrophin functions. In conclusion, this study provides the first evidence that IL-1β can reduce ACh release, which is directly correlated to rat memory deficits in the radial maze. Furthermore, the results demonstrated that glucocorticoid and NGF may involve in IL-1-induced changes in ACh release. N-3 fatty acid E-EPA by suppressing inflammation blocked IL-1 effects on ACh release and NGF expression, and significantly improved memory.

Acknowledgements

This work was supported by the Canadian Institutes for Health Research (CIHR) grant to Cai Song. We thank Amarin Neuroscience Ltd. and Atlantic Innovation Foundation for their financial supports.

Disclosure statement

The authors declare that there are no actual or potential conflicts of interests involving them or the institutions with which they are affiliated.

References


