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Involvement of cholinergic and glutamatergic functions in working memory impairment induced by interleukin-1β in rats

Yuji Matsumoto, Mayuki Yoshida, Shigenori Watanabe, Tsuneyuki Yamamoto *

Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

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Abstract

Interleukin-1β at doses of 32 and 100 ng/side, injected bilaterally into the dorsal hippocampus of rats, significantly increased the working memory errors in a three-panel runway setup, whereas interleukin-1β at doses affecting working memory errors had no effect on the number of errors in the first trial or the latency. The increase in working memory errors induced by intrahippocampal administration of 100 ng/side interleukin-1β was significantly decreased by concurrent injection (300 ng/side) of the interleukin-1 receptor antagonist. The cholinesterase inhibitor physostigmine at a dose of 3.2 μg/side and D-cycloserine (1.0 and 10 μg/side), which is a partial agonist acting at the glycine binding site of the NMDA receptor/channel complex, reduced the increase in working memory errors induced by 100 ng/side interleukin-1β. These results suggest that interleukin-1β causes disruptions of septohippocampal cholinergic and glutamatergic transmission via its high-affinity receptor, which underlie the impairment of working memory.

Keywords: Interleukin-1β; Working memory; Acetylcholine; *N*-methyl-D-aspartate receptor; Hippocampus

1. Introduction

Interleukin-1β is well known as a proinflammatory cytokine, which shows numerous responses to infectious agents. In the brain, interleukin-1β and other cytokines are produced by astrocytes or microglias, and their receptors or binding sites are distributed in many regions. The interleukin-1 receptor is distributed in the CA1 and CA3 pyramidal cells and dentate gyrus granule cells of the hippocampus with high density (Takao et al., 1990; Ban et al., 1991; Lechan et al., 1990; Cunningham et al., 1992).

Alzheimer's disease is characterized by the presence of senile plaques and neurofibrillary tangles accompanied by synaptic and neuronal loss (Coyle et al., 1983), underlying the cognitive impairments associated with dementia. The level of interleukin-1β is markedly increased in the hippocampus and frontal cortex in the brains of Alzheimer's disease patients (Cacabelos et al., 1994), suggesting that an increase in the level of interleukin-1β is related to the impairment of the cognitive function observed in Alzheimer's disease patients. The septohippocampal cholinergic system, known to be critical to the hippocampus and required for normal memory function (Smith, 1988), is extensively disrupted in the brains of Alzheimer's disease patients (Davies and Maloney, 1976; Perry and Perry, 1977). It has also been reported that interleukin-1β decreased cholinergic transmission by blocking acetylcholine release from presynaptic cells (Rada et al., 1991). These results suggest that the impairment of memory function observed in Alzheimer's disease patients is due to an increase in interleukin-1β in the brain, which causes a decrease in cholinergic transmission. However, few behavioral studies have been designed to clarify the role of interleukin-1β in memory function. In the present study, we investigated, using the three-panel runway task, the effect of intrahippocampal administration of interleukin-1β on the working memory performance of rats, considered a model of working memory in humans, which is often disrupted in the early stage of Alzheimer's disease.

In addition to the cholinergic system, the glutamatergic system plays an important role in hippocampal memory, e.g., intrahippocampal administration of the muscarinic acetylcholine receptor antagonist scopolamine or selective and competitive *N*-methyl-D-aspartate (NMDA) receptor antagonists such as (±)-3-(2-carboxypiperazin-4-yl)

* Corresponding author. Tel.: +81-92-642-6629; fax: +81-92-642-6632.

E-mail address: yamamoto@yakuri.phar.kyushu-u.ac.jp (T. Yamamoto).

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propyl-1-phosphonic acid produced a dose-dependent disruption of working memory performance in a three-panel runway task (Ohno et al., 1992), indicating that this behavior depended to some extent on the excitatory neurotransmission via not only muscarinic but also NMDA receptors in the hippocampus. Electrophysiologically, activation of the NMDA receptor is an absolute requirement for induction of long-term potentiation in the hippocampus, which models synaptic plasticity and is hypothesized to be a neurobiological mechanism underlying memory processes (Collingridge and Briss, 1987; Bliss and Collingridge, 1993). Thus, to elucidate the functional interactions between interleukin-1β in the hippocampus and the cholinergic or glutamatergic neuronal activity involved in regulating the memory process, we examined the effects of cholinergic activation by the cholinesterase inhibitor physostigmine and of glutamatergic activation by D-cycloserine, a partial agonist at the glycine binding site on the NMDA receptor/channel complex, on the working memory performance changed by intrahippocampal injection of interleukin-1β.

2. Materials and methods

2.1. Animals

Male Wistar-strain rats (8–10 weeks old) were obtained from Japan SLC. The initial free-feeding weights were 230–250 g, and the rats were placed on a deprivation schedule to maintain their weights at approximately 80% of the free-feeding level. The rats were housed in groups of four or five per cage at a constant temperature (23 ± 2 °C), with a 12-h light–dark cycle (light period 07:00–19:00 h), and with water freely available. These experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by Declaration of Helsinki and Faculty of Pharmaceutical Sciences, Kyushu University Publication, enacted 1988.

2.2. Apparatus

Working memory was assessed with a three-panel runway apparatus (Fig. 1A), as described in our previous reports (Furuya et al., 1988; Ohno et al., 1992). This apparatus (175 × 36 × 25 cm) was composed of a start box, a goal box and four consecutive choice points between them. Each choice point consisted of a gate with three panels (12 × 25 cm). The rats were prevented from passing through two of the panels in the gate by front stoppers, and prevented from returning to the start box or to a previous choice point by rear stoppers fixed to each of the panels in all the gates. When the rats reached the goal box, they received three food pellets (45 mg each; A Holton Industries, Frenchtown, NJ) as positive reinforcement.

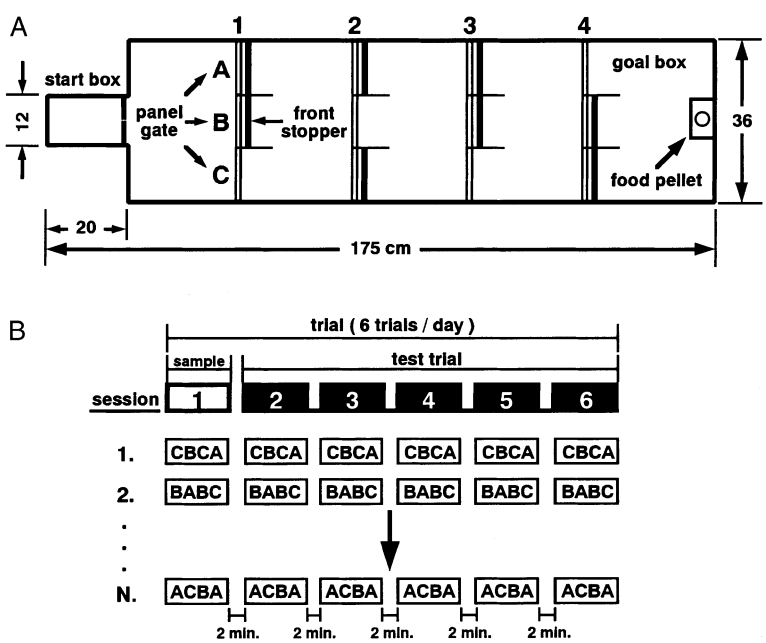

Fig. 1. Schematic drawing of the three-panel runway apparatus and experimental procedure. (A) Schematic drawing of the three-panel runway apparatus. Rats were allowed to perform the task after the guillotine door was opened in front of the start box. Rats had to pass through four consecutive choice points to obtain food pellets placed in the goal box. Each choice point consisted of a gate with three panels (A, B and C). The rats could pass through only one of the three panel gates (the correct panel gate) at each choice point. (B) Experimental procedure for the working memory task with the three-panel runway apparatus. Rats were given six consecutive trials at 2-min intervals (defined as one session) per day. In the working memory experiment, the locations of the correct panel gates were held constant within a session, but were changed from one session to the next. Thus, the rats were required to acquire new correct panel gate locations within a session in the working memory task.

2.3. Experimental procedures

The rats were made to perform the task in six consecutive trials (defined as one session) per day with removal of the front stopper of only one of the three panel gates (the correct panel gate) at each choice point. Trials were run at 2-min intervals and water was freely available between trials in the home cage. The locations of the correct panel at each gate were held constant within a session, but were changed from one session to the next (working memory procedure; Fig. 1B). Thus, in this behavioral paradigm, rats were required to learn a specific location sequence during six trials of a session. The number of times an animal attempted to pass through an incorrect panel (defined as errors) and the time required for the animal to obtain food pellets (defined as latency) were recorded for each rat during each trial of a session. The number of errors in the first trial and latency were recorded separately, from those in the second to the sixth trial of a session which were summed for the evaluation of working memory function, i.e., the ability of rats to remember the new location of the correct panel within a session. The learning criterion was less than eight errors summed from the second to sixth trial (working memory errors). A rat was used in subsequent experiments, if it achieved this criterion in three consecutive sessions. In each experiment, a repeated measures design was used, each rat was given the drug test twice in a randomly varied order, separated in 3–5 days.

In the three-panel runway task, the random performance level was four errors per trial, or 24 errors per session. The working memory errors markedly decreased with repeated training, whereas the errors in the first trial remained constant at approximately four. Approximately 20–30 training sessions were required for the rats to reach the criterion of less than eight working memory errors. Latency was also reduced during repeated sessions and was stable from the 10th session on.

2.4. Surgery

The rats that met the learning criterion were anesthetized with sodium pentobarbital (40 mg/kg i.p.), and were implanted bilaterally with guide cannulae for microinjection of drugs into the hippocampus, as described previously. The position of the injection cannula tip, which protruded 1.0 mm below the tip of the guide cannula, was aimed at the dorsal hippocampus: 3.8 mm posterior to the bregma, 2.2 mm lateral to the midline and 3.2 mm ventral to the surface of the skull measured at the bregma, according to the brain atlas of Paxinos and Watson (1989). The rats were allowed at least 5 days of postoperative recovery before runway sessions were resumed. The rats were tested after they had achieved the criterion of learning in the test of working memory after surgical manipulations. After completion of behavioral testing, the injection site in the hippocampus was verified by Cresyl violet staining, as described previously.

2.5. Drugs

The drugs used in this study were interleukin-1β (PeproTech EC, Margravine Road, London), interleukin-1 receptor antagonist (interleukin-1ra; PeproTech EC), physostigmine (Sigma, St. Louis, MO) and D-cycloserine (Research Biochemicals, Natick, MA). All the drugs were dissolved in saline. On the test day, rats received a single injection of interleukin-1β (2 μl/4 min) through the injection cannula and the session described above was given 10 min after the injection. When two drugs were injected concurrently, they were mixed together and the injection volume was 2 μl/side.

2.6. Statistical analysis

The significance of differences between the groups was determined by a one-way analysis of variance (ANOVA) followed by the two-tailed Dunnett's test. A difference was considered as significant at $P < 0.05$.

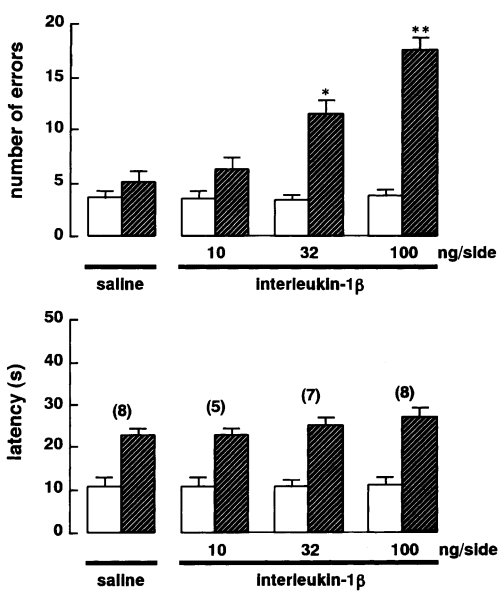

Fig. 2. Effect of intrahippocampal injection of interleukin-1β on the increase in the number of working memory errors but not latency. Rats received the runway test 10 min after interleukin-1β was administered. Each column represents the means ± S.E.M. of errors and latencies recorded in the first trial (open columns) and those summed from the second to the sixth trial within a session (hatched columns). The number of animals in each group is represented above each latency column. The significance of difference from the saline-injected group ($^{*}P < 0.05$, $^{**}P < 0.01$) was determined by a one-way ANOVA followed by Dunnett's test.

3. Results

As shown in Fig. 2, interleukin-1β at doses of 32 and 100 ng/side, administered bilaterally into the dorsal hippocampus, increased the number of working memory errors significantly and dose-dependently ($P < 0.05$ and 0.01, respectively), although it had no effect on the number of errors in the first trial and latency. The increase in working memory errors induced by intrahippocampal administration of 100 ng/side interleukin-1β was significantly and dose-dependently reduced by concurrent injection of 300 ng/side interleukin-1 receptor antagonist ($P < 0.01$; Fig. 3). However, interleukin-1 receptor antagonist had no effect on the latency in the first trial and those summed from the second to the sixth trial.

The increase in working memory errors induced by intrahippocampal administration of 100 ng/side interleukin-1β was attenuated by concurrent injection of 3.2 μg/side physostigmine ($P < 0.01$; Fig. 4). Concurrent administration of 1.0 and 10 μg/side D-cycloserine into the hippocampus counteracted the ability of 100 ng/side interleukin-1β to increase working memory errors ($P <$ 0.05 and 0.01, respectively; Fig. 4). Physostigmine or D-cycloserine at doses affecting the number of working memory errors had no effect on the latency in the first trial and those summed from the second to the sixth trial.

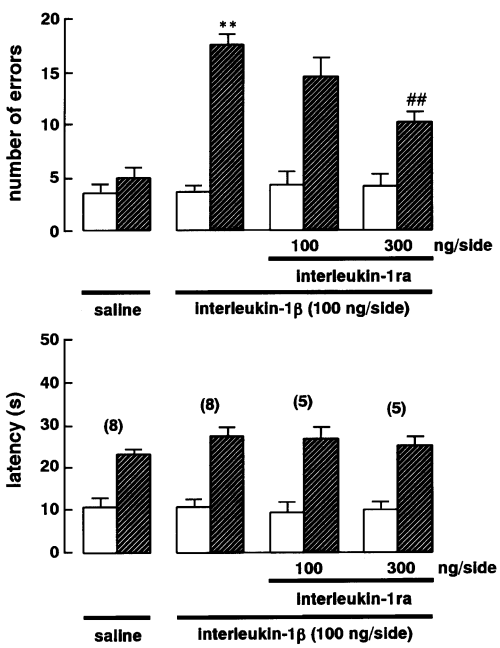

Fig. 3. Effect of concurrent injection of interleukin-1 receptor antagonist on the increase in the number of working memory errors induced by intrahippocampal administration of interleukin-1β. Rats received the runway test 10 min after drugs were administered. Each column represents the means ± S.E.M. of errors and latencies recorded in the first trial (open columns) and those summed from the second to the sixth trial within a session (hatched columns). The number of animals in each group is represented above each latency column. The significance of differences from the saline-injected group (** $P < 0.01$) and that from the interleukin-1β-injected group (## $P < 0.01$) was determined by a one-way ANOVA followed by Dunnett's test.

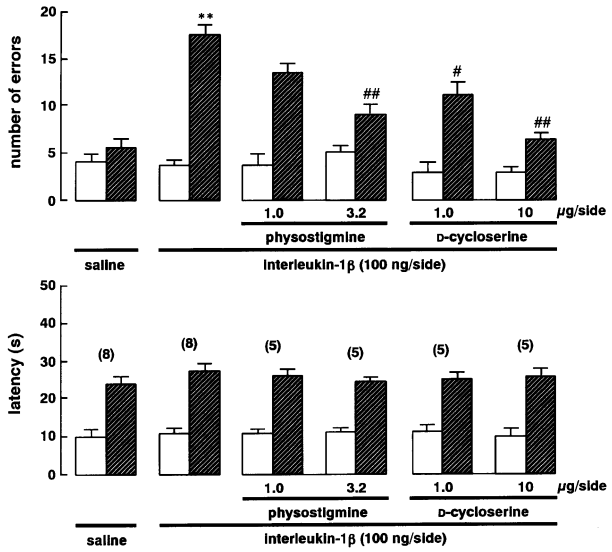

Fig. 4. Effects of concurrent injection of physostigmine and D-cycloserine on the increase in the number of working memory errors induced by intrahippocampal administration of interleukin-1β. Rats received the runway test 10 min after drugs were administered. Each column represents the means ± S.E.M. of errors and latencies recorded in the first trial (open columns) and those summed from the second to the sixth trial within a session (hatched columns). The number of animals in each group is represented above each latency column. The significance of differences from the saline-injected group (** $P < 0.01$) and that from the interleukin-1β-injected group (# $P < 0.05$, ## $P < 0.01$) was determined by a one-way ANOVA followed by Dunnett's test.

4. Discussion

The three-panel runway task allows us to assess hippocampus-dependent working memory processes in rats, since the working memory in this task is highly sensitive to disruption by dorsal hippocampal lesions (Kitajima et al., 1992). The present study demonstrated that interleukin-1β impaired working memory in rats when administered into the dorsal hippocampus and that the effect was prevented by concurrent injection of interleukin-1 receptor antagonist, interleukin-1 ra. These results indicate that interleukin-1β impaired working memory via the interleukin-1 receptor in the hippocampus. At this time, no difference was found between the saline and interleukin-1β groups in the number of errors in the first trial and the latency. Therefore, we can exclude the possibility that this working memory impairment is related to ataxia, sedation or drowsiness.

It is well documented that the hippocampus receives cholinergic intervention from the medial septum via the fimbria-fornix (Mesulam et al., 1983). Using the three-panel runway task, we previously found that intrahippocampal administration of the muscarinic receptor antag-

onist scopolamine impaired working memory and that the cholinesterase inhibitors physostigmine and tetrahydroaminoacridine alleviated the impairment of working memory in hippocampal-lesioned rats (Kitajima et al., 1992; Yamamoto et al., 1990). Judging from these findings, the septohippocampal cholinergic system plays a crucial role in working memory processes. In this regard, interleukin-1β was reported to block the release of acetylcholine in the hippocampus (Rada et al., 1991). Therefore, concurrent administration of physostigmine ameliorated the working memory deficit induced by intrahippocampal administration of interleukin-1β, suggesting that interleukin-1β causes this deficit by reducing cholinergic transmission.

The hippocampus is the main site of long-term potentiation, a cellular model of learning and memory in the mammalian nervous system, and activation of the NMDA receptor is essential for induction of hippocampal long-term potentiation in vitro (Collingridge and Briss, 1987; Collingridge et al., 1983; Teyler and DiScenna, 1987). In our previous reports using the three-panel runway task, it was demonstrated that intrahippocampal injection of NMDA receptor antagonists impaired working memory and that concurrent injection of D-cycloserine, which is known to enhance the NMDA response by acting as a partial agonist at the glycine site on the NMDA receptor/channel complex (Monahan et al., 1989; Watson et al., 1990), reversed working memory failure induced by intrahippocampal scopolamine (Ohno and Watanabe, 1996; Ohno et al., 1992). Thus, it is conceivable that NMDA receptor-dependent processes in the hippocampus are as responsible for working memory as the cholinergic system in the hippocampus. On the other hand, there is a report that interleukin-1β inhibited glutamatergic transmission in vitro (Coogan and O'Connor, 1997). In the present study, D-cycloserine, concurrently administered into the hippocampus, reversed the working memory deficit induced by interleukin-1β. These findings suggest that intrahippocampal administration of interleukin-1β induces, via the NMDA receptor, a deficiency of glutamatergic synapse responses followed by working memory impairment.

Taken together, the present results suggest that the impairment of working memory by interleukin-1β is due to decreases in certain neurotransmitters such as acetylcholine and/or glutamate in the hippocampus which have been associated with the memory loss observed in Alzheimer's disease patients. To our knowledge, this is the first study which has shown in vivo that the neurotransmissions in the hippocampus are disrupted by interleukin-1β, which often much increases in the brains of Alzheimer's disease patients.

5. Conclusion

The present study demonstrated that intrahippocampal administration of interleukin-1β induced impairment of working memory via the interleukin-1 receptor and this impairment was ameliorated by increases in cholinergic and glutamatergic transmission. Taken together, it is suggested that interleukin-1β-induced working memory impairment is due to decreases in these neural transmissions in the hippocampus.

References

Ban, E., Milon, G., Prudhomme, N., Fillion, G., Haour, F., 1991. Receptors for interleukin-1 (alpha and beta) in mouse brain: mapping and neuronal localization in hippocampus. Neuroscience 43, 21–30.

Bliss, T.V., Collingridge, G.L., 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361, 31–39.

Cacabelos, R., Alvarez, X.A., Fernandez-Novoa, L., Franco, A., Mangues, R., Pellicer, A., Nishimura, T., 1994. Brain interleukin-1beta in Alzheimer's disease and vascular dementia. Methods Find. Exp. Clin. Pharmacol. 16, 141–151.

Collingridge, G.L., Briss, T.V., 1987. NMDA receptors—their role in long-term potentiation. Trends Neurosci. 10, 288–293.

Collingridge, G.L., Kehl, S.J., McLennan, H., 1983. Excitatory amino acids in synaptic transmission in the Schaffer collateral–commissural pathway of the rat hippocampus. J. Physiol. 334, 33–46.

Coogan, A., O'Connor, J.J., 1997. Inhibition of NMDA receptor-mediated synaptic transmission in the rat dentate gyrus in vitro by interleukin-1beta. NeuroReport 8, 2107–2110.

Coyle, J.T., Price, D.L., DeLong, M.R., 1983. Alzheimer's disease: a disorder of cortical cholinergic innervation. Science 219, 1184–1190.

Cunningham Jr., E.T., Wada, E., Carter, D.B., Tracey, D.E., Battey, J.F., De Souza, E.B., 1992. In situ histochemical localization of type I interleukin-1 receptor messenger RNA in the central nervous system, pituitary, and adrenal gland of the mouse. J. Neurosci. 12, 1101–1114.

Davies, P., Maloney, A.J., 1976. Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet 2 1403.

Furuya, Y., Yamamoto, T., Yatsugi, S., Ueki, S., 1988. A new method for studying working memory by using the three-panel runway apparatus in rats. Jpn. J. Pharmacol. 46, 183–188.

Kitajima, I., Yamamoto, T., Ohno, M., Ueki, S., 1992. Working and reference memory in rats in the three-panel runway task following dorsal hippocampal lesions. Jpn. J. Pharmacol. 58, 175–183.

Lechan, R.M., Toni, R., Clark, B.D., Cannon, J.G., Shaw, A.R., Dinarello, C.A., Reichlin, S., 1990. Immunoreactive interleukin-1β localization in the rat forebrain. Brain Res. 514, 135–140.

Mesulam, M.M., Mufson, E.J., Wainer, B.H., Levey, A.I., 1983. Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1–Ch6). Neuroscience 4, 1185–1201.

Monahan, J.B., Handelmann, G.E., Hood, W.F., Cordi, A.A., 1989. D-cycloserine, a positive modulator of the *N*-methyl-D-aspartate receptor, enhances performance of learning tasks in rats. Pharmacol. Biochem. Behav. 34, 649–653.

Ohno, M., Watanabe, S., 1996. D-cycloserine, a glycine site agonist, reverses working memory failure by hippocampal muscarinic receptor blockade in rats. Eur. J. Pharmacol. 318, 267–271.

Ohno, M., Yamamoto, T., Watanabe, S., 1992. Effects of intrahippocampal injections of *N*-methyl-D-aspartate receptor antagonists and scopolamine on working and reference memory assessed in rats by a three-panel runway task. J. Pharmacol. Exp. Ther. 263, 943–950.

Paxinos, G., Watson, C., 1989. The Rat Brain in Stereotaxic Coordinates. Academic Press, Australia.

Perry, P.K., Perry, R.H., 1977. Necropsy evidence of central cholinergic deficits in senile dementia. Lancet 1, 189.

Rada, P., Mark, G.P., Vitek, M.P., Mangano, R.M., Blume, A.J., Beer, B., Hoebel, B.G., 1991. Interleukin-1beta decreases acetylcholine

measured by microdialysis in the hippocampus of freely moving rats. Brain Res. 550, 287–290.

Smith, G., 1988. Animal models of Alzheimer's disease: experimental cholinergic denervation. Brain Res. 472, 103–118.

Takao, T., Tracey, D.E., Mitchell, W.M., De Souza, E.B., 1990. Interleukin-1 receptors in mouse brain: characterization and neuronal localization. Endocrinology 127, 3070–3078.

Teyler, T.J., DiScenna, P., 1987. Long-term potentiation. Annu. Rev. Neurosci. 10, 131–161.

Watson, G.B., Bolanowski, M.A., Baganoff, M.P., Deppeler, C.L., Lanthorn, T.H., 1990. D-cycloserine acts as a partial agonist at the glycine modulatory site of the NMDA receptor expressed in *Xenopus* oocytes. Brain Res. 510, 158–160.

Yamamoto, T., Yatsugi, S., Ohno, M., Furuya, Y., Kitajima, I., Ueki, S., 1990. Minaprine improves impairment of working memory induced by scopolamine and cerebral ischemia in rats. Psychopharmacology 100, 316–322.