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Synergistic effect of combined treatment with risperidone and galantamine on phencyclidine-induced impairment of latent visuospatial learning and memory: Role of nAChR activation-dependent increase of dopamine D_1 receptor-mediated neurotransmission

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Abstract

The clinically achievable efficacy of the atypical antipsychotics on cognitive symptoms of schizophrenia is practically limited by their dose-dependent side effects. Thus, there is the need for adjuvant treatments or strategies for the cognitive impairments. Further, human autopsy and genetic data in schizophrenia have indicated the existence of the abnormality of nicotinic acetylcholine receptors (nAChR). In the present study, we aimed to investigate the synergistic effect and mechanisms of a combined treatment with an atypical antipsychotic risperidone and galantamine, which is a nAChR-allosteric modulator and a modest cholinesterase inhibitor, on the impairment of latent visuospatial learning and memory in mice resembling the cognitive impairment of schizophrenia. Repeated treatment with phencyclidine (PCP, 10 mg/kg, 14 days)-induced cognitive impairment in mice in a one trial water-finding test was used as a model of the cognitive impairment of schizophrenia. In vivo microdialysis was used to investigate the extracellular concentration of dopamine in the medial prefrontal cortex (mPFC). Combined treatment with galantamine and risperidone, at low, ineffective doses (both at 0.05 mg/kg) showed a synergistic effect to reverse cognitive impairment and increase extracellular concentration of dopamine in the mPFC. The synergistic behavioral effect was abolished by a dopamine-D_1 receptor antagonist, SCH 23390, and a nAChR antagonist, mecamylamine, but not a muscarinic AChR (mAChR) antagonist, scopolamine. Mecamylamine also blocked the synergistic effect on dopamine release in the mPFC of PCP-treated mice. The study indicates that galantamine and risperidone may have synergistic effect on the cognitive impairments in schizophrenia patients by synergistically promoting the nAChR activation-dependent increase of dopamine D_1 receptor-mediated neurotransmission.

Keywords: Phencyclidine; Cognitive impairment; Schizophrenia; Risperidone; Galantamine; Synergistic effect; nAChR; Dopamine

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1. Introduction

The symptoms of schizophrenia are classified as positive (e.g., hallucination, delusion), negative (e.g., anhedonia, social withdrawal or poor social interaction) and cognitive symptoms (e.g., deficits in attention, working memory, mental flexibility), most of which are related with dopaminergic aberration (Abi-Dargham, 2004). Among the dopaminergic projections, the mesolimbic and mesocortical pathways are tightly involved in the pathophysiology of schizophrenia. The positive symptoms are thought to arise from a subcortical hyperstimulation of dopamine D_2 receptors, especially in striatal areas, whereas the negative and cognitive symptoms arise from a cortical dopaminergic neurotransmission mediated by dopamine-D_1 receptors in the dorsolateral prefrontal cortex (PFC) in schizophrenia patients that corresponds to the medial PFC (mPFC) in rodents (Abi-Dargham, 2004; Albert et al., 2002; Fink-Jensen, 2000; Kolb, 1990).

Although the aberration of dopaminergic system is critical in the pathophysiology of schizophrenia, other neurotransmitter systems are more or less involved in the pathophysiology of schizophrenia and interact with the dopaminergic system (Fink-Jensen, 2000; Javitt and Zukin, 1991; Noda et al., 2000, 2001). PCP, a non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, induces psychotomimetic states in humans and rodents, incorporating not only the positive symptoms (e.g. hallucinations, paranoia) but also the negative symptoms (e.g. social withdrawal, motor retardation) and cognitive deficits (e.g., impairment of attention and working memory), thus, PCP-treated animals have been proposed as a preclinical model of schizophrenia (Castner et al., 2004; Javitt and Zukin, 1991; Morris et al., 2005; Noda et al., 1995, 2000, 2001).

Deficits in attention and information-processing mechanisms have been suggested to play a critical role in schizophrenia, therefore, the study of cognitive function related to sensory information or attention has been of central importance in the attempt to understand this disorder (Noda et al., 2001). The water-finding test is thought to be a latent visuospatial learning and memory paradigm related to the ability to sort visuospatial information and to attention process (Mackintosh, 1975; Ichihara et al., 1993). This test does not need any motivation to train animals, and animals are deprived of water only before the testing trial (Ichihara et al., 1993). The end of the water nozzle is set further above the floor in the testing trial than in training to decrease the probability of being found by chance. The PCP-induced behavioral deficit in mice in this paradigm best resembles the facts found in a clinical task made by Daniel et al. (2006) that schizophrenia patients have deficits in localizing the objects in the space that they previously explored, and in remembering the spatial relations among landmarks in the environment. Alternatively, the deficit in PCP-treated mice in the water-finding test resembles the poor performance in schizophrenia patients in the typical object-relocation task, independent of overall intellectual ability (Gillett, 2002; Van't Wout et al., 2006). There are also many other reports that indicate the visuospatial deficits in schizophrenia patients (Bilder et al., 2000; Brewer et al., 2005; Gabrovska et al., 1997; Glahn et al., 1997), although the precise nature of the deficit still remains unclear. Among those reports, Maruff et al. (1995) have reported the asymmetries in the covert orienting of visual spatial attention in schizophrenia, and this attentional deficit is dynamic and may reflect disruption to the neurocognitive network controlling attention at the level of the anterior cingulate cortex in the PFC. Poor performance in figure-ground segregation has also been found in schizophrenic patients in several visuospatial tests like the hidden figures test or the embedded figures test, which require the observers to identify which one of several simple figures (perceptually present) is hidden in a complex visual configuration (Loas, 2004).

Risperidone is an atypical antipsychotic drug with antagonistic properties at D_2, 5-HT_{2A} and α_1 receptors (Shayegan and Stahl, 2004). It has much better efficacy on the positive symptoms of schizophrenia than conventional neuroleptics (Khan, 1997). Risperidone also has some effects on the cognitive symptoms, however these effects are practically limited by various side effects. Therefore, there is still the need for adjuvant drugs for the cognitive symptom. A number of studies have indicated that there is a deficit with the nicotinic acetylcholine receptors (nAChRs) in the PFC of schizophrenia patients (Arnold et al., 2004; Deutsch et al., 2005), which has been postulated to be related with the cognitive symptoms (Kumari and Postma, 2005). It has been found in clinical surveys that nicotine-containing cigarettes improve cognitive function in schizophrenia patients compared with nicotine-free cigarettes, which is supposed to reflect nicotine's ability to raise dopamine levels in the PFC (Kumari and Postma, 2005). Galantamine, a medicine for Alzheimer's disease, is an allosteric modulator of nAChRs, and the weakest acetylcholinesterase (AChE) inhibitor among the three cholinesterase (ChE) inhibitors presently used in clinical trials (Samochocki et al., 2003; Sharp et al., 2004). In a clinical case report, galantamine enhanced cognition in 5 schizophrenia patients treated with clozapine (Bora et al., 2005), which is an atypical antipsychotic.

The present study was designed to test the hypothesis that co-administration of galantamine and risperidone synergistically attenuates cognitive deficit in a PCP-treated animal model of schizophrenia, and to analyze the mechanism underlying the effect.

2. Methods and materials

2.1. Animals

Male mice of the ICR strain (Japan SLC Inc., Shizuoka, Japan), 6 weeks old at the beginning of experiments, were used. They were housed in plastic cages, five mice per cage through out the research, received food (CE2; Clea Japan Inc., Tokyo, Japan) and water ad libitum, and were maintained on a 12/12-h light/dark cycle (lights on from 8:00 AM to 8:00 PM). Behavioral experiments were carried out in a sound-attenuated and air-regulated experimental room, to which mice were habituated for at least 1 h. All experiments were performed following the Guidelines for Animal Experiments of Nagoya University, which conformed to the international guidelines set out in the "Guide for the Care and Use of laboratory Animals" (ILAR-NRC publication, revised in 1996).

2.2. Drugs

Phencyclidine HCl (PCP) was synthesized by the authors according to the method of Maddox and colleagues (**Maddox et al., 1965**) and was checked for purity. Galantamine and risperidone were provided by Janssen Pharmaceutical K. K. (Tokyo, Japan). Mecamylamine hydrochloride, (−) scopolamine hydrobromide, *R*(+)-SKF 81297 hydrobromide and *R*(+)-SCH 23390 hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). All the doses were referred to the salt forms of the compounds. PCP was dissolved in 0.9% saline. Oral solution of risperidone and/or galantamine was freshly prepared by dissolving them in diluted tartaric acid solution (final pH 3.2). SKF 81297, SCH 23390, mecamylamine and scopolamine were dissolved in 0.9% saline.

2.3. Drug treatment

The doses of risperidone and galantamine used in the present study were determined in preliminary experiments. The doses of antagonists were referred to our previous publication (**Wang et al., 2007a,b**) and/or other related researches being done in the laboratory, and determined in preliminary experiments.

For the experiments that the drugs were given systemically, the mice were administered PCP (10 mg/kg per day s.c.) or saline once a day for 14 consecutive days (**Noda et al., 1995**). The saline- and PCP-treated mice were p.o. administered galantamine (0.05, 0.1 or 0.3 mg/kg) and risperidone (0.025, 0.05 or 0.1 mg/kg) 1 h before the training trial of the water-finding test, or immediately after baseline collections in microdialysis experiment. Mecamylamine (3 mg/kg) or scopolamine (0.1 mg/kg) was s.c. injected 20 min after the treatment with galantamine and/or risperidone, and SCH 23390 (0.02 mg/kg) was s.c. injected 30 min after the co-administration. All compounds were systemically administered at a volume of 0.1 ml/10 g body weight. Control mice received the same volume of saline or vehicle.

For mPFC-local microinjection, mice were anesthetized with diethyl ether and fixed on the stereotactic apparatus (Narishige, Tokyo, Japan) 20 min before the training trial of water-finding test. A L-shape injection cannula (27 gauge) with a bevel tip at the short end of it was clipped on a pincers and implanted into the mPFC (+0.3 mm mediolateral from the midpoint on the line linking the two rear canthi, −2.5 mm in depth). SKF 81297 at the dose of 0.15 μg/0.5 μL/mouse and SCH 23390 at the dose of 0.02 μg/0.5 μL/mouse were infused into the mPFC in 45 s using a Hamilton microsyringe connected to the cannula via a teflon tube, and the connection was held for another 45 s after the injection. Since the depth of the injection (−2.5 mm) was predetermined by the length of the short end of the L-shape injection cannula, a misinjection would be mainly resulted from a horizontal departure from the right position, and the misinjected brain structures would be the forces major of the corpus callosum (fmi) and the caudate putamen (CPu) partially surrounded by the fmi, as shown in the atlas of **Franklin and Paxinos (1997)**. The fmi and CPu are easy to be discriminated from the PFC by the white color and outline of the fmi. After the behavioral experiments, the mice were decapitated, and the brains were taken out. The brains were transversely cut along the direction of the vertical insertion of the cannula to confirm the injection site, which was obvious by its dark red color and easy to be recognized, as shown in **Fig. 2**. Misinjected mice were excluded from subsequent data analysis.

2.4. Water-finding test

The protocol of **Noda et al. (2001)** was used for the study. The apparatus consisted of an open field [50 × 30 × 15 (H) cm] with an alcove [10 × 10 × 10 (H) cm] in the middle of one of the long walls of the enclosure. The floor of the open field was divided into 15 identical squares. The nozzle of a drinking bottle that was identical with those used in the home cages was inserted from the ceiling of the alcove.

The water-finding test consisted of two trials: a training trial (the 1st day) and a testing trial (the 2nd day). The training trial was started 3 days after the withdrawal of PCP treatment. Mice that had not been deprived of water were placed individually into and toward one corner of the open field of the apparatus. Each mouse was given 3 min to explore the environment. The mice that did not find the drinking nozzle during the 3-min exploratory period were omitted from the testing trial. The mice were immediately returned to their home cages after the training trial, and deprived of water for 24 h before the testing trial. In the testing trial, mice were again individually put into the apparatus. Finding latency was defined as the time from entering the alcove till drinking the water.

The field of the vision of mice is limited by their moving attitude, and it is extremely harder for them to casually find a short water nozzle located at the center of the ceiling than the things that are not at the center in the small alcove. In order to decrease the possibility of being found by chance in testing trials, the tip of the water nozzle inserted from the center of the ceiling was set further from the floor in the testing trial (7.5 cm) than in the training trial (6.5 cm).

2.5. In vivo microdialysis

In vivo microdialysis was performed 3 days after the withdrawal of PCP treatment. One day before the microdialysis, mice were anesthetized with sodium pentobarbital and a guide cannula (MI-AG-6; Eicom Corp., Kyoto, Japan) was implanted into the mPFC (+1.9 mm anteroposterior, +1.0 mm mediolateral from the bregma, −1.5 mm dorsoventral from the skull, +15 degree angle from vertical) according to the atlas of **Franklin and Paxinos (1997)**. One day after the operation, a dialysis probe (A-I-6-01; 1 mm membrane length; Eicom Corp.) was inserted through the guide cannula, and perfused with artificial cerebrospinal fluid (aCSF; 147 mM NaCl, 4 mM KCl, and 2.3 mM $CaCl_2$) at a flow rate of 1.2 μL/min (**Shintani et al., 1993**). The outflow fractions were collected every 10 min. Following the collection of 3 stable baseline fractions, galantamine and/or risperidone was p.o. administered to the mice, and dialysates were collected for 90 min after the drug administration. Dopamine levels in the dialysates were analyzed using an HPLC system equipped with an electrochemical detector (**Nagai et al., 2004**).

2.6. Statistical analysis

Statistical difference among the experimental groups was tested using a one-way analysis of variance (ANOVA) for behavioral experiments, and a two-way ANOVA for microdialysis. The modified Tukey test was adopted for multiple comparisons. *P* values less than 0.05 were accepted as significant.

3. Results

3.1. Individual effects of risperidone and galantamine on impairment of latent visuospatial learning and memory in PCP-treated mice

In the testing trial, PCP (10 mg/kg 14 days)-treated mice showed a deficit of latent visuospatial learning and memory after withdrawal from PCP treatment. The treatment with risperidone (0.1 mg/kg) and galantamine (0.3 mg/kg) ameliorated the deficit of latent visuospatial learning and memory in PCP-treated mice. Risperidone at the doses of 0.025 and 0.05 mg/kg, and galantamine at the doses of 0.05 and 0.1 mg/kg failed to ameliorate the impairment of latent visuospatial learning and memory in PCP-treated mice (**Fig. 1a**, $F_{(7,118)} = 3.323$, $p < 0.01$). Galantamine at the dose of 0.2 mg/kg showed tendency to ameliorate the deficit of latent visuospatial learning and memory, without reaching the level of statistical significant difference (data not shown).

The effects of risperidone (0.1 mg/kg) and galantamine (0.3 mg/kg) at their effective doses were antagonized by mPFC-local administration of SCH 23390 at the dose of 0.02 μg/0.5 μL/mouse, which did not significantly affect the performance in saline-treated mice (**Fig. 1b**, $F_{(6,83)} = 4.497$, $p < 0.01$). The mPFC-local injection site was shown in **Fig. 2**.

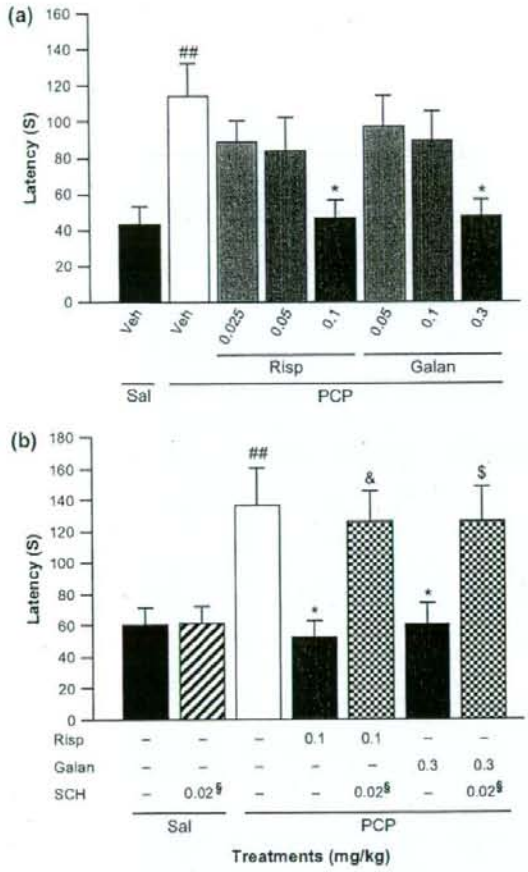

Fig. 1. Individual effects of risperidone and galantamine on impairment of latent visuospatial learning and memory in PCP-treated mice. PCP (10 mg/kg, s.c.) was injected for 14 days. Control groups were treated with same volume of saline (Sal). Galantamine (Galan, 0.05, 0.1 and 0.3 mg/kg, p.o.) and risperidone (Risp, 0.025, 0.05 and 0.1 mg/kg, p.o.) were administered 1 h before the training trial. The D_1 receptor antagonist SCH 23390 was s.c. injected at a dose of 0.02 mg/kg 30 min after the co-administration, or was mPFC-locally injected 15 min before the training trial. **a**. Effects of risperidone and galantamine on the finding latency in PCP-treated mice. **b**. Effects of risperidone and galantamine on the finding latency was antagonized by mPFC-local administration of SCH 23390. Results are expressed as means ± SEM, $n = 14–16$, and analyzed by a one-way ANOVA, followed by the modified Tukey test for multiple comparisons. $^{\#\#}p < 0.01$, compared to Sal/vehicle-treated group. $^{*}p < 0.05$, compared to PCP/vehicle-treated group. $^{\&}p < 0.05$, compared to PCP/risperidone-treated group. $^{\$}p < 0.05$, compared to PCP/galantamine-treated group. Veh: vehicle (dilute tartaric acid solution, pH 3.2). §mPFC-local administration of SCH 23390 at the dose of 0.02 μg/0.5 μL/mouse.

3.2. Synergistic effect of galantamine with risperidone on impairment of latent visuospatial learning and memory in PCP-treated mice

The co-administration of galantamine (0.05 mg/kg) and risperidone (0.025 mg/kg), both at their lowest doses, failed to ameliorate the impairment of latent visuospatial learning and memory in PCP-treated mice (data not shown). The co-administration of galantamine at the lowest dose of 0.05 mg/kg and risperidone at the sub-lowest dose of 0.05 mg/kg greatly ameliorated the impairment of latent visuospatial learning and memory in the PCP-treated mice, although they did not improve the latent visuospatial learning and memory in the saline-treated mice (**Fig. 3**, $F_{(5,95)} = 4.865$, $p < 0.01$). Since the individual treatment with risperidone and galantamine both at the dose of 0.05 mg/kg failed to improve the PCP-induced impairment of latent visuospatial learning and memory by themselves, but showed synergistic effect in the water-finding test, these doses of risperidone and galantamine were used in the subsequent experiments.

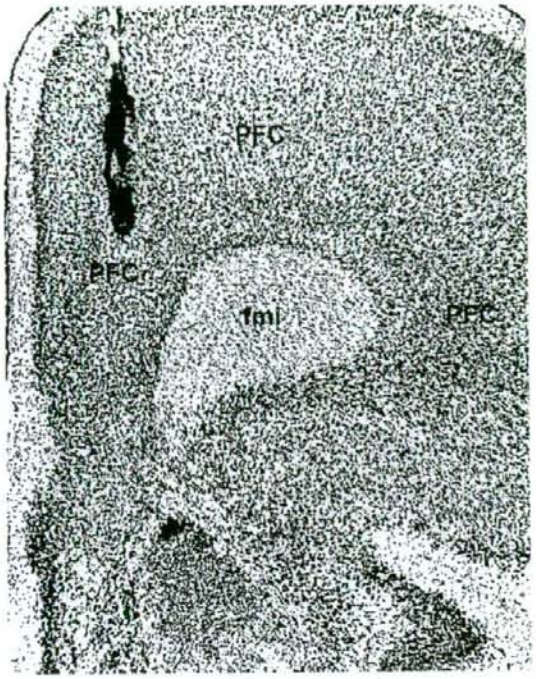

Fig. 2. Representative figure of mPFC-local injection site. PFC, prefrontal cortex. fmi, forces major of the corpus callosum.

The treatment with donepezil at the doses of 0.6 and 1.2 mg/kg ameliorated the impairment of latent visuospatial learning and memory in PCP-treated mice. However, the combined treatment with risperidone (0.05 mg/kg) and donepezil (0.3 mg/kg) did not show a synergistic effect on the cognitive impairment induced by PCP (**Fig. 4**, $F_{(6,100)} = 3.647$, $p < 0.01$).

3.3. Nicotinic, but not muscarinic, AChR is involved in the synergistic effect of galantamine with risperidone

We investigated whether nAChR or mAChR is involved in the synergism of galantamine with risperidone, by investigating whether mecamylamine (3 mg/kg) or scopolamine (0.1 mg/kg)

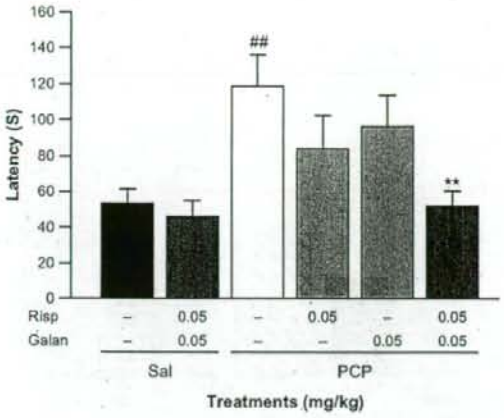

Fig. 3. Synergistic effect of galantamine with risperidone on impairment of latent visuospatial learning and memory in PCP-treated mice. PCP (10 mg/kg, s.c.) was injected for 14 days. Control groups were treated with same volume of saline (Sal). Galantamine (G, 0.05 mg/kg, p.o.) and risperidone (R, 0.05 mg/kg, p.o.) were administered 1 h before the training trial. Results are expressed as means ± SEM, $n = 12-17$, and analyzed by a one-way ANOVA, followed by the modified Tukey test for multiple comparisons. $^{\#\#}p < 0.01$, compared to Sal/vehicle-treated group. $^{**}p < 0.01$, compared to PCP/vehicle-treated group.

could block the behavioral effect of the co-administration. Mecamylamine (3 mg/kg) was s.c. injected to the mice 20 min after the co-administration of galantamine (0.05 mg/kg) and risperidone (0.05 mg/kg). Forty minutes after the injection, mecamylamine blocked the effects of the co-administration on the performance in the PCP-treated mice in the water-finding test

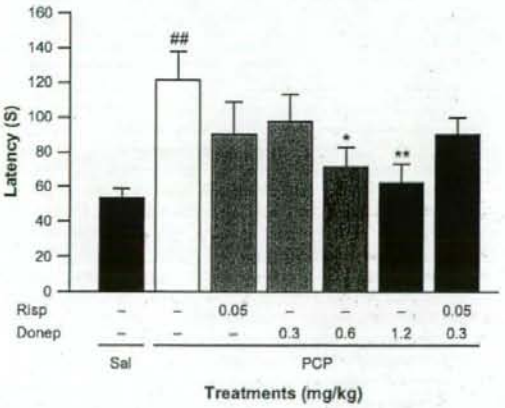

Fig. 4. Combined treatment with donepezil and risperidone did not show synergistic effect on latent visuospatial learning and memory in PCP-treated mice. PCP (10 mg/kg, s.c.) was injected for 14 days. Control groups were treated with same volume of saline (Sal). Donepezil (Donep, 0.3, 0.6 and 1.2 mg/kg, p.o.) and risperidone (Risp, 0.05 mg/kg, p.o.) were administered 1 h before the training trial. Results are expressed as means ± SEM, $n = 12-17$, and analyzed by a one-way ANOVA, followed by the modified Tukey test for multiple comparisons. $^{\#\#}p < 0.01$, compared to Sal/vehicle-treated group. $^{*}p < 0.05$, $^{**}p < 0.01$, compared to PCP/vehicle-treated group.

at the dose (3 mg/kg) that did not inhibit latent visuospatial learning and memory in saline-treated mice (Fig. 5a), $F_{(4,57)} = 6.203$ ($p < 0.01$).

Scopolamine (0.1 mg/kg) was s.c. injected to the mice 20 min after the co-administration. The performance in saline-treated mice was impaired by scopolamine at the relatively low dose of 0.1 mg/kg (Fig. 5b). However, scopolamine at this dose failed

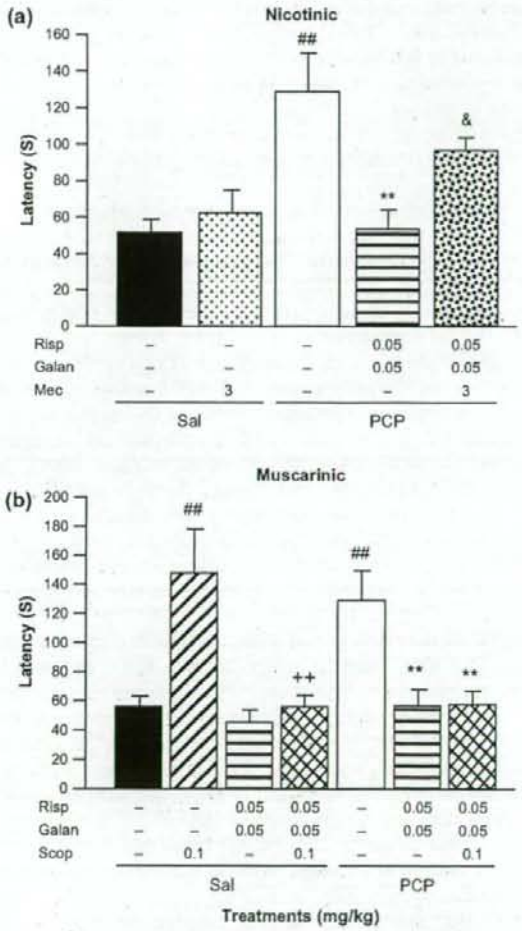

Fig. 5. Nicotinic, but not muscarinic, AChRs are critical for the synergism of galantamine with risperidone. PCP (10 mg/kg, s.c.) was injected for 14 days. Control groups were treated with same volume of saline (Sal). Galantamine (Galan, 0.05 mg/kg, p.o.) and risperidone (Risp, 0.05 mg/kg, p.o.) were administered 1 h before the training trial, and mecamylamine (Mec, 3 mg/kg, s.c.) or scopolamine (Scop, 0.1 mg/kg, s.c.) was injected 20 min after the co-administration. **a**. Synergistic effect of galantamine with risperidone on finding latency was antagonized by mecamylamine. **b**. Synergistic effect of galantamine with risperidone on finding latency was not antagonized by scopolamine. Results are expressed as means ± SEM, $n = 10-12$, and analyzed by a one-way ANOVA, followed by the modified Tukey test for multiple comparisons. $^{\#\#}p < 0.01$, compared to Sal/vehicle-treated group. $^{++}p < 0.01$, compared to Sal/vehicle/Scop-treated group. $^{**}p < 0.01$, compared to PCP/vehicle-treated group. $^{\&}p < 0.05$, compared to PCP/Risp/Galan-treated group.

to antagonize the synergistic effects of the co-administration of galantamine (0.05 mg/kg) and risperidone (0.05 mg/kg) in the PCP-treated mice (Fig. 5b), $F_{(6,77)} = 6.226$ ($p < 0.01$). In order to understand it well, we also investigated the effects of the co-administration followed by the injection of scopolamine (0.1 mg/kg) in saline-treated mice. The treatment with scopolamine at the dose of 0.1 mg/kg also failed to antagonize the effect of the co-administration of these two drugs on the performance in saline-treated mice (Fig. 5b). In other words, the impairing effect of scopolamine at the relatively low dose of 0.1 mg/kg was compensated by the co-administration of galantamine (0.05 mg/kg) and risperidone (0.05 mg/kg) in saline-treated mice.

3.4. The synergistic effect of galantamine with risperidone is mediated by dopamine D_1 receptors

Since the mesocortical dopaminergic neurotransmission through D_1 receptors is thought to be involved in the cognitive symptoms of schizophrenia (Abi-Dargham, 2004; Albert et al., 2002; Fink-Jensen, 2000; Kolb, 1990), we investigated whether the synergistic cognitive effect of galantamine with risperidone is mediated by D_1 receptors.

The effects of the co-administration of galantamine and risperidone on the performance in the PCP-treated mice were abolished by systemic administration of a D_1 receptor antagonist, SCH 23390, at the dose of 0.02 μg/kg that did not significantly impair the latent visuospatial learning and memory in saline-treated mice (Fig. 6a), $F_{(4,75)} = 5.660$ ($p < 0.01$).

The mesocortical dopaminergic system that correlated with the cognitive symptoms projects to the PFC. In order to know whether the synergistic effect of galantamine with risperidone on the impairment of latent visuospatial learning and memory is mediated by the D_1 receptors in the mPFC, the strategy of mPFC-local microinjection of the dopamine D_1 receptor agonist SKF 81297 and the antagonist SCH 23390 was used in the present study. SKF 81297 (0.15 μg/0.5 μL/mouse) and SCH 23390 (0.02 μg/0.5 μL/mouse) were injected into the mPFC of the mice 15 min before the training trial. The microinjection of the D_1 receptor agonist SKF 81297 ameliorated the impairment of latent visuospatial learning and memory in PCP-treated mice (Fig. 6b). In contrast to SKF 81297, the dopamine D_1 receptor antagonist SCH 23390 blocked the effect of the co-administration of the drugs on the latent visuospatial learning and memory ($p < 0.05$) in the PCP-treated mice at the dose (0.02 μg/0.5 μL/mouse) that did not significantly impair the latent visuospatial learning and memory in saline-treated mice (Fig. 6b), $F_{(5,83)} = 4.292$ ($p < 0.01$).

3.5. Performance in mice in training trial of water-finding test

In the training trial, the statistical differences of the discovering latency and ambulation counts in mice were not found among the groups treated with saline or other compounds. All of the treatments used in the study at the present doses did not significantly affect the exploratory activity in the mice in the training trial (Table 1), $F_{(20,272)} = 0.628$ (for discovering

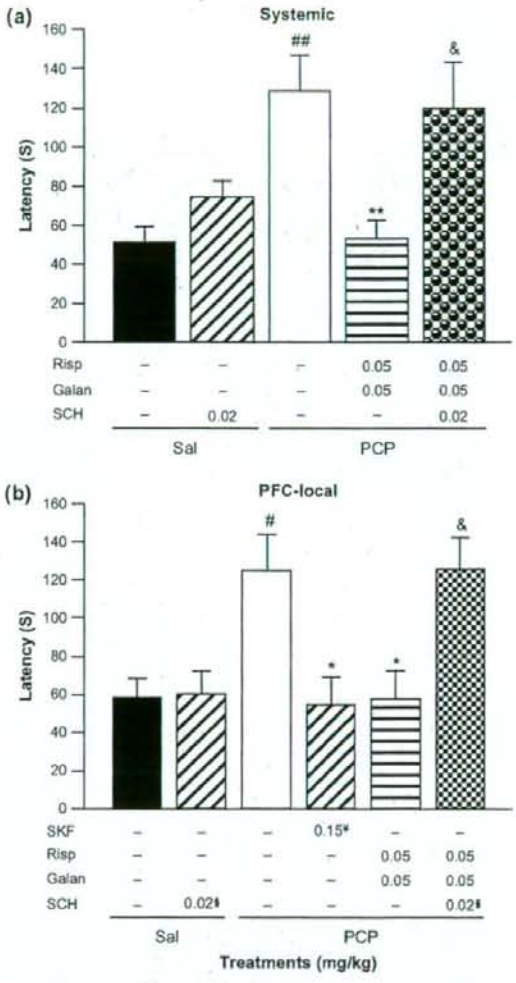

Fig. 6. Synergistic effect of galantamine with risperidone is mediated by D_1 receptors. PCP (10 mg/kg, s.c.) was injected for 14 days. Control groups were treated with same volume of saline (Sal). Galantamine (Galan, 0.05 mg/kg, p.o.) and risperidone (Risp, 0.05 mg/kg, p.o.) were administered 1 h before the training trial. The Dopamine D_1 receptor agonist SKF 81297 was mPFC-locally injected 15 min before the training trial. The D_1 receptor antagonist SCH 23390 was s.c. injected at a dose of 0.02 mg/kg 30 min after the co-administration, or was mPFC-locally injected 15 min before the training trial. **a**. Synergistic effect of galantamine with risperidone on finding latency was blocked by systemic administration of SCH 23390. **b**. Synergistic effect of galantamine with risperidone on finding latency was blocked by mPFC-local administration of SCH 23390. Results are expressed as means ± SEM, $n = 11–17$, and analyzed by a one-way ANOVA, followed by the modified Tukey test for multiple comparisons. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared to Sal/vehicle-treated group, $^{*}p < 0.05$, $^{**}p < 0.01$, compared to PCP/vehicle-treated group, $^{\&}p < 0.05$, compared to PCP/Risp/Galan-treated group. $^{¥}$mPFC-local administration of SKF 81297 at a dose of 0.15 μg/0.5 μL/mouse. §mPFC-local administration of SCH 23390 at a dose of 0.02 μg/0.5 μL/mouse.

Table 1
Discovering latency and ambulation counts in mice in training trial of the water-finding test

Treatments (mg/kg)		DL (s)	Amb (counts)
Saline control		52.0 ± 9.78	25.4 ± 9.78
Saline/Risp (0.05) + Galan (0.05)		53.9 ± 8.17	35.2 ± 6.65
Saline/SCH (0.02)		55.1 ± 6.55	21.4 ± 3.27
Saline/Mec (3)		52.0 ± 6.29	30.4 ± 6.98
Saline/Scop (0.1)		60.6 ± 12.6	38.5 ± 5.69
Saline/Risp (0.05) + Galan (0.05)/Scop (0.1)		67.5 ± 10.9	36.9 ± 5.25
PCP control		45.5 ± 6.97	28.0 ± 4.21
PCP/Risp	(0.025)	47.1 ± 7.80	35.1 ± 6.58
	(0.05)	55.3 ± 6.98	26.9 ± 3.92
	(0.1)	60.6 ± 12.2	35.7 ± 7.36
PCP/Galan	(0.05)	55.3 ± 6.74	31.0 ± 3.37
	(0.1)	45.5 ± 6.97	26.5 ± 4.30
	(0.3)	47.1 ± 7.80	24.9 ± 3.62
PCP/Donep	(0.3)	53.2 ± 6.41	26.9 ± 3.38
	(0.6)	50.6 ± 7.17	27.3 ± 4.12
	(1.2)	51.9 ± 6.47	25.9 ± 3.39
PCP/Risp (0.05) + Galan (0.05)		56.3 ± 6.61	32.2 ± 3.05
PCP/Risp (0.05) + Donep (0.3)		53.9 ± 6.83	28.9 ± 3.26
PCP/Risp (0.05) + Galan (0.05)/SCH (0.02)		53.4 ± 8.96	25.4 ± 3.52
PCP/Risp (0.05) + Galan (0.05)/Mec (3)		60.0 ± 12.6	33.0 ± 4.63
PCP/Risp (0.05) + Galan (0.05)/Scop (0.1)		58.2 ± 11.0	36.7 ± 6.51

PCP, phencyclidine (10 mg/kg, s.c. injected for 14 d); Risp, risperidone; Galan, galantamine; Donep, donepezil; SCH, SCH 23390; Mec, mecamylamine; Scop, scopolamine; DL, the latency to discover the water nozzle for the first time after being put into the apparatus, the discovery of the water nozzle is defined as exploring (approaching, sniffing or touching) it with their noses or mouths, which is a part of their environmental exploratory activity and a behavioral characteristic of mice when they newly find an immobile object in the environment; Amb: the ambulation counts from start till discovering the water nozzle. Values are means ± SEM, $n = 10–17$; statistically analyzed by one-way ANOVA. Significant differences were not found among the groups.

latency), $p = 0.890$, $F_{(20,272)} = 0.958$ (for ambulation counts), $p = 0.514$.

3.6. Synergistic effect of galantamine with risperidone on extracellular concentration of dopamine in mPFC of PCP-treated mice

It has been reported that the extracellular dopamine concentration decreased in the PFC of repeated PCP-treated mice (**Jentsch et al., 1998a,b; Jentsch and Roth, 1999**). The change in the basal level was mirrored in the dopaminergic response to potassium stimulation at high concentration, which is observed in another study in our laboratory, in which the release of dopamine in the mPFC of PCP-treated (10 mg/kg, 14 days) mice was insensitive to the potassium stimulation (data not shown).

The extracellular concentration of dopamine was not significantly increased in the mPFC of the PCP-treated mice by the individual treatment with galantamine (0.05 mg/kg) or risperidone (0.05 mg/kg) at their non-effective doses. In contrast to the individual treatments, the co-administration of galantamine (0.05 mg/kg) and risperidone (0.05 mg/kg) significantly increased extracellular concentration of dopamine in the mPFC (**Fig. 7**), $F_{\text{group}(5,357)} = 25.093$ ($p < 0.01$), $F_{\text{time}(12,357)} = 3.635$ ($p < 0.01$).

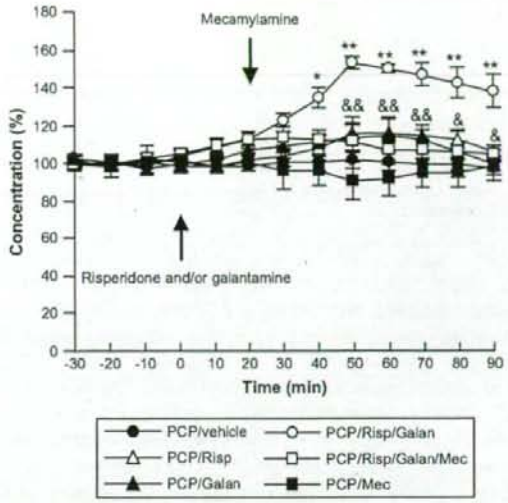

Fig. 7. Synergistic effect of galantamine with risperidone on extracellular concentration of dopamine in mPFC of PCP-treated mice. PCP (10 mg/kg, s.c.) was injected for 14 days. Galantamine (Galan, ▲, 0.05 mg/kg, p.o.) and risperidone (Risp, △, 0.05 mg/kg, p.o.) were administered individually or together (○, p.o.). The control group was given equivalent amount of vehicle (●). Mecamylamine (Mec, 3 mg/kg, s.c.) was injected 20 min after the co-administration (□), or injected individually as a control group (■). Results are expressed as means ± SEM, $n = 4–5$, and analyzed by a two-way ANOVA, followed by the modified Tukey test for multiple comparisons $^{*}p < 0.05$, $^{**}p < 0.01$, compared between PCP/vehicle-treated and PCP/Risp/Galan-treated groups. $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, compared between PCP/Risp/Galan-treated and PCP/Risp/Galan/Mec-treated groups.

3.7. Nicotinic AChR antagonist, mecamylamine, blocked synergistic effect of galantamine with risperidone on extracellular concentration of dopamine in mPFC of PCP-treated mice

It has been reported by **Ichikawa et al. (2002)** that risperidone increases cortical acetylcholine release, which in turn promotes dopamine release. In the present study, the increasing effect of the co-administration of galantamine and risperidone on extracellular concentration of dopamine was antagonized by the nAChR antagonist mecamylamine (3 mg/kg, s.c. injected 20 min after the co-administration) at the dose that did not significantly change the extracellular concentration of dopamine in the mPFC (**Fig. 7**). These findings indicated the mechanism how the dopamine release was increased by the co-administration of galantamine and risperidone, but not by their individual treatments.

3.8. Effects of co-administration of risperidone and galantamine on extracellular concentration of dopamine in mPFC of saline-treated mice

The individual treatment with risperidone (0.05 mg/kg) or galantamine (0.05 mg/kg) did not significantly affect the extracellular concentration of dopamine in the mPFC of

saline-treated mice (Fig. 8). However, the co-administration of risperidone (0.05 mg/kg) and galantamine (0.05 mg/kg) increased the extracellular concentration of dopamine in the mPFC of saline-treated mice 40 and 50 min after the co-administration (Fig. 6). $F_{group(3,151)} = 9.742$ ($p < 0.01$), $F_{time(12,151)} = 2.490$ ($p < 0.01$).

4. Discussion

PCP induces psychomimetic state in human and behavioral changes in animals that closely resemble schizophrenia (Javitt and Zukin, 1991; Nabeshima et al., 1989; Noda et al., 1995). Dopamine release in the striatal area including nucleus accumbens increases soon after the acute treatment with PCP (Balla et al., 2001; Greenslade and Mitchell, 2004). The activation of the dopamine-D_2 receptors in the striatum accounts for the positive symptoms of schizophrenia (Abi-Dargham, 2004). In stark contrast to the acute PCP exposure, repeated treatment with PCP reduces the basal extracellular concentration of dopamine and the response of dopamine release to potassium stimulation in the mPFC as shown in our and other researchers' studies (Jentsch et al., 1998a,b; Jentsch and Roth, 1999; Wang et al., 2007b). In the present study, the effects of the individual treatment with risperidone or galantamine at their effective doses and the combined treatment at their non-effective doses were abolished by mPFC-local administration of the D_1 receptor antagonist SCH 23390, indicating the involvement of dopaminergic system in the effects. The present results are consistent with the notion that the dopaminergic dysfunction in the PFC is a characteristic of the neurochemical changes in schizophrenia patients that accounts for the cognitive symptoms (Abi-Dargham, 2004; Albert et al., 2002; Fink-Jensen, 2000; Kolb, 1990).

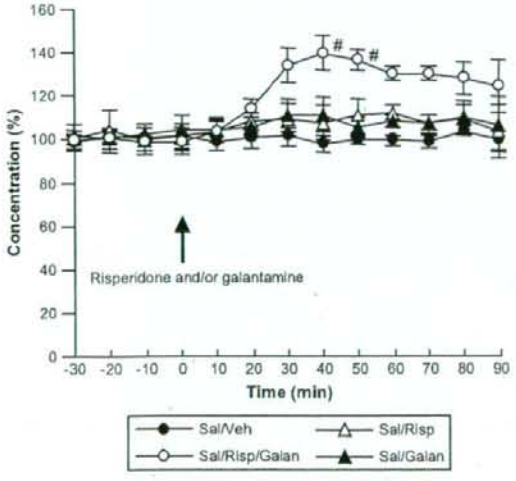

Fig. 8. Synergistic effect of galantamine with risperidone on extracellular concentration of dopamine in mPFC of saline-treated mice. Saline/vehicle-treated mice were used as a control group (Sal/Veh, ●). Galantamine (Galan, ▲, 0.05 mg/kg, p.o.) and risperidone (Risp, △, 0.05 mg/kg, p.o.) were administered individually or together (○, p.o.). Results are expressed as means ± SEM, $n = 4–5$, and analyzed by a two-way ANOVA, followed by the modified Tukey test for multiple comparisons. $^{\#}p < 0.05$, compared between Sal/Veh-treated and Sal/Risp/Galan-treated groups.

The cognitive symptoms of schizophrenia are often the most debilitating and difficult to treat in clinical trials. Deficits in attention and information processing mechanisms have been suggested to play a critical role in the pathology of schizophrenia (Noda et al., 2001), and schizophrenia might involve an inability to properly filter incoming sensory information and thus result in sensory inundation and subsequent cognitive fragmentation (McGhie and Chapman, 1961; Noda et al., 2001; Venables, 1960). The water-finding test, which is a latent visual learning paradigm, relates to attention and the ability to sort sensory information (Noda et al., 2001). Repeated treatment with PCP significantly prolonged the finding latency in the water-finding test at the dose of 10 mg/kg, indicating an impairment of latent visuospatial learning and memory that engages attention processes.

Repeated treatment with PCP impaired the latent visuospatial learning and memory in mice as shown in the water-finding test. In the present study, a medicine for Alzheimer's disease, galantamine, ameliorated the cognitive impairment in the PCP-treated mice, and the co-administration of galantamine and risperidone at their non-effective doses showed significant effect on the cognitive impairment in the mice. These results clearly indicated that galantamine may be effective in treating the cognitive symptom of schizophrenia and the combined treatment with galantamine and risperidone may have synergistic effect on the symptom. Since the synergy between galantamine and risperidone in ameliorating the cognitive impairment was blocked by the systemic and mPFC-local administration of the D_1 receptor antagonist SCH 23390, it indicated that D_1 receptor-mediated neurotransmission in the mPFC plays an important role in the synergistic effect of these two drugs on the impairment of latent visuospatial learning and memory induced by repeated PCP treatment.

Ichikawa et al. (2002) have reported that risperidone increases acetylcholine release in the cortex, but not in the striatal areas. This effect of risperidone on the release of acetylcholine may be indispensable for the synergy between galantamine and risperidone in ameliorating the cognitive impairment induced by repeated PCP treatment. Other antipsychotics without an acetylcholine-releasing effect may have none or less synergistic effect with galantamine as recently reported (Lee et al., 2007). Activation of nAChRs promotes the release of DA (Cao et al., 2005; Salminen et al., 2004; Wang et al., 2007a; Wonnacott, 1997; Zhang et al., 2004). In the present study, the effect of the co-administration on dopamine release in the mPFC was antagonized by the nAChR antagonist mecamylamine. These findings indicated the pivotal role of nAChR in regulating dopaminergic neurotransmission in the synergistic effect of galantamine and risperidone. This notion is supported by the result in the present study that donepezil, an AChE inhibitor without the nAChR-potentiating effect, actually did not show synergistic effect with risperidone. At low doses, galantamine potentiates the function of

nAChRs. This acting mode of galantmine at low doses not only makes it possible to avoid the problematic rapid desensitization of receptors generally induced by receptor agonists (Deutsch et al., 2005; Friedman, 2004) but also makes the receptor-mediated cognitive effect be receptor use-dependent, which may be better for learning and memory than general activation of all the nAChRs in the brain caused by agonists. Besides being an allosteric potentiator of nAChR, galantamine is a rapidly reversible and rather modest AChE inhibitor (IC_{50} in the frontal cortex and the hippocampus of mouse and human in the range from 2.8 to 3.9 μM) (Bickel et al., 1991b; Samochocki et al., 2003; Sharp et al., 2004). At the doses used in the present study that are far below those required to reach its IC_{50} value for AChE inhibition (Bickel et al., 1991a; Farlow, 2003; Scott and Goa, 2000; Samochocki et al., 2003), the effect of galantamine on the release of dopamine in the mPFC was mainly resulted from its potentiation of the nAChR, but not from the inhibition of AChE. This notion is supported by the publication that there is only 1–12% brain AChE inhibition 1 h after s.c. injection of 3 mg/kg galantamine (Geerts et al., 2005; Thomsen et al., 1991), and by the fact observed in the study that galantamine at the dose of 3 mg/kg was not more effective than that of 0.3 mg/kg (data not shown).

It has been reported that the repeated treatment with risperidone (0.5 mg, co-administered after 0.5 mg twice daily for 6 days) had no effect on the bioavailability and disposition of galantamine, and the systemic exposure of risperidone active moiety, the most clinically relevant component of risperidone treatment, was not affected by galantamine co-administration. The plasma concentration of risperidone was not changed within 10 h after the co-administration, while the systemic exposure was increased by approximately 10% for risperidone, and mean peak plasma concentration of risperidone active moiety decreased by approximately 10% after co-administration with galantamine (12 mg, co-administered after a serial pretreatment for 6 days) (Huang et al., 2002).

SCH 23390 displays an 800 times higher affinity for D_1 receptor than D_2 receptor (Christensen et al., 1984). The fact that SCH 23390 blocks dopamine-stimulated adenylate cyclase at concentrations ($IC_{50} \approx 0.01$ μM) about 2000 times lower than that needed to block spiperone binding ($IC_{50} \approx 24$ μM) and three times lower than that needed to block ketanserin binding ($IC_{50} \approx 0.03$ μM) suggests a more specific antagonism for D_1-receptor than 5-HT_2 receptor (Bischoff et al., 1986; Iorio et al., 1983). SCH 23390 also possesses weak affinities for 5-HT_{1A} ($IC_{50} \approx 2.6$ μM), 5-HT_{1B} ($IC_{50} \approx 0.5$ μM) and α_1-adrenergic receptors ($IC_{50} \approx 4.4$ μM) (Bischoff et al., 1986, 1988). SKF 81297 is an agonist for D_1 receptor with high selectivity. The rank order for dopamine D_1:D_2 receptor selectivity in rhesus striata is: SKF 81297 > SKF 38393 ≫ SKF 82958 > SKF 77434 > $R(+)$ 6-BrAPB > $S(-)$ 6-BrAPB > dopamine, among which SKF 81297 is the only agonist that displays more than 100 times selectivity for D_1 receptor than D_2 receptor (Weed et al., 1998). Mecamylamine antagonizes all types of nAChRs, but not mAChRs, with greatest affinity for α4β2 receptor, and at higher doses it can also antagonize NMDA receptor (Rabenstein et al., 2006; Xiao and Kellar, 2004). The rank order of nAChR ligands for nAChR-binding affinity in rat forebrain is mecamylamine (K_i > 500,000) ≫ choline ($K_i \approx$ 43,000) ≫ methyllcaconitine ($K_i \approx$ 6600) ≫ carbachol > DHβE > DMPP ≫ acetylcholine ($K_i \approx$ 45) > (−)-Nicotine ($K_i \approx$ 12) > cytosine ($K_i \approx$ 1.9) > A-85380 > (±)-I-epibatidine > I-A-85380 > (±)-epibatidine (Xiao and Kellar, 2004). Scopolamine is a competitive antagonist for all mAChR subtypes with high affinities (Goudie et al., 2004). By citing an unpublished paper, Goudie et al. (2004) reported that scopolamine may also have relatively weak affinity for D_2 receptor. In the present study, the doses that we used are very low, therefore, the effects of the compounds attribute to their principal activity at the receptors, matching the purpose for using them in the present study.

Although galantamine is only a rather modest AChE inhibitor ($IC_{50} \approx 2.8–3.9$ μM) compared with other AChE inhibitors presently used in clinical trials, such as rivastigmine ($IC_{50} \approx 4$ nM) and donepezil ($IC_{50} \approx 15–24$ nM), it increases extracellular concentration of acetylcholine at relatively high doses. In addition, similar as donepezil and rivastigmine, galantamine blocks ACh-activated channels at very high concentration (>10 μM) (Samochocki et al., 2003; Sharp et al., 2004). Based on the above reasons and the fact that galantamine can improve latent visuospatial learning and memory synergistically with risperidone by allosterically potentiating nAChR at relatively low doses, it is preferable to use galantamine at low doses for treating the cognitive symptom in schizophrenia.

The present study indicates that the combined treatment with galantamine and risperidone may have synergistic effect on repeated PCP treatment-induced impairment of latent visuospatial learning and memory by promoting the nAChR activation-dependent increase of dopamine D_1 receptor-mediated neurotransmission, and the combined treatment may be used as a new strategy for treating the cognitive symptom of schizophrenia.

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The Allosteric Potentiation of Nicotinic Acetylcholine Receptors by Galantamine Ameliorates the Cognitive Dysfunction in Beta Amyloid$_{25-35}$ I.c.v.-Injected Mice: Involvement of Dopaminergic Systems

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Galantamine, a drug for Alzheimer's disease, is a novel cholinergic agent with a dual mode of action, which inhibits acetylcholinesterase and allosterically modulates nicotinic acetylcholine receptors (nAChRs), as a result stimulates catecholamine neurotransmission. In the present study, we investigated whether galantamine exerts cognitive improving effects through the allosteric modulation of nAChR in the intracerebroventricular beta amyloid ($A\beta$)$_{25-35}$-injected animal model of Alzheimer's disease. Galantamine (3 mg/kg p.o.) significantly increased the extracellular dopamine release in the hippocampus of saline- and $A\beta_{25-35}$-injected mice. The effects of nicotine on the extracellular dopamine release were potentiated by galantamine, but antagonized by mecamylamine, a nAChR antagonist. $A\beta_{25-35}$-injected mice, compared with saline-injected mice, could not discriminate between new and familiar objects in the novel object recognition test and exhibited less freezing response in the fear-conditioning tasks, suggesting $A\beta_{25-35}$ induced cognitive impairment. Galantamine improved the $A\beta_{25-35}$-induced cognitive impairment in the novel object recognition and fear-conditioning tasks. These improving effects of galantamine were blocked by the treatment with mecamylamine, SCH-23390, a dopamine-D1 receptor antagonist, and sulpiride, a dopamine-D2 receptor antagonist, but not by scopolamine, a muscarinic acetylcholine receptor antagonist. This study provides the first *in vivo* evidence that galantamine augments dopaminergic neurotransmission within the hippocampus through the allosteric potentiation of nAChRs. The improving-effects of galantamine on the $A\beta_{25-35}$-induced cognitive impairment may be mediated through the activation of, at least in part, dopaminergic systems, and the enhancement of dopamine release may be one of multiple mechanisms underlying the therapeutic benefit of galantamine.
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INTRODUCTION

Alzheimer's disease is a progressive and neurodegenerative disorder that is associated with a global impairment of higher mental function, with confusion, loss of memory, and impairment of cognitive function (Palmer, 2002). There is evidence that the common pathological features of the deposition of β-amyloid peptide ($A\beta$) and cholinergic degeneration may play an important role in the pathogenesis of Alzheimer's disease (Nordberg, 2001). One of the most prominent cholinergic dysfunction in Alzheimer's disease is the reduced number of nicotinic acetylcholine receptors (nAChRs) in the hippocampus and cortex, correlating well with the severity of Alzheimer's disease (Schroder *et al*, 1991; Burghaus and Schutz, 2000). This dysfunction results in reduced nicotinic cholinergic excitation including postsynaptic depolarization, presynaptic neurotransmitter release, and intracellular signaling.

Because Alzheimer's disease is associated with a dysfunction in nicotinic neurotransmission, a novel approach to

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drug treatment in Alzheimer's disease is the application of allosteric modulators of nAChRs, which up-modulates (potentiates) the channel activity of nAChRs in response to acetylcholine. Such properties are displayed by a novel class of nAChR ligands, named 'allosteric potentiating ligands' (APLs) (Maelicke and Albuquerque, 2000; Maelicke *et al*, 2000). Galantamine, an approved medication for Alzheimer's disease, has a dual mechanism of action, which inhibits acetylcholinesterase and allosterically modulates nAChR as a potent APL (Eisele *et al*, 1993; Santos *et al*, 2002). In contrast to pure agonist nicotine, the stimulation of the nAChRs by galantamine is synchronized with physiological presynaptic cholinergic activity, thereby avoiding overstimulation and desensitization, and probably leading to an optimal activation of the downstream intracellular pathways (Geerts, 2005). Galantamine augments catecholamine neurotransmission within the hippocampus by augmenting the stimulative effects of endogenous nicotinic cholinergic circuits (Sharp *et al*, 2004) Thus, galantamine has been demonstrated to have potential cognitive improving effects on Alzheimer's disease since the allosteric action of galantamine may be related, in part, to the stimulation of catecholamine neurotransmission in addition to its enhancing effects on cholinergic systems by inhibition of acetylcholinesterase.

In the hippocampus, a critical area involved in attention and memory, the significance of nicotinic–dopaminergic interactions for cognitive function has been well documented (Hefco *et al*, 2004; Levin and Simon, 1998). Some cognitive and noncognitive aspects of Alzheimer's disease arise from the dysfunction of the dopaminergic and serotonergic systems rather than the cholinergic systems (Assal and Cummings, 2002; Erkinjuntti, 2002). Acetylcholine, or its hydrolysis product choline, would activate presynaptic nAChRs, leading to a Ca^{2+}-dependent enhancement of dopamine release (Turner, 2004). From the data mentioned above, it is very important to confirm whether galantamine can improve cognitive dysfunction of Alzheimer's disease by allosterically potentiating nicotinic–dopaminergic pathway.

The present study was designed to test the hypothesis that galantamine improves cognitive dysfunction in the $A\beta_{25-35}$-injected animal model of Alzheimer's disease (Maurice *et al*, 1996), and such cognitive improving effects of galantamine are mediated via activation of nAChR-dopaminergic systems. We attempted to investigate: (1) whether cognitive improving effects of galantamine are mediated via nAChRs in the $A\beta_{25-35}$-injected mice and (2) whether galantamine augments dopamine neurotransmission within the hippocampus by activation of nAChRs.

MATERIALS AND METHODS

Subjects

Male mice of the ICR strain (Japan SLC Inc., Shizuoka, Japan), aged 5 weeks at the beginning of experiments, were used. They were housed in plastic cages, received food (CE2; Clea Japan Inc., Tokyo, Japan) and water *ad libitum*, and were maintained on a 12/12-h light/dark cycle (lights on from 0800 to 2000 hours). Behavioral experiments were carried out in a sound-attenuated and air-regulated experimental room, to which mice were habituated for at least 1 h. All experiments were performed by a blind manner and in accordance with the Guidelines for Animal Experiments of Nagoya University Graduate School of Medicine. The procedures involving animals and their care conformed to the international guidelines set out in the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.

Drugs

Galantamine (4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-6H-benzofuro [3a,3,2-ef]benzazepin-6-ol hydrobromide) was supplied by Janssen Pharmaceutical KK (Tokyo, Japan). (−)-Nicotine di-[+]tartrate, SCH-23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl- 2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride), *S*(−)-sulpiride, and mecamylamine were purchased from Sigma (St Louis, MO, USA). Sulpiride was initially dissolved in a minimum volume of 0.1N HCl and was then diluted with saline. $A\beta_{25-35}$ was obtained from Wako (Osaka, Japan), and was dissolved in saline at the concentration of 3 mM and stored at −20°C. The $A\beta_{25-35}$ (3 mM) were aggregated, or 'aged', by incubating in saline at 37°C for 4 days according to previous report (Maurice *et al*, 1996).

Drug Treatments

The intracerebroventricular injection of $A\beta_{25-35}$ was performed according to the protocol of Maurice *et al* (1996). Briefly, a microsyringe with a specially made 28-gauge stainless-steel needle, 3 mm in length, was used for microinjection. Mice were anesthetized lightly with ether, and inserted needle unilaterally 1 mm to the right of the midline point equidistant from each eye, at an equal distance between the eyes and the ears and perpendicular to the plane of the skull. $A\beta_{25-35}$ (9 nmol/3 μl) or saline (3 μl) was delivered gradually within 30 s. Mice exhibited normal behavior within 1 min after injection. The injection site was confirmed by injecting Indian ink in preliminary experiments. Neither insertion of the needle nor injection of the saline had a significant influence on survival, and behavioral responses or cognitive functions (Maurice *et al*, 1996).

The saline- and $A\beta_{25-35}$-injected mice were administered galantamine (p.o.), mecamylamine (s.c.), SCH-23390 (s.c.), and sulpiride (s.c.) 60, 30, 30, and 60 min, respectively, before the training session of the novel object recognition and the conditioning phase of the cued and contextual fear-conditioning tasks. The dosages used in the present study were referred to and converted from the clinical doses, our previous publications, and/or other related researches being done in the laboratory, and determined in the preliminary researches for this study. All compounds were systemically administered at a volume of 0.1 ml/10 g body weight.

Behavioral Procedures

Previous reports have shown that acute exposure of hippocampal cultures to aged $A\beta_{25-35}$ induced an apoptotic-mediated neuronal toxicity during a 6-day incubation and that acute injection of aged $A\beta_{25-35}$ also induced cognitive dysfunction in several learning and memory tests

in mice (Lockhart *et al*, 1994). In the present study, the behavioral tests started on 6 days after $A\beta_{25-35}$ injection, and were carried out sequentially according to the experimental schedule shown in Figure 1.

Novel Object Recognition Test

The task was carried out on the days 6–8 after $A\beta_{25-35}$ injection according to the protocol of Nagai *et al* (2003) and Kamei *et al* (2006) with a minor modification. The experimental apparatus consisted of a Plexiglas open-field box (40 × 40 × 29 (H) cm), the floor of which was covered with paper bedding (Japan SLC Inc., Shizuoka, Japan). The apparatus was placed in a sound-isolated room. A light bulb, fastened in the upper part of the room and cannot be seen directly by the mice, provided a constant illumination of about 40 lux at the level of the task apparatus.

The novel-object recognition task procedure consisted of three sessions: habituation, training, and retention sessions. Each mouse was individually habituated to the box, with 10 min of exploration in the absence of objects on the day 6 (habituation session). During the training session on the day 7, two objects (A and B) were placed in the back corner of the box, 10 cm from the side wall. A mouse was then placed in the middle front of the box and the total time spent in exploring the two objects was recorded for 10 min by the experimenter with two stopwatches. Exploration of an object was defined as directing the nose to the object at a distance of less than 2 cm and/or touching it with the nose. During the retention session on the day 8 (24 h after the training session), the animals were placed back into the same box, in which one (eg object A) of the familiar objects used during training was replaced by a novel object C. The animals were then allowed to explore freely for 10 min and the time spent exploring each object was recorded. Throughout the experiments, the objects were used in a counterbalanced manner in terms of their physical complexity and emotional neutrality. A preference index, a ratio of the amount of time spent exploring any one of the two objects (training session) or the novel object (retention session) over the total time spent exploring both objects, was used to measure cognitive function (eg A or B/(B + A) × 100 (%) in the training session, and B or C/(B + C) × 100 (%) in the retention session).

Cued and Contextual Fear-Conditioning Tests

The cued and contextual fear-conditioning tasks were carried out on the days 9–10 after $A\beta_{25-35}$ infusion according to a previous report (Enomoto *et al*, 2005) with a minor modification. For measuring basal levels of freezing response (preconditioning phase), on the day 9, mice were individually placed in a neutral cage (23 × 23 × 12 cm) for 1 min and then in the conditioning cage (25 × 31 × 11 cm) for 2 min. For training (conditioning phase), mice were placed in the conditioning cage, and then a 15-s tone (80 dB) was delivered as a conditioned stimulus. During the last 5 s of the tone stimulus, a foot shock of 0.8 mA was delivered as an unconditioned stimulus through a shock generator (Neuroscience Idea Co. Ltd, Osaka, Japan). This procedure was repeated four times with 15-s intervals. Cued and contextual tests were carried out 24 h after fear conditioning on the day 10. For the cued test, the freezing response was measured in the neutral cage for 1 min in the presence of a continuous tone stimulus identical with the conditioned stimulus. For the contextual test, mice were placed in the conditioning cage, and the freezing response was measured for 2 min in the absence of the tone and the unconditioned stimulus. The freezing response was defined as that all the paws of a mouse stayed still and stooped down with fear.

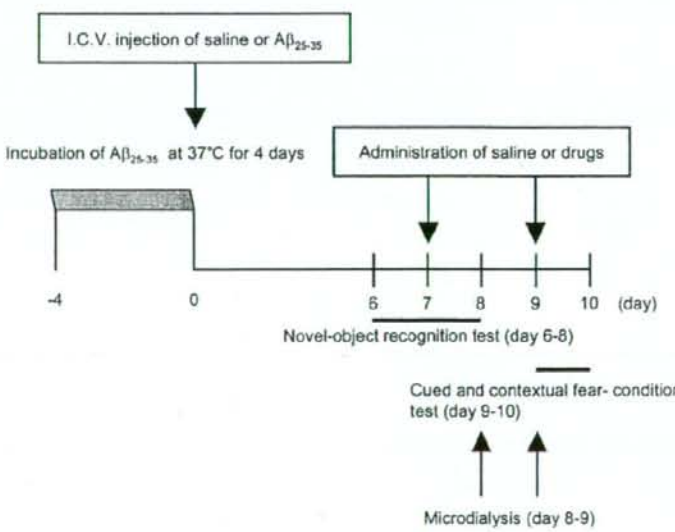

Figure 1 Behavioral experimental schedule. i.c.v., intracerebroventricular.

Determination of Extracellular Dopamine Levels in the Hippocampus

We examined the effect of galantamine on the extracellular level of dopamine in the hippocampus of saline- and Aβ_{25-35}-injected mice. At 7 or 8 days after Aβ_{25-35} infusion, the mice were anesthetized with sodium pentobarbital (40 mg/kg) and a guide cannula (MI-AG-4; Eicom, Kyoto, Japan) was implanted into the hippocampus (coordinates: anteroposterior (AP): +3.05 mm, mediolateral (ML): +3.03 mm from bregma, dorsoventral (DV): 2.00 mm from the skull), according to the atlas of Paxinos and Franklin (2004). At 8 or 9 days after Aβ_{25-35} infusion (24 h after the implantation of the guide cannula), the dialysis probe (A-I-4-02; membrane length 2 mm, Eicom) was implanted into the hippocampus and perfused with Ringer's solution (147 mM NaCl, 4 mM KCl, and 2.3 mM $CaCl_2$) at a flow rate of 1.2 µl/min. The outflow fractions were collected every 10 min. After the collection of three stable baseline fractions, mice were treated with galantamine, nicotine, and/or mecamylamine, and the dialysates were collected every 10 min for 90 min. Dopamine levels in the dialysates were assayed by HPLC equipped with Eicompak PP-ODS column and electrochemical detector (ECD-300, Eicom).

Statistical Analysis

Results are expressed as means ± SEM. Statistical difference among the experimental groups was tested using one-way analysis of variance (ANOVA) for behavioral tests or two-way ANOVA for microdialysis, and Tukey–Kramer *post hoc* test was employed for multiple comparisons. *P*-values less than 0.05 were accepted as significant.

RESULTS

Effects of Galantamine on the Impairments of Performance in Novel Object Recognition and Cued/Contextual Fear-Conditioning Tasks in the Aβ_{25-35}-Injected Mice

During the training session of the novel object recognition task, saline- and Aβ_{25-35}-injected mice treated with saline or galantamine (1 and 3 mg/kg) spent equal amount of time in exploring either of the two objects (Figure 2a), and thus there was no biased exploratory preference in the five groups without affecting total exploring time in the exploration of the objects (data not shown). When retention performance was tested 24 h after the training session, the

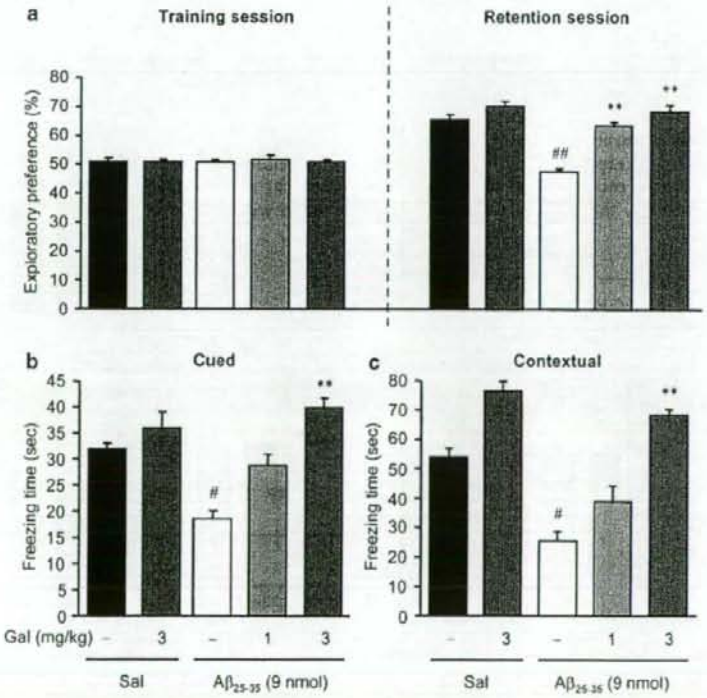

Figure 2 Effects of galantamine on behavioral deficits in Aβ_{25-35}-injected mice in novel object recognition, and cued and contextual fear-conditioning tasks. Galantamine (1 and 3 mg/kg p.o.) was administrated to saline- and Aβ_{25-35} (9 nmol/3 µl)-injected mice 60 min before the training session of the novel object recognition task and the conditioning phase of the cued and contextual fear-conditioning tasks. (a) Novel object recognition task. In training session, $F(3,61) = 0.14$ ($p = 0.94$). In retention session, $F(3,61) = 27.44$ ($p < 0.01$). (b) Cued conditioning task, $F(3,61) = 8.68$ ($p < 0.01$). (c) Contextual conditioning task, $F(3,61) = 6.97$ ($p < 0.01$). Results were expressed as means ± SEM ($n = 13$–17), and analyzed by a one-way ANOVA, followed by Tukey–Kramer test for multiple comparisons. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs* saline-treated, saline-injected mice; $^{**}p < 0.01$ *vs* saline-treated, Aβ_{25-35}-injected mice.

level of exploratory preference for the novel object in the saline-treated $A\beta_{25-35}$-injected mice was significantly decreased compared to that in the saline-treated, saline-injected mice ($p<0.01$ by *post hoc* (Figure 2a)). Galantamine (1 and 3 mg/kg)-treated $A\beta_{25-35}$-injected mice spent a significantly longer time in exploring novel object than the saline-treated $A\beta_{25-35}$-injected mice ($p<0.01$ by *post hoc*) (Figure 2a), indicating that galantamine improved the recognition of novelty in mice impaired by $A\beta_{25-35}$ infusion.

In the preconditioning phase of the cued and contextual fear-conditioning task, the saline- and $A\beta_{25-35}$-injected mice treated with saline or galantamine (1 and 3 mg/kg) hardly showed the freezing response (data not shown), and there were no differences in the basal levels of freezing response among the five groups (data not shown). In the cued and contextual fear-testing trial, saline-treated, saline-injected mice exhibited marked cued and contextual freezing response, indicating that the associative learning ability in these mice is better than that in saline-treated $A\beta_{25-35}$-injected mice, which showed a significant decrease of cued and contextual freezing response 24 h after fear conditioning ($p<0.01$ by *post hoc*) (Figure 2b and c). The performance of saline-treated, $A\beta_{25-35}$-injected mice was completely reversed by the treatment with galantamine at the dose of 3 mg/kg, but not 1 mg/kg (Figure 2b and c). Galantamine (3 mg/kg) tended to increase the cognition in both asks in saline-injected mice, but not significant. No alterations of nociceptive response were found in all the groups: the minimal current required to elicit flinching/running, jumping, or vocalization was same in all the groups (data not shown). As galantamine (1 and 3 mg/kg) significantly improved the $A\beta_{25-35}$-induced cognitive impairment in dose-dependent manner, the dose of 3 mg/kg was used in subsequent experiments.

Antagonistic Effects of Mecamylamine, an nAChR Antagonist, on the Cognitive Improving Effects of Galantamine in the $A\beta_{25-35}$-Injected Mice

To determine whether the improving effects of galantamine on $A\beta_{25-35}$-induced cognitive impairments are mediated via nAChRs, we examined the antagonism by mecamylamine, an nAChRs antagonist, against the cognitive improving effects of galantamine in $A\beta_{25-35}$-injected mice.

In the training session of the novel object recognition task or the preconditioning phase of cued/contextual fear-conditioning tasks, there were no differences in the exploratory preference for the objects or the basal levels of freezing response, respectively, among all the groups. Galantamine (3 mg/kg) improved cognitive dysfunction in novel object recognition and tone cue- and context-

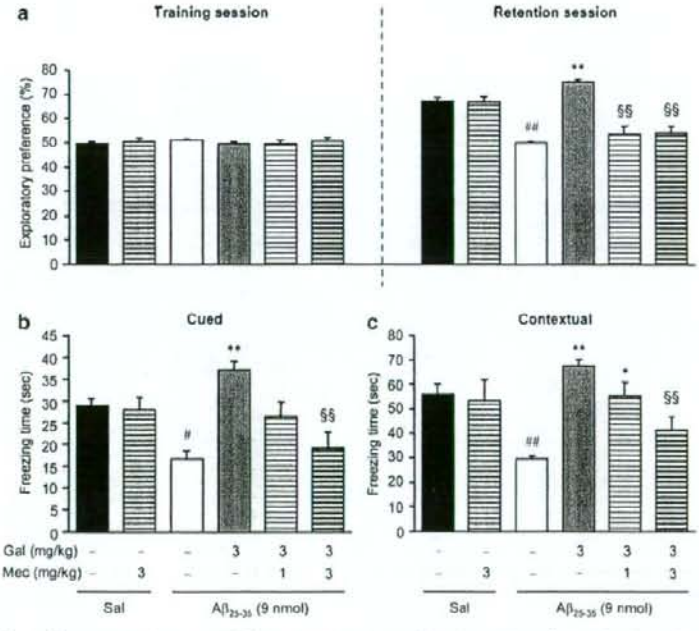

Figure 3 Antagonistic effects of mecamylamine, an nAChR antagonist, on cognitive improving effects of galantamine in $A\beta_{25-35}$-injected mice. Galantamine (3 mg/kg p.o.) and mecamylamine (Mec: 1 and 3 mg/kg) were administrated to saline- and $A\beta_{25-35}$ (9 nmol/3 μl)-injected mice 60 and 30 min, respectively, before the training session of novel object recognition task and the conditioning phase of cued and contextual fear-conditioning tasks. (a) Novel object recognition task. In training session, $F(5,63)=0.32$ ($p=0.90$). In retention session, $F(5,63)=25.83$ ($p<0.01$). (b) Cued conditioning task, $F(5,63)=7.73$ ($p<0.01$). (c) Contextual conditioning task, $F(5,63)=7.90$ ($p<0.01$). Results were expressed as means ± SEM ($n=8$–12), and analyzed by a one-way ANOVA, followed by Tukey–Kramer test for multiple comparisons. $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs* saline-treated, saline-injected mice; $^{**}p<0.01$ *vs* saline-treated, $A\beta_{25-35}$-injected mice; $^{\S\S}p<0.01$ *vs* galantamine-treated, $A\beta_{25-35}$-injected mice. Sal, saline; Gal, galantamine; Mec, mecamylamine.

conditioned fear-learning tasks in $A\beta_{25-35}$-injected mice. The nAChR antagonist mecamylamine (3 mg/kg) significantly and completely blocked the improving effects of galantamine on the impairment of recognition ($p<0.01$ by *post hoc*, Figure 3a) and cued/contextual-dependent fear learning ($p<0.01$ by *post hoc*, Figure 3b and c) in the $A\beta_{25-35}$-injected mice. In saline-injected mice, mecamylamine (3 mg/kg) by itself had no effect on the novel object recognition and cued/contextual fear-conditioning performances (Figure 3).

Effects of Scopolamine, a Muscarinic Receptor Antagonist, on the Cognitive Improving Effects of Galantamine in the $A\beta_{25-35}$-Injected Mice

To determine whether muscarinic receptors are involved in the effects of galantamine on the performance of $A\beta_{25-35}$-injected mice in the cognitive tasks, the muscarinic receptor antagonist, scopolamine (0.1 and 0.2 mg/kg), was s.c. injected to the mice 30 min after the p.o. administration of galantamine (3 mg/kg). Scopolamine at the dose of 0.2 mg/kg impaired the performance of saline-i.c.v.-injected mice in both novel object recognition and tone cue- and context-conditioned fear-learning tasks (Figure 4a–c). Galantamine (3 mg/kg) improved cognitive dysfunction in novel object recognition and tone cue- and context-conditioned fear-learning tasks in $A\beta_{25-35}$-injected mice. In the novel object recognition test, scopolamine (0.1 and 0.2 mg/kg) failed to prevent the effect of galantamine (3 mg/kg) (Figure 4a). In the cued and contextual conditioning tasks, the effects of galantamine (3 mg/kg) were not significantly prevented by scopolamine at the dosage that induces behavioral impairments in the task in saline-i.c.v.-injected mice (Figure 4b and c). In order to well understand the effects of scopolamine on the performance of mice in these tasks, we added the galantamine (3 mg/kg)-treated and galantamine (3 mg/kg)/scopolamine (0.2 mg/kg)-treated saline-i.c.v.-injected control groups. Scopolamine (0.2 mg/kg) failed to affect the performance in the novel object recognition task in galantamine-treated mice (Figure 4a). In the cued and contextual conditioning tasks, there is the tendency to inhibit the performance in galantamine (3 mg/kg)-treated mice by scopolamine (2 mg/kg), but not significant (Figure 4b and c). Although the effects of galantamine was not significantly prevented by scopolamine in the cued and contextual conditioning tasks, the performance of mice is more sensitive to scopolamine in this task than in the novel object recognition task (Figure 4a–c).

Effects of Galantamine on the Extracellular Dopamine Level in the Hippocampus of the Saline- and $A\beta_{25-35}$-Injected Mice

We examined whether galantamine at the dose of 3 mg/kg, which ameliorated the cognitive dysfunction in the $A\beta_{25-35}$-injected mice, facilitated the dopamine release in the hippocampus of saline- and $A\beta_{25-35}$-injected mice.

As shown in Figure 5a, the basal extracellular level of dopamine in the hippocampus of $A\beta_{25-35}$-injected mice

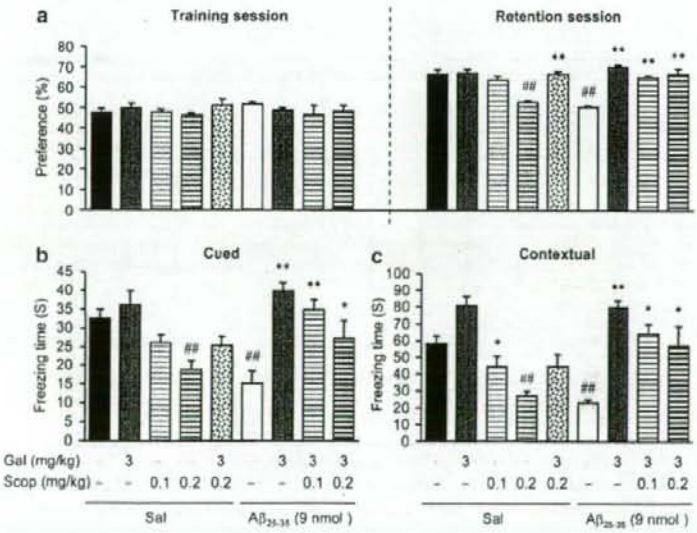

Figure 4 Effects of scopolamine, a muscarinic antagonist, on cognitive improving effects of galantamine in $A\beta_{25-35}$-injected mice. Galantamine (Gal: 3 mg/kg p.o.) and scopolamine (Scop: 0.1 and 0.2 mg/kg) were administrated to saline- and $A\beta_{25-35}$ (9 nmol/3 μl)-injected mice 60 and 30 min, respectively, before the training session of novel object recognition task and the conditioning phase of cued and contextual fear-conditioning tasks. (a) Novel object recognition task. In training session, $F(5,63)=0.32$ ($p=0.90$). In retention session, $F(8,90)=60.10$ ($p<0.01$). (b) Cued conditioning task, $F(8,90)=5.33$ ($p<0.01$). (c) Contextual conditioning task, $F(8,90)=8.04$ ($p<0.01$). Results were expressed as means ± SEM ($n=8$–12), and analyzed by a one-way ANOVA, followed by Tukey–Kramer test for multiple comparisons. $^{\#\#}p<0.01$ *vs* saline-treated saline-injected mice; $^{*}p<0.05$, $^{**}p<0.01$ *vs* saline-treated, $A\beta_{25-35}$-injected mice. Sal, saline; Gal, galantamine; Scop, scopolamine.

were significantly decreased compared to that of saline-injected mice. Galantamine (3 mg/kg) caused a marked increase in the extracellular level of dopamine in the hippocampus of saline- (Figure 5b) and $A\beta_{25-35}$-injected mice (Figure 5c). The significant increase in the extracellular level of dopamine was observed from about 20 min after galantamine administration ($p<0.01$ by *post hoc*, Figure 5b and c). When mecamylamine (3 mg/kg) was injected to saline- and $A\beta_{25-35}$-injected mice 30 min after galantamine administration, the galantamine-induced elevation of extracellular dopamine levels was significantly diminished (Figure 5b and c). However, mecamylamine by itself did not significantly affect the extracellular dopamine levels in the saline- and $A\beta_{25-35}$-injected mice (Figure 5b and c).

To confirm that dopamine release is facilitated through nAChR stimulation by galantamine, we measured extracellular dopamine level induced by nicotine in combination with galantamine in the hippocampus of $A\beta_{25-35}$-injected mice. The individual administration with nicotine at the dose of 0.4 mg/kg or galantamine at the dose of 1 mg/kg does not affect the extracellular level of dopamine in the hippocampus of $A\beta_{25-35}$-injected mice, However, the combination of nicotine (0.4 mg/kg) with galantamine (1 mg/kg) significantly increased the extracellular level of dopamine in the hippocampus of $A\beta_{25-35}$-injected mice (Figure 6). The potentiating effect of galantamine on dopamine release was antagonized by mecamylamine (3 mg/kg) administration (Figure 6). The synergistic effects of nicotine and galantamine at low doses, and the fact that

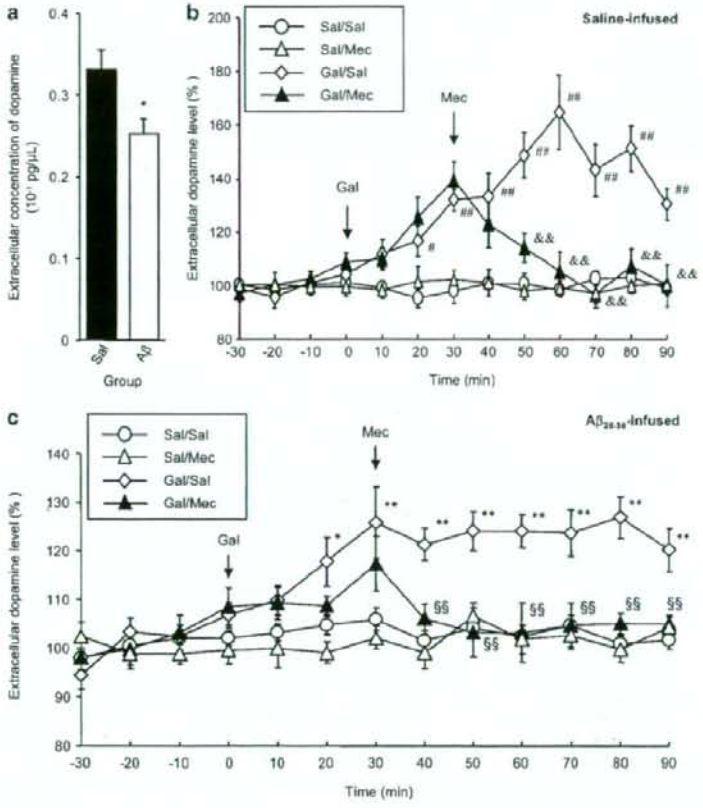

Figure 5 Effects of galantamine on extracellular dopamine level in the hippocampus of saline- and $A\beta_{25-35}$-injected mice. At 8 or 9 days after $A\beta_{25-35}$ infusion (24 h after the implantation of the guide cannula), the dialysis probe was implanted into the hippocampus. Saline- and $A\beta_{25-35}$-injected mice were treated with galantamine (Gal: 3 mg/kg p.o.) and/or mecamylamine (Mec: 3 mg/kg s.c.), and dialysates were collected every 10 min for 90 min. Dopamine levels in the dialysates were assayed by HPLC with electrochemical detection. (a) Spontaneous extracellular dopamine levels in the hippocampus of saline- and $A\beta_{25-35}$-injected mice. Results were expressed as means ± SEM, $n=8$. $^{*}p<0.05$ *vs* saline-i.c.v.-injected mice, by Student's *t*-test. (b) Effects of galantamine on extracellular dopamine level in the hippocampus of saline-injected mice. Results were expressed as means ± SEM, $n=5$–8, and analyzed by a two-way ANOVA, followed by Tukey–Kramer test for multiple comparisons, $F_{time}(12,259)=3.74$ ($p<0.01$); $F_{group}(3,259)=37.71$ ($p<0.01$). (c) Effects of galantamine on the extracellular dopamine level in the hippocampus of $A\beta_{25-35}$-injected mice. Results were expressed as means ± SEM, $n=5$–8, and analyzed by a two-way ANOVA, followed by Tukey–Kramer test for multiple comparisons, $F_{time}(12,324)=5.79$ ($p<0.01$); $F_{group}(3,324)=50.62$ ($p<0.01$). $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs* saline-treated saline-injected mice. $^{\&\&}p<0.01$ *vs* galantamine-treated saline-injected mice. $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs* saline-treated, $A\beta_{25-35}$-injected mice; $^{\S\S}p<0.01$ *vs* galantamine-treated, $A\beta_{25-35}$-injected mice. Sal, saline; Gal, galantamine; Mac, mecamylamine.

the synergy was antagonized by mecamylamine, indicated that galantamine indeed potentiates an nAChR-mediated effect.

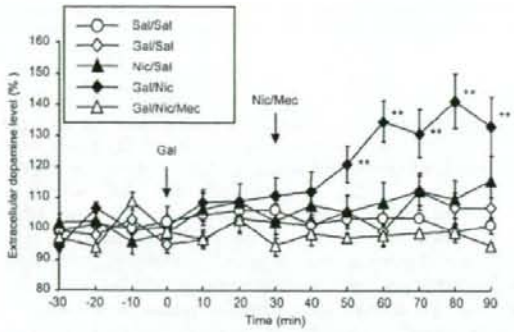

Figure 6 Effects of combined treatment with nicotine and galantamine at their non-effective doses on extracellular dopamine level in the hippocampus of Aβ_{25-35}-injected mice. Aβ_{25-35}-injected mice were administered nicotine (Nic: 0.4 mg/kg s.c.) and mecamylamine (Mec: 3 mg/kg s.c.) 30 min after galantamine (Gal: 1 mg/kg, p.o.) treatment, and dialysates were collected every 10 min for 90 min. Results were expressed as means ± SEM, $n = 5–8$, and analyzed by a two-way ANOVA, followed by Tukey-Kramer test for multiple comparisons, $F_{time}(12,376) = 4.56$ ($p < 0.01$); $F_{group}(3,376) = 22.71$ ($p < 0.01$). **$p < 0.01$ *vs* saline-treated Aβ_{25-35}-injected mice.

Involvement of Dopaminergic Systems in the Cognitive Improving Effects of Galantamine in Aβ_{25-35}-Injected Mice

To clarify whether the improving effects of galantamine on Aβ_{25-35}-induced cognitive impairments are mediated through the activation of dopamine receptors, we investigated the antagonism of the cognitive improving effects of galantamine on Aβ_{25-35}-injected mice by SCH-23390, a dopamine-D1 receptor antagonist, and sulpiride, a dopamine-D2 receptor antagonist.

SCH-23390 (0.02 mg/kg) and sulpiride (12.5 mg/kg) significantly and completely antagonized the improving effects of galantamine on Aβ_{25-35}-induced cognitive impairment without affecting the exploratory preference for the objects in the training session of the novel object recognition task. SCH-23390 and sulpiride showed no effect on the total exploration time in either the training or retention sessions of the novel object recognition task. In addition, SCH-23390 and sulpiride by themselves had no effect on novel object recognition performance in saline-injected mice (Figure 7a).

SCH-23390 (0.02 mg/kg) and sulpiride (12.5 mg/kg) significantly blocked the ameliorating effects of galantamine on the impairments of both cued and contextual fear conditioning induced by infusion of Aβ_{25-35}. SCH-23390 and sulpiride by themselves had no effects on the cued and contextual freezing response in saline-injected mice (Figure 7b and c).

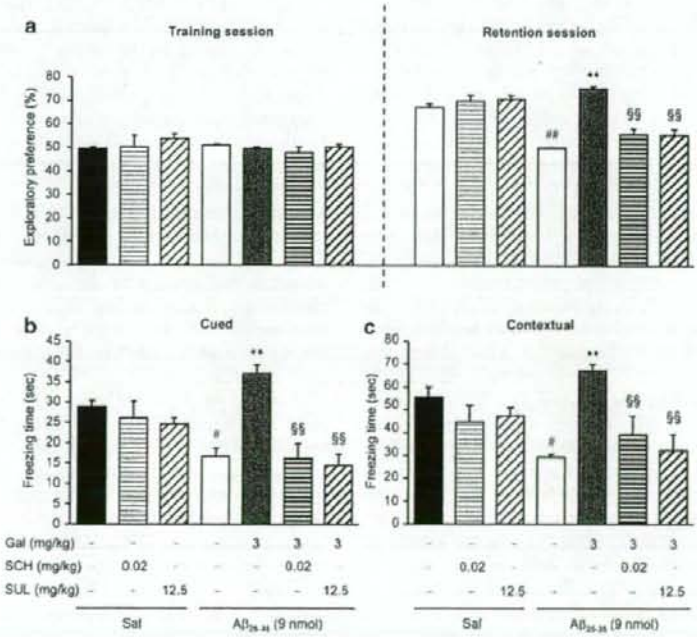

Figure 7 Involvement of dopaminergic systems in the cognitive improving effects of galantamine in Aβ_{25-35}-injected mice. Galantamine (p.o.), SCH-23390 (s.c.) and sulpiride (s.c.) were administrated to saline- and Aβ_{25-35} (9 nmol/3 μl)-injected mice 60, 30 and 60 min respectively before the training session of novel object recognition task, and the conditioning phase of cued and contextual fear-conditioning task. (a) Novel-object recognition task. In training session, $F(6,72) = 0.73$ ($p = 0.63$). In retention session, $F(6,72) = 27.13$ ($p < 0.01$). (b) Cued conditioning task. $F(6,72) = 9.61$ ($p < 0.01$). (c) Contextual conditioning task. $F(6,72) = 6.36$ ($p < 0.01$). Results were expressed as means ± SEM, $n = 8–12$, and analyzed by a one-way ANOVA, followed by Tukey-Kramer test for multiple comparisons. #$p < 0.05$, ##$p < 0.01$ *vs* saline-treated saline-injected mice; **$p < 0.01$ *vs* saline-treated Aβ_{25-35}-injected mice; §§$p < 0.01$ *vs* galantamine-treated Aβ_{25-35}-injected mice. Sal: saline, Gal: galantamine, SCH: SCH-23390, SUL: sulpiride.

DISCUSSION

We found that $A\beta_{25-35}$ infusion impaired novelty-discriminating ability in the novel object recognition task and associative learning and memory in the fear-conditioning tasks. It is unlikely that the impairment of performance of the $A\beta_{25-35}$-injected mice in these tasks is due to changes in motivation or sensorimotor function, as various motivations are involved in these behavioral tasks, and different skills are required for better performance in each task. Actually, there was no difference of the total exploration time in the training session of novel object recognition task and freezing response in preconditioning phase of cued and contextual fear-conditioning tasks between the saline- and $A\beta_{25-35}$-injected mice, indicating no changes in motor function and exploratory activity. In addition, no difference in pain threshold was found between the saline- and $A\beta_{25-35}$-injected mice. Therefore, the impairment of performance in the $A\beta_{25-35}$-injected mice is due to learning and memory deficits.

Galantamine, a medication for Alzheimer's disease, has a dual mechanism of action, which inhibits acetylcholinesterase and allosterically modulates nAChR as a potent APL (Eisele *et al*, 1993; Santos *et al*, 2002). Previous paper has reported that galantamine reverses nAChR antagonist-induced deficits in delay classical conditioning of the eye blink reflex in young rabbits (Woodruff-Pak *et al*, 2003) and impairment of spatial accuracy of APP23 transgenic mouse during probe trial of Morris water maze (Van Dam and De Deyn, 2006). In the present study, galantamine significantly ameliorated the cognitive impairments induced by $A\beta_{25-35}$ infusion in the novel object recognition and fear-conditioning tasks. Galantamine at 3 mg/kg had no effect on the total exploration time in the training session of novel object recognition task and freezing response in preconditioning phase of cued and contextual fear-conditioning tasks, and sensitivity to electric footshock in the fear-conditioning phase of cued and contextual fear-conditioning tasks in the $A\beta_{25-35}$-injected mice. Therefore, it is unlikely that the observed improvement of performance by galantamine in both tasks is due to changes in sensorimotor function and/or motivation in the $A\beta_{25-35}$-injected mice, and it is apparently valid that galantamine ameliorates learning and memory deficits caused by the infusion of $A\beta_{25-35}$ into the cerebral ventricle in mice. The improving effects of galantamine on the performance of $A\beta_{25-35}$-injected mice were prevented by the treatment with mecamylamine, an nAChR antagonist, at the dose that did not significantly affect the performance of saline-i.c.v.-injected mice. These findings support the notion that galantamine improves $A\beta_{25-35}$-induced cognitive impairment via activation of nAChRs. The roles of muscarinic receptors in the effects of galantamine were also investigated in the present study. The effects of galantamine on the performance of $A\beta_{25-35}$-injected mice in the novel object recognition task were not prevented by scopolamine at the dose which impaired the performance of saline-i.c.v.-injected mice. This indicated that muscarinic receptors are not very important for effects of galantamine on this cognitive task. Our conclusion is supported by the reports that there is only 1–12% brain AChE inhibition 1 h after s.c. injection of 3 mg/kg galantamine (Geerts *et al*, 2005), and that galantamine is an nAChR-allosteric modulator (Eisele *et al*, 1993; Santos *et al*, 2002). In other words, although the brain concentration of acetylcholine is only weakly increased by galantamine at the dose of 3 mg/kg, it shows effects mainly by allosterically modulating the function of nAChR. Our results also indicated that the potentiation of the nAChR function can compensate the hypofunction of muscarinic receptors in the present cognitive tasks, especially in the novel object recognition task. As scopolamine at a dose of 0.2 mg/kg showed some effect, but not significant, on the performance of galantamine-treated mice in the cued and contextual conditioning tasks, it was indicated that muscarinic receptors may differentially regulate the observed effects of galantamine, depending on specific behavioral tasks. Although mecamylamine blocks the effects of galantamine more strongly than scopolamine, it is hard to entirely exclude the role of muscarinic receptors in the effects of galantamine, as nAChR antagonists not only block the function of nAChRs but also eliminate the desensitization of nAChR-induced increase of the function of muscarinic receptors (Wan *et al*, 2003).

The mechanism of learning and memory impairments in the $A\beta_{25-35}$-injected mice is still not clear. Harkany *et al* (1998) have shown that bilateral injection of $A\beta[Phe(SO_3H)^{24}]_{25-35}$ peptide, a metabolically stable analog of $A\beta_{25-35}$, into the rat nucleus basalis magnocellularis causes a reduction of cortical acetylcholinesterase-positive projections. In PC12 cells, $A\beta$ has been found to suppress the expression of nAChRs, such as the decrease of nAChR-binding sites, subunit proteins, and mRNA levels (Guan *et al*, 2001, 2003). In the present study, *in vivo* microdialysis experiment revealed that the basal extracellular level of dopamine in the hippocampus of $A\beta_{25-35}$-injected mice was decreased compared to that of saline-injected mice. Furthermore, we have previously demonstrated by *in vivo* microdialysis that continuous infusion of $A\beta_{1-40}$ markedly decreased high potassium- and nicotine-induced release of acetylcholine and dopamine in the hippocampus, cerebral cortex, and striatum, respectively (Itoh *et al*, 1996). These findings confirmed that the deposition of $A\beta$ in the brain is in some way related to the impairment of cognition and cholinergic–dopaminergic degeneration and suggest that dysfunctions of cholinergic and dopaminergic systems are responsible, at least in part for the $A\beta$-induced learning and memory deficits.

It has been reported that galantamine (10 μM, 4 days) dose not significantly affect the mRNA level and protein expression of nAChR subunits (Kume *et al*, 2005). Galantamine increases cholinergic function mainly in two ways: (a) increasing the concentration of acetylcholine through a competitive reversible inhibition of acetylcholine hydrolysis by acetylcholinesterase, which will increase the extracellular acetylcholine concentration and (b) allosteric modulation of nAChRs (Woodruff-Pak and Santos, 2000). The potential cognitive improving effects of galantamine on Alzheimer's disease may be related, in part, to the stimulation of dopamine neurotransmission in addition to its enhancing effects on cholinergic systems by inhibition of acetylcholinesterase. The *in vivo* microdialysis experiment showed that galantamine significantly increased the dopamine release in the hippocampus of saline- and $A\beta_{25-35}$-injected mice. The effect of nicotine on dopamine release