continued drinking water for more than 5 s, and there was no difference in drinking behavior among the groups.

Impairment of Learning-Associated CaMKII Activation in the Prefrontal Cortex on Repeated PCP Treatment in the Water-Finding Test. Because the NMDA/CaMKII signaling pathway plays an important role in learning and memory (Cammarota et al., 2002), we examined the learning-associated activation of CaMKII (i.e., phosphorylation of threonine 286 of the α-subunit; CaMKII phosphorylation) in the prefrontal cortex after the training trial for latent learning. Levels of phosphorylated CaMKII in the prefrontal cortex of the saline-treated mice (n = 8) were significantly increased immediately after the training trial, compared with those in the nontrained, saline-treated mice (n = 8) ($p < 0.01$; Fig. 3). However, they did not increase in the trained, PCP-treated mice (n = 8) above the basal level on exposure to the apparatus (n = 7) (p = 0.99, Fig. 3), and were significantly lower than those of the trained, saline-treated mice ($p < 0.01$, Fig. 3).

Impairment of Latent Learning by a CaMKII Inhibitor in the Water-Finding Test. To examine the relationship between the activation of CaMKII and latent leaning, we evaluated the effect of a CaMKII inhibitor on water-finding performance in mice. The mice microinjected with KN93, a CaMKII inhibitor, at 10 (n = 13) but not 1 (n = 10) nmol/mouse, bilaterally, into the prefrontal cortex before the training trial showed a significantly prolonged finding latency in the test trial, compared with the mice treated with vehicle (n = 10) (10 nmol of KN93; $p < 0.01$, 1 nmol of KN93; p = 0.26, Fig. 4A) or KN92 (n = 13; 10 nmol/mouse bilaterally), an inactive inhibitor (10 nmol of KN93; $p < 0.01$; 1 nmol of KN93, p = 0.89; Fig. 4A). State-dependent learning denotes the fact that information that has been learned while an animal is under the influence of a certain drug (state) can only be recalled when the animal is in the same state in which the information was learned, not in a different (i.e., undrugged) state (Zarrindast et al., 2006). The mice infused with 10 nmol of KN93 before both the training and test trials showed a significantly prolonged finding latency in the test trial compared with the mice treated with vehicle (data not shown). This prolonged finding latency was not different from that of the mice infused with KN93 only before the training trial (data not shown). It was suggested that there were no state-dependent effects of KN93 on latent learning in the water-finding task. Treatment with 10 nmol of KN93 (n = 7) significantly decreased the level of phosphorylated CaMKII after exposure to the apparatus, compared with the mice treated with vehicle (n = 7) ($p < 0.01$; Fig. 4B) or KN92 (n = 7) ($p < 0.01$; Fig. 4B).

Impairment by Repeated PCP Treatment of CaMKII Activation through NMDA Receptor Stimulation in Slices of the Prefrontal Cortex. To confirm that the activation of CaMKII is facilitated after stimulation of the NMDA receptor, we measured the amount of phosphorylated CaMKII in slices of the prefrontal cortex stimulated with NMDA (100 μM). Under our experimental conditions, an increase in phosphorylated CaMKII (n = 6) was detected 5 min after the stimulation compared with the basal level (n = 6) (without stimulation) in the prefrontal cortex prepared from the saline-treated mice ($p < 0.05$, Fig. 5). In the prefrontal cortex of the PCP-treated mice, however, stimulation with NMDA (n = 6) did not increase the level of phosphorylated CaMKII, which was significantly lower than that of the saline-treated mice ($p < 0.05$, Fig. 5).

Microinjection of Glycine into the Prefrontal Cortex Reversed the Impairment of Latent Learning and of Learning-Associated CaMKII Phosphorylation in the PCP-Treated Mice. Glycine is known as an agonist of glycine sites that stimulates NMDA receptors (Johnson and Ascher, 1987) and improves the symptoms of schizophrenia (Coyle and Tsai, 2004). Therefore, we investigated the effects of infusing glycine into the prefrontal cortex on the PCP-induced impairment of latent learning and CaMKII's activation. Glycine (1 μmol/mouse, bilaterally) treatment (n = 9) significantly shortened the prolonged finding latency induced by repeated PCP treatment (n = 10) ($p < 0.05$; Fig. 6A). In the Western blot analysis, the bilateral injection of glycine (n = 12) significantly increased the amount of phosphorylated CaMKII in the prefrontal cortex immediately after training in the PCP-treated mice (n = 12) ($p < 0.01$; Fig. 6B). The bilateral injection into the prefrontal cortex failed to affect the finding latency (n = 7–9) (p = 0.33; Supplemental Data 1A) and the learning-associated phosphorylation of CaMKII in the saline-treated mice (n = 8–10) (p = 0.35; Supplemental Data 1B).

Changes in the Phosphorylation and Expression of NMDA Receptor NR1 Subunits in the Prefrontal Cortex on Repeated PCP Treatment. To investigate whether the impairment of CaMKII's activation in the PCP-treated mice is dependent on a malfunction of NMDA receptors, the expression and activation of NR1 were examined. The NR1 expression level in the total tissue ex-

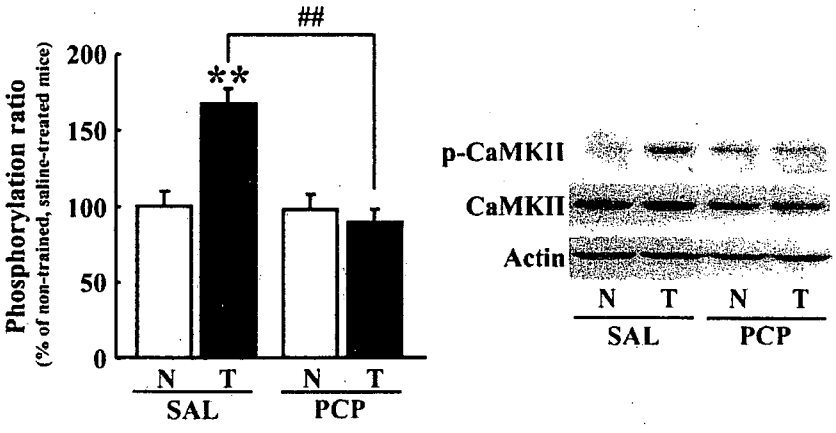

Fig. 3. Impairment of learning-associated CaMKII activation in the prefrontal cortex on repeated PCP treatment in the water-finding test. A training trial was performed 4 days after cessation of the repeated PCP treatment (10 mg/kg s.c. once a day for 14 days). Immediately after the training trial, mice were sacrificed by decapitation and CaMKII phosphorylation (Thr286; p-CaMKII) and αCaMKII expression (CaMKII) in the prefrontal cortex were detected by Western blotting. Loaded protein was normalized to actin. The phosphorylation ratio was calculated as CaMKII phosphorylation versus CaMKII expression. Each column represents the mean ± S.E.M. (n = 7–8). Results with the one-way ANOVA were: $F_{3,27}$ = 13.11; $p < 0.01$. **, $p < 0.01$ compared with corresponding nontrained mice. ##, $p < 0.01$ compared with trained, saline-treated mice. N, nontrained mice; T, trained mice; SAL, saline.

tracts in the prefrontal cortex was significantly increased in the PCP-treated mice (n = 6) compared with the saline-treated mice (n = 6) ($p < 0.01$, Fig. 7B), whereas there was no significant difference in the NR1 expression level in the membrane-enriched extracts between the saline-treated (n = 8) and PCP-treated (n = 7) mice (p = 0.61; Fig. 7E). There was no significant difference in the level of phosphorylated NR1 (Ser^{897}) in the total tissue extracts between the saline-treated (n = 6) and PCP-treated mice (n = 6) (p = 0.08; Fig. 7A), whereas the level in the membrane-enriched extracts was significantly decreased in the PCP-treated mice (n = 7) compared with the saline-treated mice (n = 8) ($p < 0.01$, Fig. 7D). Thus, the NR1 phosphorylation ratio in total tissue and membrane-enriched extracts was significantly decreased in the PCP-treated mice compared with the saline-treated mice ($p < 0.01$, n = 6, Fig. 7C, $p < 0.01$, n = 7–8, Fig. 7F). A single PCP treatment affected neither the phosphorylation, the expression, nor the phosphorylation ratio of NR1 (data not shown).

Infusion of NR1 Antisense Oligonucleotide into the Prefrontal Cortex Impaired the Latent Learning and Learning-Associated Phosphorylation of CaMKII in the Water-Finding Test. We examined the role of the prefrontal cortical NR1 subunit in the latent learning of the water-finding task and relation to the learning-associated phosphorylation of CaMKII because the repeated PCP treatment impaired latent learning and learning-associated

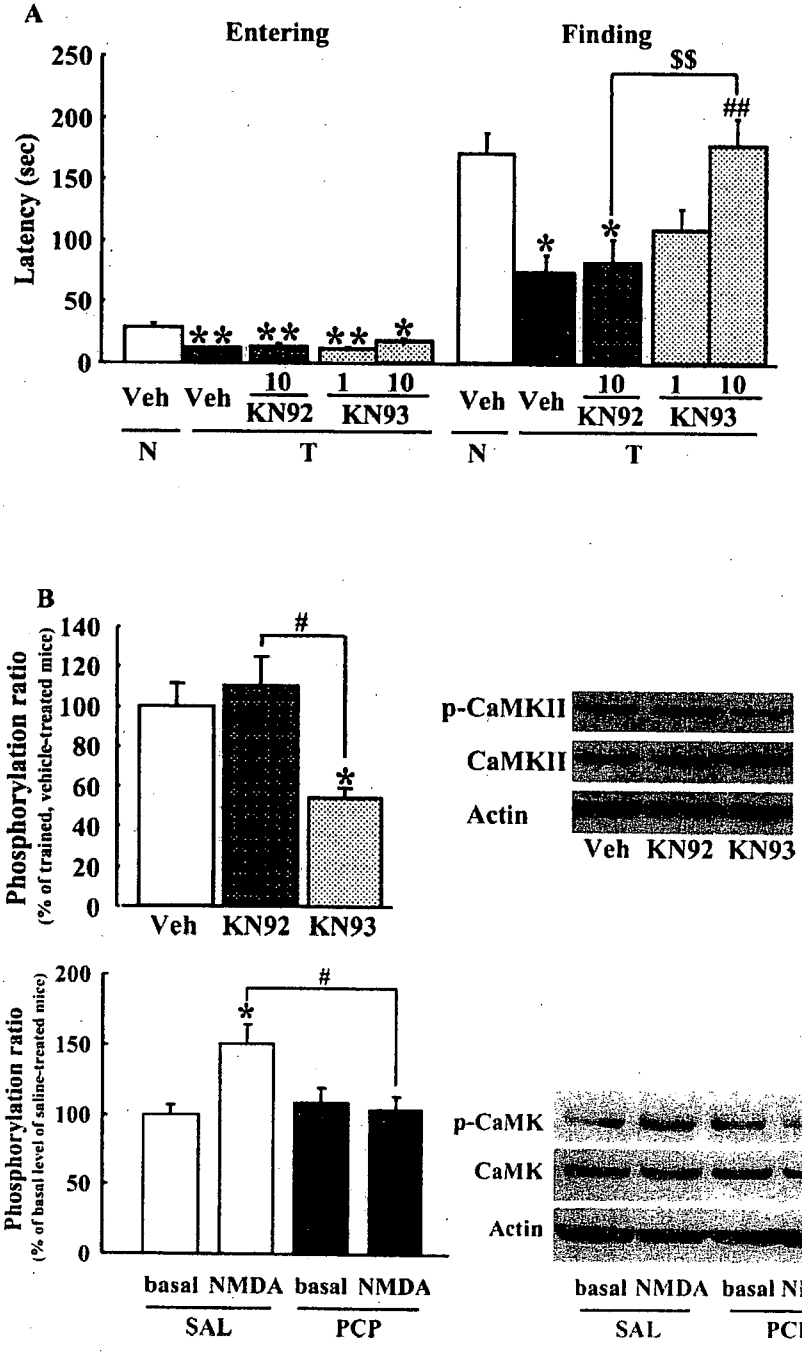

Fig. 4. Impairment of latent learning by a CaMKII inhibitor in the water-finding test. A, effect of the infusion of a CaMKII inhibitor into the prefrontal cortex on latent learning. Mice were administered a CaMKII inhibitor (KN93; 1 and 10 nmol/mouse bilaterally) 10 min before the training trial. The entering and finding latencies were measured in the test trial 24 h after the training trial of the water-finding task. Each column represents the mean ± S.E.M. (n = 10–13). Results with the one-way ANOVA were: entering latency, $F_{4,51}$ = 66.43, $p < 0.01$; finding latency, $F_{4,51}$ = 7.59, $p < 0.01$. **, $p < 0.01$; *, $p < 0.05$ compared with the nontrained, vehicle-treated mice. ##, $p < 0.01$ compared with the trained, vehicle-treated mice. $$, $p < 0.01$ compared with trained, inactive CaMKII inhibitor (KN92; 10 nmol)-treated mice. B, effect of the infusion of a CaMKII inhibitor into the prefrontal cortex on the learning-associated phosphorylation of CaMKII. Mice were administered KN93 (10 nmol) 10 min before the training trial. Immediately after the training trial, mice were sacrificed by decapitation and CaMKII phosphorylation (Thr286; p-CaMKII) and αCaMKII expression (CaMKII) in the prefrontal cortex were detected by Western blotting. Loaded protein was normalized to actin. The phosphorylation ratio was calculated as CaMKII phosphorylation versus CaMKII expression. Each column represents the mean ± S.E.M. (n = 7). Results with the one-way ANOVA were: $F_{2,18}$ = 8.10; $p < 0.01$. *, $p < 0.05$ compared with the trained, vehicle-treated mice. #, $p < 0.05$ compared with the trained, KN92 (10 nmol)-treated mice. N, nontrained mice; T, trained mice; SAL, saline; Veh, vehicle.

Fig. 5. Impairment by repeated PCP treatment of CaMKII activation through NMDA receptor stimulation in slices of the prefrontal cortex. Slices of the prefrontal cortex were incubated in the absence or presence of 100 μM NMDA for 5 min. NMDA-stimulated phosphorylation of αCaMKII (Thr286; p-CaMKII) was detected by Western blotting. Loaded protein was normalized to actin. The phosphorylation ratio was calculated as CaMKII phosphorylation versus CaMKII expression. Each column represents the mean ± S.E.M. (n = 6). Results with the one-way ANOVA were: $F_{3,20}$ = 4.80; $p < 0.05$. *, $p < 0.05$ compared with corresponding non-stimulated group. #, $p < 0.05$ compared with the saline-treated mice stimulated with NMDA. SAL, saline.

NMDA-CaMKII signaling through a malfunction of NR1. The performance in the water-finding test of mice that received the antisense or sense NR1 oligonucleotide in the prefrontal cortex is shown in Fig. 8A. Treatment with the antisense oligonucleotide (n = 9) significantly prolonged the finding latency compared with treatment with the sense oligonucleotide (n = 9) ($p < 0.05$; Fig. 8A). Infusion of the antisense (n = 7), but not sense (n = 7), oligonucleotide into the prefrontal cortex markedly reduced NR1 expression levels in the prefrontal cortex ($p < 0.01$, Fig. 8B) but not in other areas of the brain (hippocampus; p = 0.96, n = 7, Supplemental Data 2A, striatum; p = 0.97, n = 7, Supplemental Data 2B). It is noteworthy that the latent learning-associated phosphorylation of CaMKII was significantly decreased by treatment with the antisense NR1 oligonucleotide (n = 7), whereas the sense oligonucleotide (n = 7) had no effect ($p < 0.01$, n = 7; Fig. 8C).

Decrease of High Potassium- and PCP-Induced Dopamine Release from the Prefrontal Cortex in the Mice Treated Repeatedly with PCP. A hypofunctioning dopaminergic neuronal system in the prefrontal cortex is one of the causes of schizophrenia, and the dopaminergic neuronal system plays an important role in memory (Winterer and Weinberger, 2004). Therefore, changes in the amount of dopamine released in the prefrontal cortex were investigated by microdialysis in the PCP-treated mice. The amount of dopamine released in response to high potassium (50 mM) in the prefrontal cortex was significantly lower in the PCP-treated mice (n = 6) than in the saline-treated mice (n = 5) ($p < 0.05$, Fig. 9A). To clarify the cause of the reduced sensitivity to the potassium-evoked release in the PCP-treated mice, we investigated the release evoked by PCP (10 mg/kg s.c) in the prefrontal cortex. The administration of PCP significantly increased the amount of dopamine release in the mice treated repeatedly with saline (n = 6) but not in the mice treated repeatedly with PCP (n = 6) ($p < 0.01$, Fig. 9B).

Infusion of a Dopamine-D1 Receptor Agonist into the Prefrontal Cortex Rescued Impairment of Latent Learning and Learning-Associated Phosphorylation of NR1 Induced by Repeated PCP Treatment. In the next experiment, we investigated whether the infusion of a dopamine D1 receptor agonist reverses the hypofunctioning of the glutamatergic neuronal system in the prefrontal cortex and impairment of latent learning in the PCP-treated mice. Infusion of the agonist SKF81297 (n = 8) (10 nmol/mouse bilaterally) into the prefrontal cortex significantly shortened the prolonged finding latency in the PCP-treated mice (n = 9) ($p < 0.05$; Fig. 10A). This dose of SKF81297 (n = 8) failed to affect the finding latency of the saline-treated mice (n = 9) (p = 0.96, Supplemental Data 3A). The decrease in the learning-associated NR1 phosphorylation ratio in the PCP-treated mice (n = 8) was also reversed by the local infusion of SKF81297 (n = 7) into the prefrontal cortex ($p < 0.05$; Fig. 10B). The infusion of SKF81297 (n = 6) into the prefrontal cortex also elevated levels of phosphorylated NR1 in the saline-treated mice (n = 6) ($p < 0.01$, Supplemental Data 3B).

Discussion

In the present study, we investigated whether repeated PCP treatment causes an impairment of latent learning via a malfunction of dopaminergic-glutamatergic signaling in a

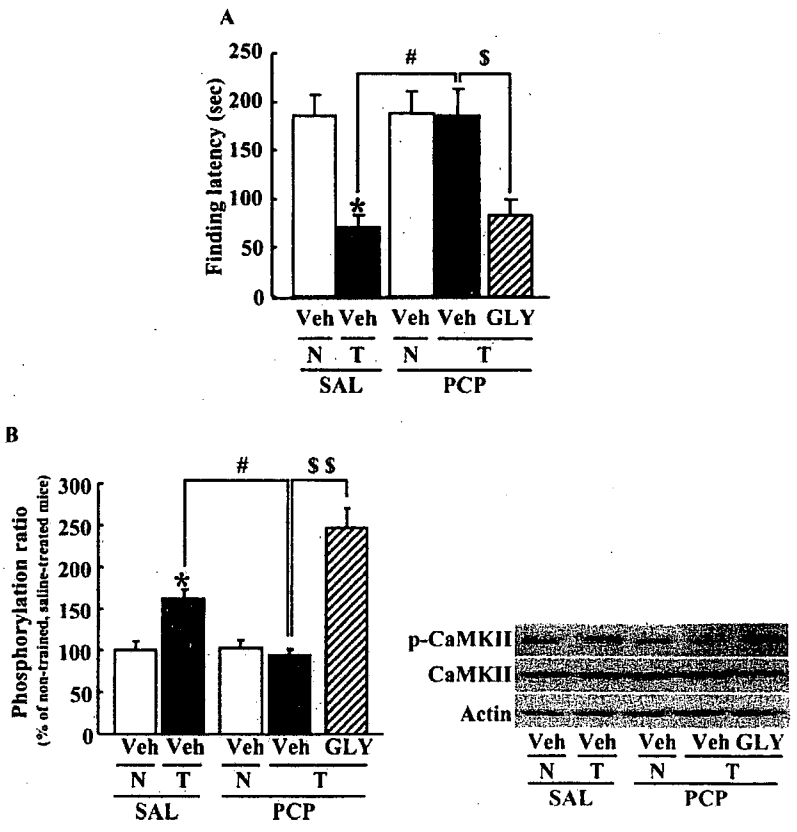

Fig. 6. Microinjection of glycine into the prefrontal cortex reversed the impairment of latent learning and of learning-associated CaMKII phosphorylation in the PCP-treated mice. A, effect of infusing glycine into the prefrontal cortex on the impairment of latent learning induced by repeated PCP treatment. The mice treated repeatedly with PCP (10 mg/kg s.c. once a day for 14 days; withdrawal 4 days) were administered glycine (1 μmol/mouse, bilaterally) 10 min before the training trial of the water-finding test. The test trial was performed 1 day after the training trial. Each column represents the mean ± S.E.M. (n = 9–10). Results with the one-way ANOVA were: $F_{4,44} = 8.12$; $p < 0.01$. *, $p < 0.05$ compared with the corresponding nontrained mice. #, $p < 0.05$ compared with the trained, saline-treated mice. $, $p < 0.05$ compared with the trained, PCP-treated mice. B, effect of infusing glycine into the prefrontal cortex on the impairment of learning-associated CaMKII phosphorylation in the prefrontal cortices of mice treated repeatedly with PCP. Repeated PCP-treated (10 mg/kg s.c. once a day for 14 days; withdrawal 4 days) mice were administered glycine (1 μmol/mouse, bilaterally) 10 min before the training trial of the water-finding test. Immediately after the training trial, mice were sacrificed by decapitation and CaMKII phosphorylation (Thr286; p-CaMKII) and αCaMKII expression (CaMKII) in the prefrontal cortex were detected by Western blotting. Loaded protein was normalized to actin. The phosphorylation ratio was calculated as CaMKII phosphorylation versus CaMKII expression. Each column represents the mean ± S.E.M. (n = 11–12). Results with the one-way ANOVA were: $F_{4,54} = 21.47$, $p < 0.01$. *, $p < 0.05$ compared with corresponding nontrained, mice. #, $p < 0.05$ compared with the trained, saline-treated mice. $$, $p < 0.01$ compared with the trained, PCP-treated mice. N, nontrained mice; T, trained mice; SAL, saline; Veh, vehicle.

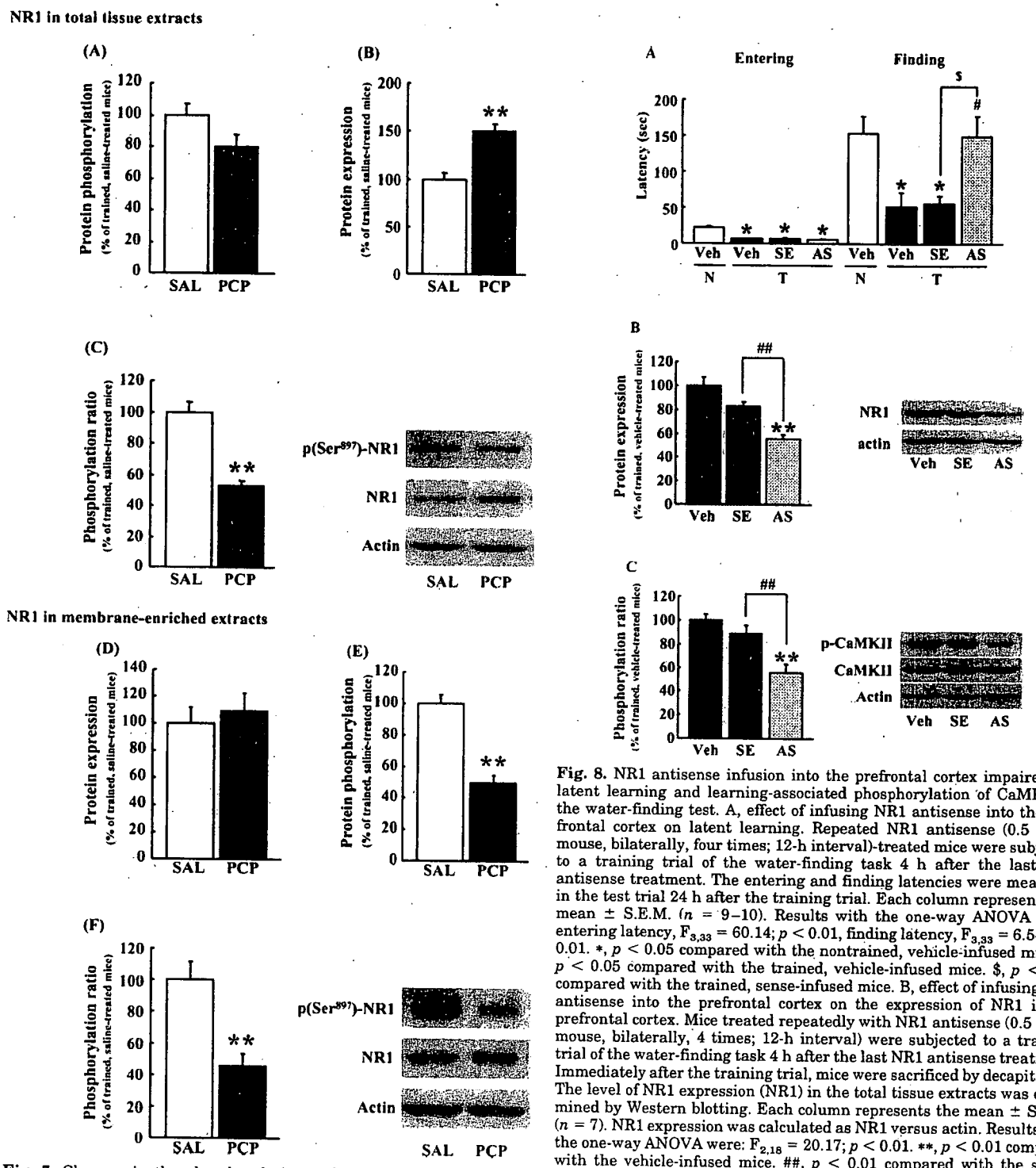

Fig. 7. Changes in the phosphorylation and expression of the NMDA receptor subunit NR1 in the prefrontal cortex on repeated PCP treatment. Immediately after the training trial, the mice treated repeatedly with PCP (10 mg/kg s.c. once a day for 14 days; withdrawal 4 days) were sacrificed by decapitation and NR1 phosphorylation (Ser^{897}; *p*-NR1) and NR1 expression (NR1) in the total tissue extracts (A–C) and membrane-enriched extracts (D–F) were detected by Western blotting. Loaded protein was normalized to actin. The phosphorylation ratio was calculated as NR1 phosphorylation versus NR1 expression. Each column represents the mean ± S.E.M. (A–C, $n = 6$; D–F, $n = 7$–8). Results are presented as the level of NR1 expression (A and D), level of NR1 phosphorylation (Ser897) (B and E), and the ratio of NR1 phosphorylation versus NR1 expression (C and F). **, $p < 0.01$ compared with the saline-treated mice (Student's *t* test). SAL, saline.

Fig. 8. NR1 antisense infusion into the prefrontal cortex impaired the latent learning and learning-associated phosphorylation of CaMKII in the water-finding test. A, effect of infusing NR1 antisense into the prefrontal cortex on latent learning. Repeated NR1 antisense (0.5 nmol/mouse, bilaterally, four times; 12-h interval)-treated mice were subjected to a training trial of the water-finding task 4 h after the last NR1 antisense treatment. The entering and finding latencies were measured in the test trial 24 h after the training trial. Each column represents the mean ± S.E.M. ($n = 9$–10). Results with the one-way ANOVA were: entering latency, $F_{3,33} = 60.14$; $p < 0.01$, finding latency, $F_{3,33} = 6.54$; $p < 0.01$. *, $p < 0.05$ compared with the nontrained, vehicle-infused mice. #, $p < 0.05$ compared with the trained, vehicle-infused mice. $, $p < 0.05$ compared with the trained, sense-infused mice. B, effect of infusing NR1 antisense into the prefrontal cortex on the expression of NR1 in the prefrontal cortex. Mice treated repeatedly with NR1 antisense (0.5 nmol/mouse, bilaterally, 4 times; 12-h interval) were subjected to a training trial of the water-finding task 4 h after the last NR1 antisense treatment. Immediately after the training trial, mice were sacrificed by decapitation. The level of NR1 expression (NR1) in the total tissue extracts was determined by Western blotting. Each column represents the mean ± S.E.M. ($n = 7$). NR1 expression was calculated as NR1 versus actin. Results with the one-way ANOVA were: $F_{2,18} = 20.17$; $p < 0.01$. **, $p < 0.01$ compared with the vehicle-infused mice. ##, $p < 0.01$ compared with the sense-infused mice. C, effect of infusing NR1 antisense into the prefrontal cortex on the learning-associated CaMKII phosphorylation. Mice treated repeatedly with NR1 antisense (0.5 nmol/mouse, bilaterally, 4 times; 12-h interval) were subjected to a training trial of the water-finding task 4 h after the last NR1 antisense treatment. Immediately after the training trial, mice were sacrificed by decapitation and CaMKII phosphorylation (Thr286; p-CaMKII) and αCaMKII expression (CaMKII) in the prefrontal cortex were detected by Western blotting. Loaded protein was normalized to actin. The phosphorylation ratio was calculated as CaMKII phosphorylation versus CaMKII expression. Each column represents the mean ± S.E.M. ($n = 7$). Results with the one-way ANOVA were: $F_{2,18} = 12.71$; $p < 0.01$. **, $p < 0.01$ compared with the vehicle-infused mice. ##, $p < 0.01$ compared with the sense-infused mice. N, nontrained mice; T, trained mice; Veh, vehicle; SE, sense; AS, antisense.

water-finding test after drug withdrawal. The mice treated repeatedly with saline took less time to find the water tube than did nontrained, saline-treated mice that were not exposed to the apparatus in a training trial. However, the PCP-treated mice had a prolonged finding latency, indicating an impairment of latent learning, in the water-finding task. Because their performance in the training trial and entering performance and drinking behavior in the test trial did not differ from that of saline-treated mice, it is unlikely that the impairment is attributable to altered anxiety processes (for example, avoidance of open fields), motor dysfunction, and/or thirst for water. The PCP-induced prolonged finding latency was attenuated by the infusion of glycine or the dopamine-D1 agonist into the prefrontal cortex only before the training trial, not before the test trial. It is suggested that repeated PCP treatment impaired latent learning (attention) of the location of the water tube in the training trial rather than a recall of memory in the test trial. The same dose of glycine or the dopamine-D1 agonist did not facilitate latent learning in the saline-treated mice, suggesting that the enhancement of the NMDA receptor and dopaminergic neuronal activities in saline-treated mice may have reached a ceiling as to acquired latent learning. The latent learning of PCP-treated mice in the water-finding task would make an excellent pharmacological model of schizophrenic cognitive dysfunction, which is related to latent learning (Exner et al., 2006) and attention (Nuechterlein and Dawson, 1984)

Accumulating evidence implicates the CaMKII pathway in cognitive functions such as learning and memory formation as well as in behavioral responses to NMDA receptor antagonists. For instance, the autophosphorylation of αCaMKII at Thr^{286} is critical for long-term potentiation and spatial memory (Giese et al., 1998) and fear memory in Pavlovian fear conditioning (Rodrigues et al., 2004). The infusion of a NMDA antagonist (2-amino-5-phosphonovalerate) into the

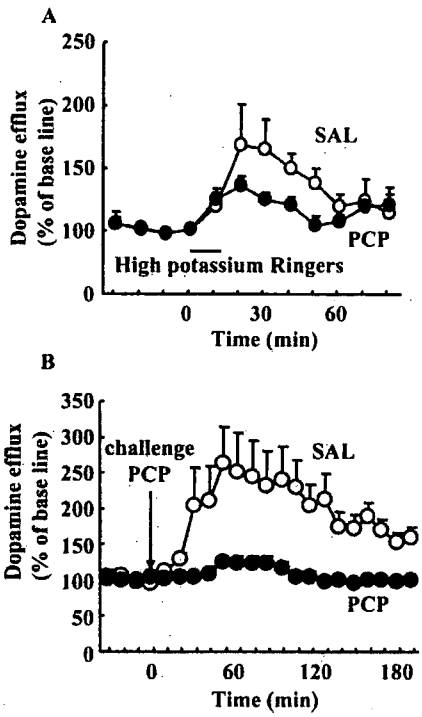

Fig. 9. Decrease of high potassium- and PCP-induced dopamine release from the prefrontal cortex in the mice treated repeatedly with PCP. A, high potassium-evoked dopamine release from the prefrontal cortex in the mice treated repeatedly with PCP. High potassium-induced dopamine release from the prefrontal cortex was measured in the mice treated repeatedly with PCP (10 mg/kg s.c. once a day for 14 days; withdrawal 4 days). Values correspond to the mean ± S.E.M. (n = 5–6). Results with the two-way ANOVA were: $F_{1,72}$ = 6.93; $p < 0.05$. B, the change of PCP-induced dopamine release from the prefrontal cortices of the mice treated repeatedly with PCP. PCP (10 mg/kg s.c.)-induced dopamine release was measured in the prefrontal cortices of mice treated repeatedly with PCP (10 mg/kg s.c. once a day for 14 days; withdrawal 4 days). Values correspond to the mean ± S.E.M. (n = 6). Results with the two-way ANOVA were: $F_{1,190}$ = 103.83; $p < 0.01$. SAL, saline. The basal levels of dopamine in the prefrontal cortex of the saline- and PCP-treated mice were 0.25 ± 0.06 and 0.27 ± 0.04 pmol/12 μl/10 min, respectively.

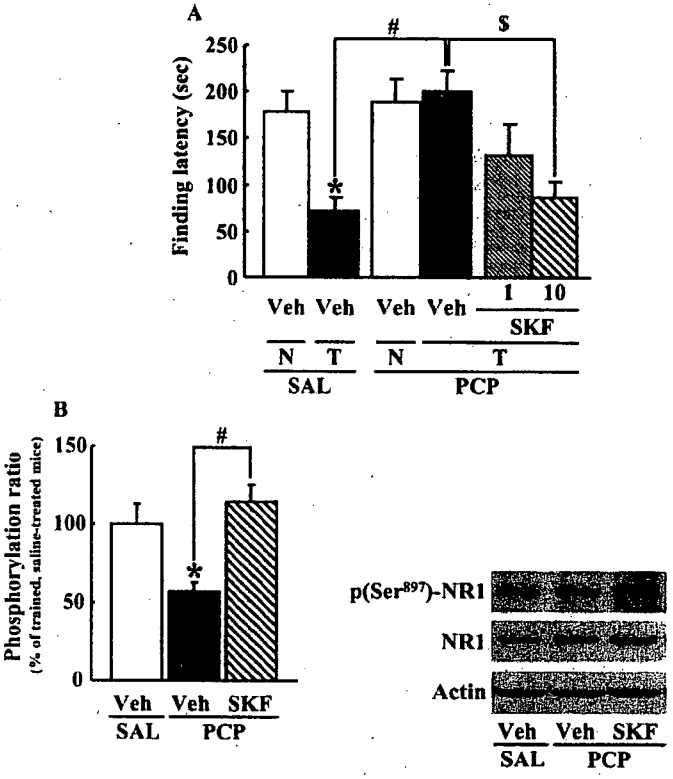

Fig. 10. Rescue, by infusion of a dopamine-D1 receptor agonist into the prefrontal cortex, of impairment of latent learning and learning-associated phosphorylation of NR1 induced by repeated PCP treatment. A, effect of the infusion of a dopamine-D1 agonist into the prefrontal cortex on the impairment of latent learning induced by repeated PCP treatment. Mice treated repeatedly with PCP (10 mg/kg s.c. once a day for 14 days; withdrawal 4 days) were administered SKF81297 (1 and 10 nmol/mouse, bilaterally) 10 min before a training trial of the water-finding test. The test trial was performed 1 day after the training trial. Each column represents the mean ± S.E.M. (n = 7–10). Results with the one-way ANOVA were: $F_{5,46}$ = 6.25; $p < 0.01$. *, $p < 0.05$ compared with the corresponding nontrained mice. #, $p < 0.05$ compared with the trained, saline-treated mice. $, $p < 0.05$ compared with the trained, PCP-treated mice. B, effect of the infusion of a dopamine-D1 agonist into the prefrontal cortex on the impairment of the NR1 phosphorylation ratio induced by repeated PCP treatment. Mice treated repeatedly with PCP (10 mg/kg s.c. once a day for 14 days; withdrawal 4 days) were administered SKF81297 (10 nmol/mouse, bilaterally) 10 min before the training trial of the water-finding test. Immediately after the training trial, mice were sacrificed by decapitation and NR1 phosphorylation (Ser897; p-NR1) and NR1 expression (NR1) in the total tissue extracts was detected by Western blotting. Loaded protein was normalized to actin. The phosphorylation ratio was calculated as NR1 phosphorylation versus NR1 expression. Each column represents the mean ± S.E.M. (n = 7–8). Results with the one-way ANOVA were: $F_{2,20}$ = 8.45; $p < 0.01$. *, $p < 0.05$ compared with the trained, saline-treated mice. #, $p < 0.05$ compared with the trained, PCP-treated mice. N, nontrained mice; T, trained mice; SAL, saline; Veh, vehicle; SKF, SKF81297.

hippocampus impaired memory consolidation in an inhibitory avoidance learning task and learning-associated phosphorylation of CaMKII (Bevilaqua et al., 2005). Although there was no difference in performance in the training trial, the present study showed that αCaMKII (Thr^{286}) was phosphorylated after the training trial in the prefrontal cortex of saline-treated mice but not PCP-treated mice and that the infusion of a CaMKII inhibitor into the prefrontal cortex of saline-treated mice impaired the latent learning. It was suggested that the phosphorylation of CaMKII in the training trial is related not to searching behavior itself but rather to attention associated with searching behavior, because the water-finding task is a latent learning task. Taken together, these results suggest that the prefrontal cortical CaMKII activation in the training trial is critical to the acquisition of latent learning, and the impairment of latent learning in PCP-treated mice is due to a failure to activate CaMKII.

A previous report has demonstrated that αCaMKII undergoes rapid phosphorylation at a threonine residue (Thr286) after the influx of Ca^{2+} mediated by the NMDA receptor, which is a ligand-gated Ca^{2+} channel (Xia and Storm, 2005). The prefrontal cortical glutamatergic transmission, particularly that meditated by NMDA receptors, participates in cognitive function (Wang, 1999). We investigated NMDA-CaMKII signaling after stimulation with exogenous NMDA in slices of the prefrontal cortex. In the prefrontal cortex prepared from saline-treated mice, levels of phosphorylated CaMKII were increased after the simulation, whereas stimulation with NMDA failed to increase the amount of phosphorylated CaMKII in the prefrontal cortex prepared from the PCP-treated mice. The prefrontal cortical infusion of glycine, which is a positive allosteric modulator for the NMDA receptor (Johnson and Ascher, 1987), alleviated the PCP-induced impairment of latent learning and learning-associated phosphorylation of CaMKII. Our findings clearly demonstrate that repeated PCP treatment disrupts the activation of CaMKII mediated via NMDA receptors and that the impairment of latent learning in the PCP-treated mice is due to dysfunctional NMDA-CaMKII signaling.

We investigated whether the dysfunctional NMDA-CaMKII signaling is accompanied by changes in the NMDA receptor subunit NR1 in the prefrontal cortex, because the level of phosphorylated NR1 (Ser897) is decreased in the frontal cortex of patients with schizophrenia (Emamian et al., 2004). In the present study, NR1 expression in total tissue extracts was enhanced in the prefrontal cortex of the PCP-treated mice, whereas NR1 (Ser897) phosphorylation in the membrane-enriched extracts and the NR1 (Ser897) phosphorylation ratio in both extracts were decreased. The phosphorylation of NR1 (Ser897) modulates the function of the NMDA receptors by facilitating expression at the cell surface of NMDA receptors from the endoplasmic reticulum (Scott et al., 2003). It is suggested that the decreased phosphorylation ratio of NR1 caused by the repeated PCP treatment results in suppressed trafficking of the NMDA receptor to the cellular surface. Furthermore, the decreased phosphorylation ratio of NR1 may be associated with the impairment of latent learning, because the infusion of the NR1-antisense oligonucleotide into the prefrontal cortex impaired latent learning and learning-associated activation of CaMKII, which were observed in the PCP-treated mice. In genetic animal experiments, mutant mice exhibited reduced NR1 functioning with schizophrenic-like behavior: NR1 knockdown mice, which express 5 to 10% of the normal level of NR1, showed an increase

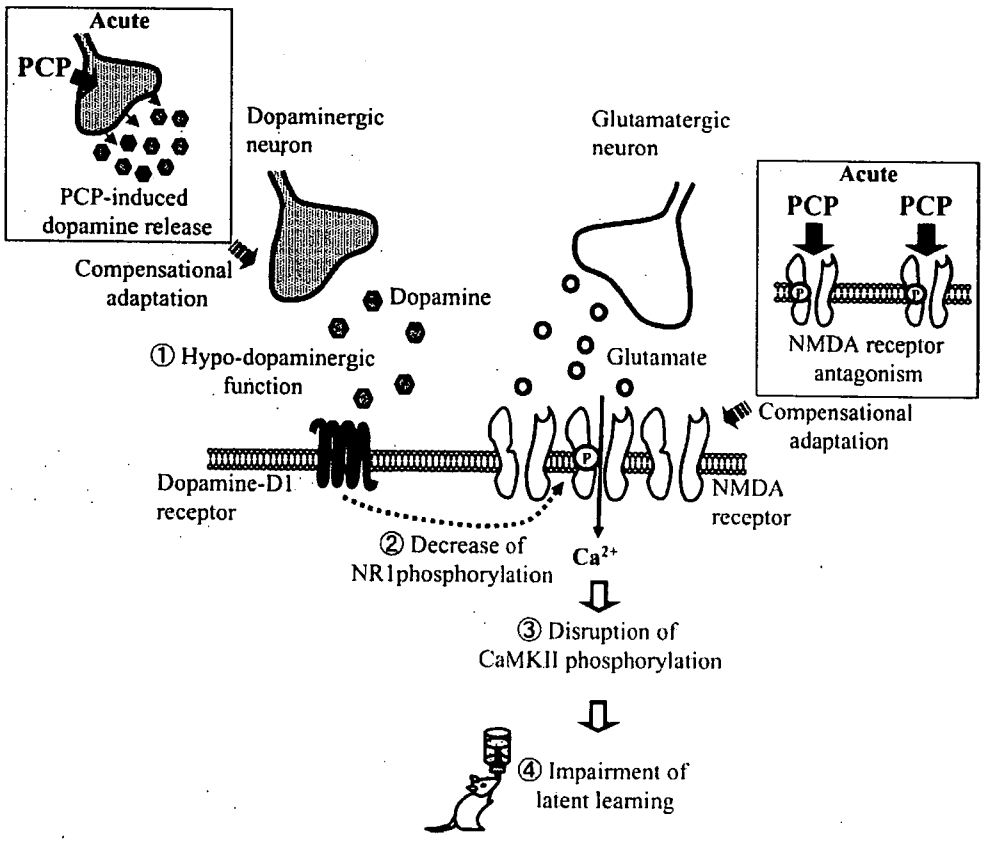

Fig. 11. Schematic representation of the molecular mechanism of latent learning impairment caused by repeated PCP treatments. A, with PCP-induced antagonism of the NMDA receptor, prefrontal cortical NR1 expression was enhanced and dopamine release was diminished. The diminished release of extracellular dopamine in the prefrontal cortex decreases dopamine-D1 signaling (①). Nevertheless, NR1 expression is enhanced, and dopamine-D1 signaling is decreased by repeated PCP treatment. Thus, NR1 (Ser897) phosphorylation is reduced (②) in the prefrontal cortex. The decreased NR1 (Ser897) phosphorylation induces a failure of learning-associated NMDA-CaMKII signaling (③), which is critical to the acquisition of latent learning (④) Thus, repeated PCP treatment impairs latent learning through a prefrontal cortical dysfunction of NMDA-CaMKII signaling, which is associated with dopaminergic hypofunction.

in locomotor activity and deficits of social and sexual interaction (Mohn et al., 1999). NR1 point-mutated mice, which have point mutations of NR1 glycine-binding site, showed hyperactivity (Ballard et al., 2002). Taken together with our reports, these results suggest that repeated PCP treatment induces impairment of latent learning by decreasing the NR1 (Ser897) phosphorylation ratio in the prefrontal cortex, and NR1 deregulation is one of the major factors in the pathogenesis of schizophrenia.

It is well established that the sensitivity of NMDA receptors is regulated by dopamine at the postsynaptic level in the prefrontal cortex. Electrophysiological experiments have indicated that NMDA-mediated excitation was enhanced by a dopamine-D1 receptor agonist in the prefrontal cortical pyramidal neurons through PKA-dependent mechanisms (Wang and O'Donnell, 2001). The dopamine-D1 receptor is coupled to G proteins, activating adenyl cyclase, increasing the level of cAMP, and phosphorylating PKA and Ser897 of NR1, which is a substrate of PKA (Tingley et al., 1997). Snyder et al. (1998) have also reported that a dopamine-D1, but not -D2, receptor agonist phosphorylated NR1 via the activation of PKA. We found that the infusion of a dopamine-D1 receptor agonist into the prefrontal cortex not only induced the phosphorylation in the NR1 (Ser^{897}) in the saline-treated mice but also attenuated the impairment of latent learning and the decrease of NR1 phosphorylation (Ser^{897}) ratio in the PCP-treated mice. These results suggest that dopaminergic function, especially dopamine-D1 receptor signaling, in the prefrontal cortex is critical for the regulation of latent learning-associated NMDA-CaMKII signaling.

Not only glutamatergic but also dopaminergic innervations of the prefrontal cortex play an important role in cognitive functions in schizophrenia (Winterer and Weinberger, 2004). In the animals treated repeatedly with PCP, dysfunctional dopaminergic transmission in the prefrontal cortex is associated with cognitive deficits (Jentsch et al., 1997a,b). In the present in vivo microdialysis experiments, the PCP-treated mice failed to release dopamine in response to high potassium stimulation or a challenge of PCP in the prefrontal cortex. Thus, it is possible that repeated PCP treatment impairs latent learning through a malfunction of NMDA-CaMKII signaling in the prefrontal cortex, which depends on the presynaptic hypofunction of dopaminergic systems.

Although short-term PCP treatment impaired latent learning (Noda et al., 2001), it failed to impair latent learning after drug withdrawal. These findings indicate that the effects of short-term PCP treatment on latent learning, neurotransmission, and/or intracerebral signaling are transient. With PCP-induced antagonism of the NMDA receptor and dopamine release, however, an enhancement of NR1 expression and a diminishment of dopamine release were observed in the prefrontal cortex of PCP-treated mice even after withdrawal (Fig. 11). These compensatory neuronal adaptations to repeated treatment might induce a malfunction of the NMDA receptor associated with hypofunctioning dopaminergic neurons in the prefrontal cortex, which is responsible for the impairment of latent learning (Fig. 11), because the infusion of the dopamine-D1 agonist into the prefrontal cortex attenuated the PCP-induced decrease in the NR1 phosphorylation ratio. Alterations in the circuitry of the prefrontal cortex may contribute to the impairments of cognitive function that are commonly observed in persons with schizophrenia (Lewis and Lieberman, 2000). These observations suggested that the neuronal changes induced by repeated PCP treatment might be more consistent with schizophrenia than the transient antagonism of the NMDA receptor induced by acute PCP treatment.

In conclusion, our results suggest that the impaired functioning of the glutamatergic and dopaminergic nervous systems in the pathogenesis of schizophrenia is mechanically linked. This repeated PCP-treated animal model will contribute to further understanding of the mechanism of cognitive dysfunction in schizophrenia.

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Neuropharmacology 53 (2007) 379–389
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

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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Received 22 March 2007; received in revised form 2 May 2007; accepted 25 May 2007

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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0028-3908/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2007.05.026

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.

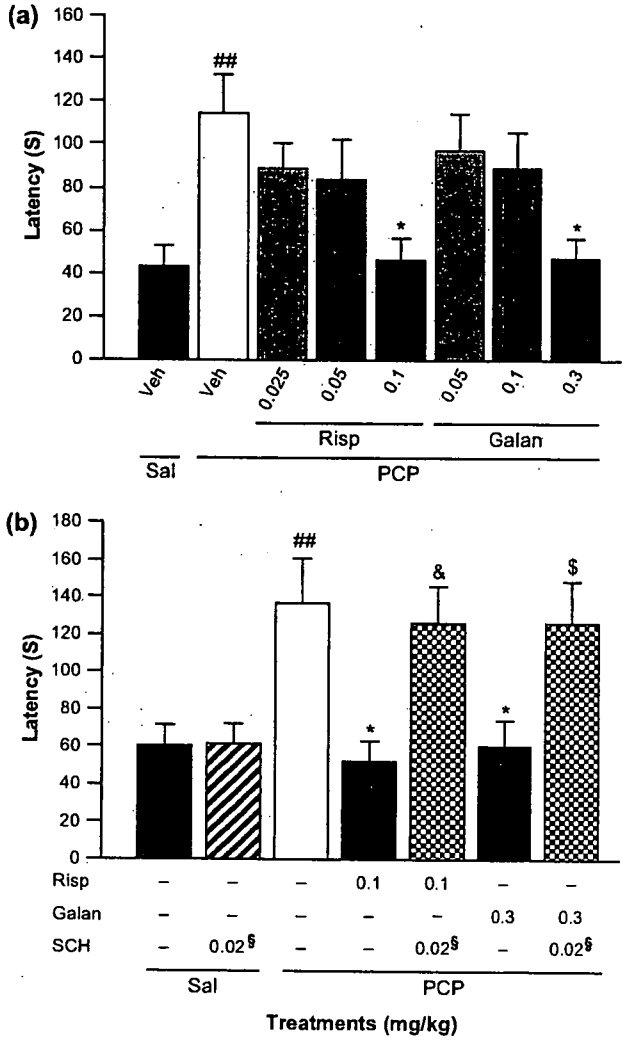

Fig. 1. Individual effects of risperidone and galantamine on impairment of latent visuospatial learning and memory in PCP-treated mice. PCP (10 mg/kg, s.c.) was injected for 14 days. Control groups were treated with same volume of saline (Sal). Galantamine (Galan, 0.05, 0.1 and 0.3 mg/kg, p.o.) and risperidone (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)

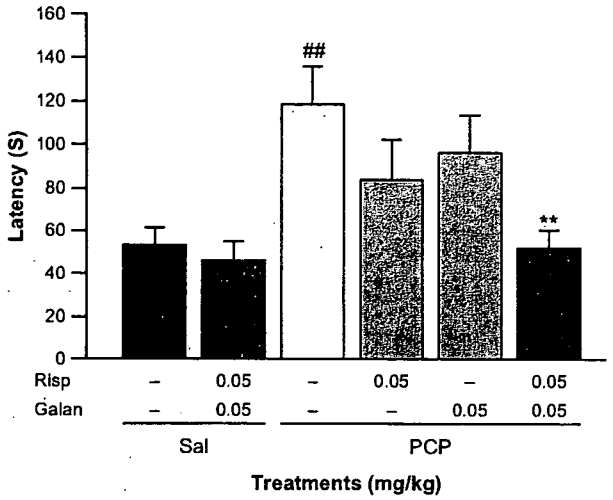

Fig. 3. Synergistic effect of galantamine with risperidone on impairment of latent visuospatial learning and memory in PCP-treated mice. PCP (10 mg/kg, s.c.) was injected for 14 days. Control groups were treated with same volume of saline (Sal). Galantamine (G, 0.05 mg/kg, p.o.) and risperidone (R, 0.05 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

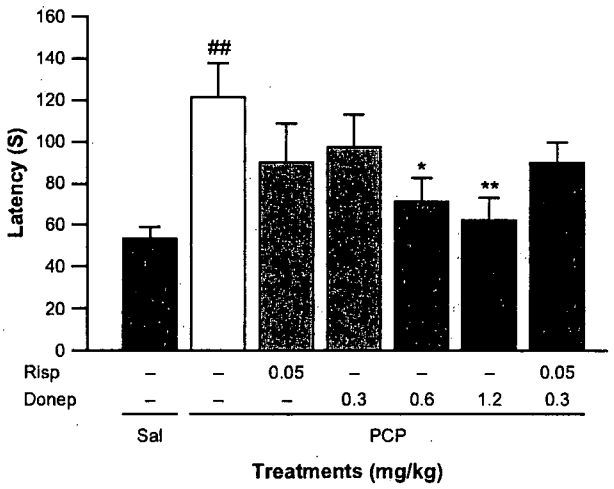

Fig. 4. Combined treatment with donepezil and risperidone did not show synergistic effect on latent visuospatial learning and memory in PCP-treated mice. PCP (10 mg/kg, s.c.) was injected for 14 days. Control groups were treated with same volume of saline (Sal). Donepezil (Donep, 0.3, 0.6 and 1.2 mg/kg, 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

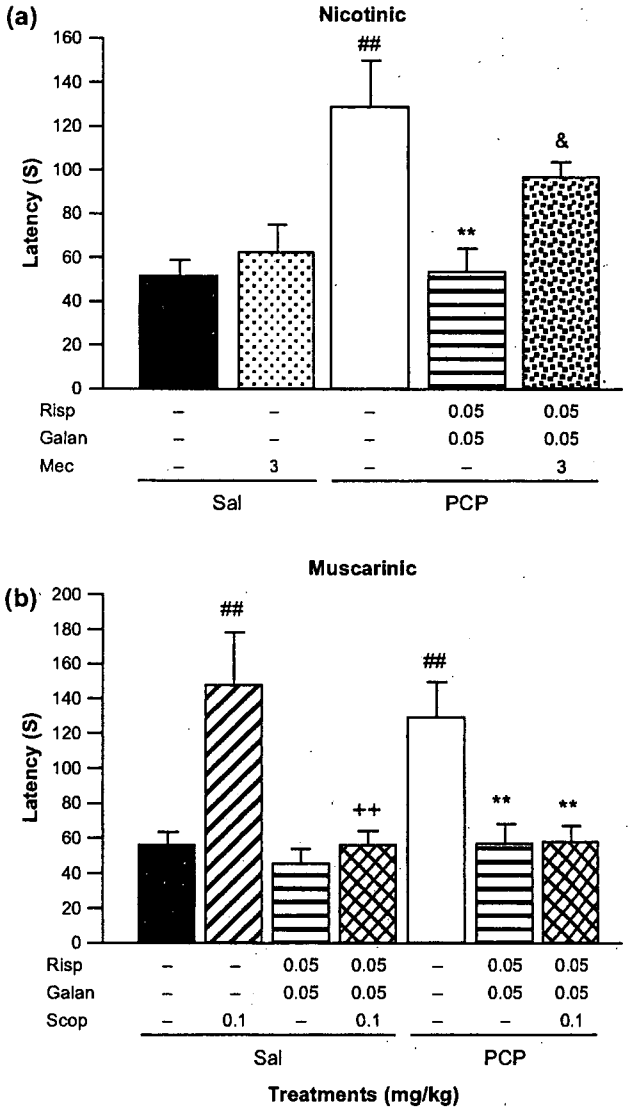

Fig. 5. Nicotinic, but not muscarinic, AChRs are critical for the synergism of galantamine with risperidone. PCP (10 mg/kg, s.c.) was injected for 14 days. Control groups were treated with same volume of saline (Sal). Galantamine (Galan, 0.05 mg/kg, p.o.) and risperidone (Risp, 0.05 mg/kg, p.o.) were 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

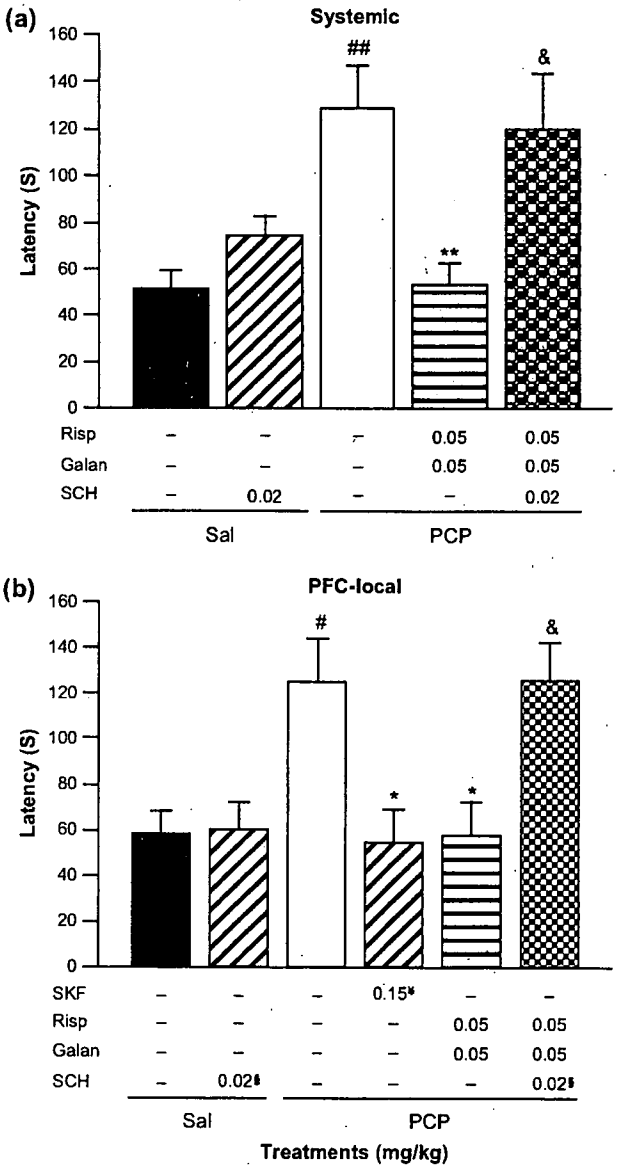

Fig. 6. Synergistic effect of galantamine with risperidone is mediated by D_1 receptors. PCP (10 mg/kg, s.c.) was injected for 14 days. Control groups were treated with same volume of saline (Sal). Galantamine (Galan, 0.05 mg/kg, p.o.) and risperidone (Risp, 0.05 mg/kg, p.o.) were administered 1 h before 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.

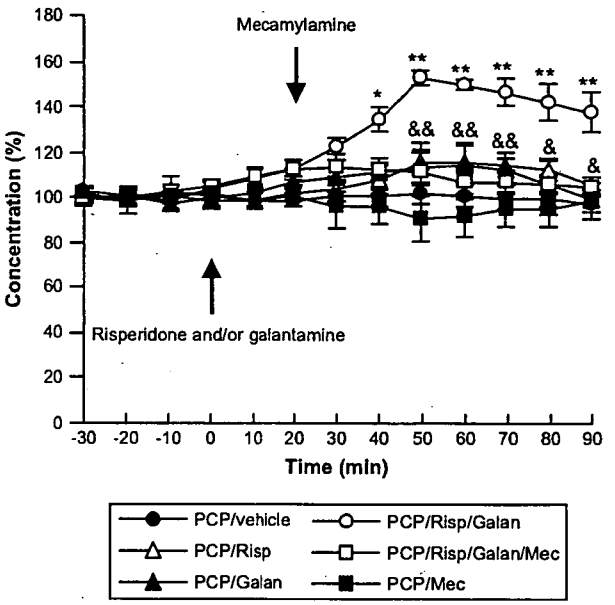

Fig. 7. Synergistic effect of galantamine with risperidone on extracellular concentration of dopamine in mPFC of PCP-treated mice. PCP (10 mg/kg, s.c.) was injected for 14 days. Galantamine (Galan, ▲, 0.05 mg/kg, p.o.) and risperidone (Risp, △, 0.05 mg/kg, p.o.) were administered individually or together (○, 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.

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), $F_{\text{group}(5,357)} = 25.093$ ($p < 0.01$), $F_{\text{time}(12,357)} = 3.635$ ($p < 0.01$).

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).

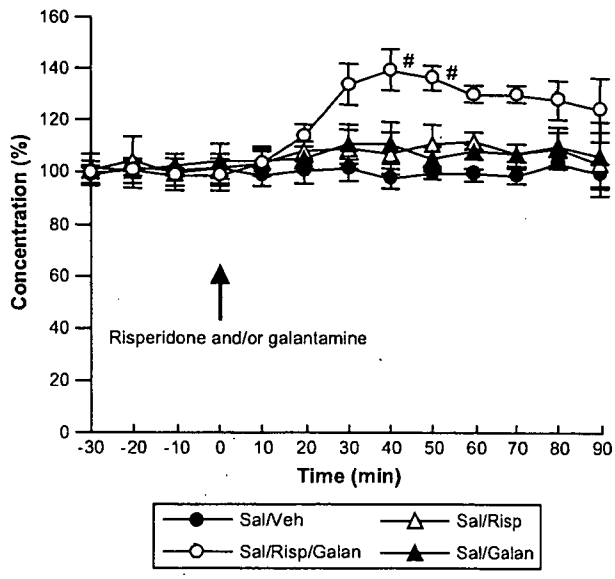

Fig. 8. Synergistic effect of galantamine with risperidone on extracellular concentration of dopamine in mPFC of saline-treated mice. Saline/vehicle-treated mice were used as a control group (Sal/Veh, ●). Galantamine (Galan, ▲, 0.05 mg/kg, p.o.) and risperidone (Risp, △, 0.05 mg/kg, p.o.) were administered 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.

Acknowledgements

We appreciate Janssen Pharmaceutical K.K. (Tokyo 101-0065, Japan) for providing purified galantamine and risperidone. This work was supported, in part, by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (14370031) (15922139) (16922036) (17390018), by a Grant-in-aid from the Ministry of Education, Culture, Sports, Science and Technology to promote multidisciplinary research projects, by a Grant-in-aid for Scientific Research on Priority Areas on "Elucidation of glia-neuron network-mediated information processing systems" from the Ministry of Education, Culture, Sports, Science and Technology (16047214), by Funds from Integrated Molecular Medicine for Neuronal and Neoplastic Disorders (21st Century COE program), by the Japan Brain Foundation, by the Mitsubishi Pharma Research Foundation, by an SRF Grant for Biomedical Research, and by Brain Research Center of the 21st Century Frontier Research Program of the Ministry of Science and Technology, Republic of Korea. There is no conflict of interest to be disclosed for any of the authors.

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Neuropsychopharmacology (2007) 32, 1261–1271

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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.
Neuropsychopharmacology (2007) **32,** 1261–1271. doi:10.1038/sj.npp.1301256; published online 29 November 2006

Keywords: Alzheimer's disease; galantamine; nicotinic acetylcholine receptor; allosteric potentiating ligand; beta amyloid$_{25-35}$; dopamine release

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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Received 7 June 2006; revised 6 September 2006; accepted 2 October 2006