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Phencyclidine Impairs Latent Learning in Mice: Interaction between Glutamatergic Systems and Sigma$_1$ Receptors

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The effect of phencyclidine (PCP) on latent learning was investigated using a one-trial water-finding task in mice. Mice without water deprivation were given PCP or saline before a training trial, which consisted of exposure to a novel open-field environment with an alcove containing a water tube. Twenty to twenty-four hours after water deprivation, animals were placed in the same apparatus and the time required to find the water tube measured (test trial). Saline-treated trained mice showed a significantly shorter time to find the water tube during the test trial (finding latency) than naive mice that had not been trained. When PCP (1mg/kg i.p.) was administered before the training trial, the finding latency was significantly prolonged in comparison with that in the saline-treated mice, indicating that PCP induced impairment of latent learning. 1-(3,4-Dimethoxy-phenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride (SA4503: 0.3 mg/kg s.c.) and (+)-pentazocine (1 mg/kg s.c.), selective sigma$_1$ receptor agonists, or D-cycloserine (10 and 30mg/kg, s.c.), a glycine binding site agonist, significantly counteracted the PCP-induced impairment of latent learning, whereas (+)-SKF-10,047 (0.1–3 mg/kg s.c.), a putative sigma$_1$ receptor agonist, did not. The ameliorating effects of SA4503 and (+)-pentazocine were antagonized by N,N-dipropyl-2-(4-methoxy-3-(2-phenylethoxy) phenyl) ethylamine (NE-100: 1 mg/kg i.p.), a selective sigma$_1$ receptor antagonist. SA4503 also ameliorated the impairment of latent learning induced by dizocilpine, a non-competitive N-methyl-D-aspartate receptor antagonist, the effect being antagonized by NE-100. These results suggest that PCP induces an impairment of latent learning, this effect being mediated via glutamatergic systems, and that activation of sigma$_1$ receptors ameliorates impairment of latent learning induced by PCP.
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Deficits in attention and information processing mechanisms have been suggested to play a critical role in disorders such as schizophrenia (Braff and Geyer 1990; Kietzman et al. 1985; Nuechterlein and Dawson 1985). This view is consistent with the earlier notion that 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; Venables 1960). Thus, the study of cognitive function related to sensory information or attention has been of central importance in the attempt to understand this disorder.

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The water-finding task provides a measure of memory related mainly to the spatial and attentional construction of the test apparatus and to the specific objects in it. Further, this task is thought to be a latent learning paradigm and to be related to the ability to sort sensory information and to attention (Ettenberg et al. 1983; Ichihara et al. 1993; Mamiya et al. 1998; Nabeshima and Ichihara 1993). Namely, this task does not need any motivation to train animals. Animals are deprived of water only before the test trial in order to promote recall of the location of the water tube in the test apparatus in which they have been exposed in the training trial. The end of the water tube is set further above the floor in the test trial than in training to decrease the probability of it being found by chance. Thus, finding latency provides a measure of latent learning and/or spatial attention in animals.

Non-competitive N-methyl-D-aspartate (NMDA) antagonists such as phencyclidine (PCP), dizocilpine and ketamine markedly induce the sensorimotor gating and cognitive deficits in animals. Interestingly, PCP exacerbates schizophrenic symptoms and in normal subjects produces behavior that in some ways resembles schizophrenic symptomatology (Allen and Young 1978; Balster 1987; Luisada 1978; Snyder 1988). PCP-induced psychosis is distinguishable from amphetamine-induced psychosis in that both positive and negative symptoms of schizophrenia are elicited by PCP, whereas only the former are elicited by amphetamine (Javitt 1987). Further, PCP causes cognitive dysfunction in normal humans (Javitt and Zukin 1991; Pearlson 1981), and cognitive deficits have also been observed in PCP-treated animals (Boyce et al. 1991; Nabeshima et al. 1986; Ogawa et al. 1994). Despite the potential importance of PCP in understanding schizophrenia, the pharmacological mechanisms underlying the effects of PCP on latent learning remain unclear.

In the present study, we investigated the effect of PCP on latent learning using the water-finding task in mice. PCP induces an impairment of latent learning in mice, and we attempted to investigate whether $sigma_1$ receptor agonists, (+)-SKF-10,047, (+)-pentazocine and SA4503, attenuate this impairment, since $sigma_1$ receptors interact with NMDA mediating neurotransmission and this interaction plays a role in NMDA-dependent learning and memory processes in rodents.

METHODS

Animals

Male mice of the ddY strain (Japan SLC Inc., Shizuoka, Japan), weighing 25–27g at the beginning of the experiments were used. The animals were housed in plastic cages and were kept in a regulated environment (23 ± 1°C, 50 ± 5% humidity), with a 12:12 hr light:dark cycle (lights on at 0730). Food (CE2, Clea Japan Inc., Tokyo, Japan) and tap water were available ad libitum.

All experiments were performed in accordance with the Guidelines for Animal Experiments of the Nagoya University School of Medicine. The procedures involving animals and their care were conducted in conformity with the international guidelines "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985).

Drugs

1-(3,4-Dimethoxy-phenethyl)-4-(3-phenylpropyl)piperazine dihydrochloride (SA4503), (+)-pentazocine and N,N-dipropyl-2-(4-methoxy-3-(2-phenylethoxy) phenyl) ethylamine (NE-100) were supplied by Santen Pharmaceutical (Osaka, Japan), Dainippon Pharmaceutical Co. Ltd. (Osaka, Japan), and Taisho Pharmaceutical (Tokyo, Japan), respectively. (+)-N-allyl-normetazocine hydrochloride ((+)-SKF-10,047), (+)-4-amino-3-isoxazolidinone (D-cycloserine), and (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,b]cyclohepten-5,10-imine maleate (dizocilpine) were purchased from Research Biochemicals Inc. (Natick, MA). Phencyclidine hydrochloride [1-(1-phenylcyclohexyl) piperidine hydrochloride: (PCP)] was synthesized by the authors according to the method of Maddox et al. (1965) and was checked for purity. All compounds were dissolved in distilled water and were administered in a volume of 0.1 ml/10 g body weight.

Apparatus

The apparatus consisted of an open field (30 × 50 × 15 cm high) with an alcove (10 × 10 × 10 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 for measuring locomotor activity. A drinking tube, identical with those used in the home cages, was inserted into the center of the alcove ceiling with its tip 6.5 cm (in the training trial) or 7.5 cm (in the test trial) above the floor.

Procedure

The test consisted of two trials: a training trial (the 1st day) and test trial (the 2nd day). In the training trial, mice not deprived of water were placed individually into one corner of the open field of the apparatus. Each mouse was allowed 3 min to explore the environment; the 3 min was counted from the time the mouse started to explore. During this time, ambulation was measured by counting the number of times the animals crossed from one square to another in the open field. The frequency of touching, sniffing, or licking of the water tube in the alcove (number of approaches) was also recorded. Animals that did not start exploring after 3 min had elapsed or that did not find the drinking tube during the 3 min exploratory period were omitted from the test trial. Non-trained mice (naive mice) were prepared

for comparison with the trained mice (control mice) in terms of their ability to find the water source in the same environment. The mice were immediately returned to their home cages after the training trial.

In the test trial, mice were again individually placed on the test apparatus 20–24 hr after the single test trial. The mice were deprived of water for 20–24 hr before the test trial. The time until the mouse moved out of the corner was measured as the starting latency. In addition, the time taken to enter the alcove (entering latency) and the time between entering the alcove and drinking the water (finding latency) were scored. Thus, the drinking latency consisted of the sum of the entering and finding latencies.

Drug Treatment

PCP (0.3 and 1 mg/kg i.p.), dizocilpine (0.05 mg/kg i.p.), D-cycloserine (3–30 mg/kg s.c.), (+)-SKF-10,047 (0.1–3 mg/kg s.c.), SA4503(0.1 and 0.3 mg/kg s.c.), (+)-pentazocine (0.3 and 1 mg/kg s.c.), and NE-100 (0.3 and 1 mg/kg i.p.) were administered 30, 15, 30, 30, 30, 30 and 45 min, respectively, before the training trial.

Statistical Analysis

All results were expressed as the mean ± S.E.M. for each group. Statistical analysis of the difference between each group was determined with ANOVA, followed by the Dunnett multiple comparisons test.

RESULTS

Effect of PCP Given Before the Training Trial on the Water Finding Performance in the Test Trial

In the training trial, there were no significant effects on number of approaches to the water tube and ambulatory behavior in the open field in mice treated with PCP (0.3 and 1 mg/kg), compared with those in control (data not shown).

The water finding performance of mice trained after receiving PCP is shown in Figure 1. Vehicle-treated mice (control) showed significantly shorter latencies to start exploring the environment (data not shown), to enter the alcove, to find the water tube, and to drink the water than did naive mice that had no exposure to the apparatus, indicating the occurrence of latent learning. PCP (1 mg/kg i.p.) significantly increased the finding and drinking latencies, compared with control, indicating that PCP induced an impairment of latent learning in mice.

Effect of D-cycloserine on the PCP-induced Impairment of Latent Learning in Mice

To determine whether the PCP-induced impairment of the water finding performance is mediated via glutamatergic systems, the effect of D-cycloserine, an agonist of glycine binding sites at the NMDA receptor ionophore complex, was investigated. Table 1 shows the effect of D-cycloserine (3–30 mg/kg) on exploratory behavior in mice given PCP (1 mg/kg) during the training trial. D-cycloserine (3–30 mg/kg) had no effect on exploratory behaviors.

Figure 2 shows the effect of the combination of D-cycloserine and PCP, given before the training trial, on the water finding performance of mice in the test trial. D-cycloserine (10 and 30 mg/kg) fully counteracted the increase in finding and drinking latencies induced by PCP (Figure 2). D-Cycloserine (30 mg/kg) itself had no effect on number of approaches to the water tube and ambulatory behavior in the open field, compared with those in control in the training trial (Table 2), although it showed a tendency to prolong finding latency, but not drinking latency, in the test trial (Figure 5).

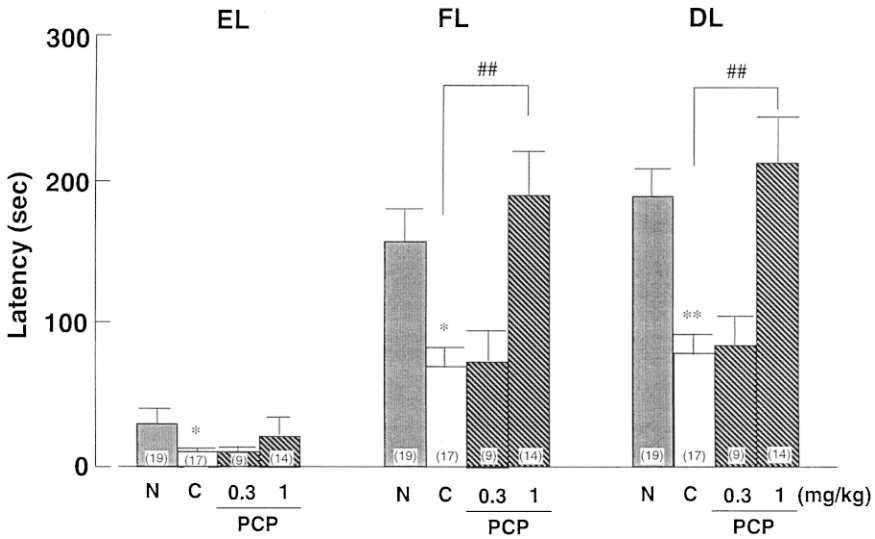

Figure 1. Effects of phencyclidine (PCP) in the water-finding task in the test trail. PCP was administered i.p. 30 min before the training trial. Figure shows latencies in entering the alcove (EL), finding water after first entering the alcove (FL) and drinking water (DL). Each value is the mean ± S.E.M. of the number of animals shown in parentheses. Results with one-way ANOVA were: EL; $F(3,55) = 3.22$ ($p < 0.05$), FL; $F(3,55) = 6.42$ ($p < .01$), DL; $F(3,55) = 8.02$ ($p < .01$). $^{*}p < .05$, $^{**}p < .01$ compared to naive mice (N). $\#\#p < 0.01$ compared to control mice (C).

Table 1. Exploratory Behavior in Mice Given PCP with Several Compounds during Training for the Water-finding Task

Treatment	Dose (mg/kg)	n(Ns,Nf)	SL (sec)	Number of Approaches (counts)	Ambulation (counts)
Control		24(0,1)	7.3 ± 1.0	3.6 ± 0.4	58.8 ± 6.3
PCP + saline		21(0,2)	4.9 ± 0.7	2.9 ± 0.3	89.3 ± 10.4
PCP + D-cycloserine	3	10(0,0)	5.8 ± 1.1	4.0 ± 0.7	73.5 ± 8.7
	10	18(0,1)	6.2 ± 0.8	3.8 ± 0.4	83.6 ± 6.8
	30	16(0,2)	5.4 ± 0.4	3.9 ± 0.4	59.3 ± 7.8
PCP + SA4503	0.1	10(0,0)	4.1 ± 0.7	2.6 ± 0.5	92.2 ± 18.0
	0.3	19(0,3)	5.0 ± 0.7	4.0 ± 0.5	107.2 ± 9.7**
PCP + SA4503 + NE-100	0.3	9(0,1)	7.7 ± 1.3	4.4 ± 0.8	71.8 ± 9.3
	1	14(0,2)	4.6 ± 0.5	2.8 ± 0.5	83.2 ± 6.8
PCP + (+)-pentazocine	0.3	9(0,1)	10.8 ± 2.0	3.1 ± 0.6	83.9 ± 15.1
	1	8(0,2)	5.3 ± 1.4	2.5 ± 0.5	82.3 ± 7.7
PCP + (+)-pentazocine + NE-100	1	13(0,2)	7.3 ± 1.9	2.1 ± 0.4	94.7 ± 9.3
PCP + (+)-SKF-10,047	0.1	10(0,0)	4.7 ± 0.6	2.0 ± 0.3	79.6 ± 6.7
	0.3	9(0,1)	6.0 ± 1.1	2.7 ± 0.6	99.3 ± 12.2
	1	10(0,0)	13.5 ± 4.4*	1.6 ± 0.2*	110.5 ± 12.8**
	3	8(0,2)	5.3 ± 0.8	2.1 ± 0.5	73.8 ± 11.8

PCP (1 mg/kg i.p.), D-cycloserine (3–30 mg/kg s.c.), SA4503 (0.1 and 0.3 mg/kg s.c.), (+)-pentazocine (0.3 and 1 mg/kg s.c.), (+)-SKF-10,047 (0.1–3 mg/kg s.c.), and NE-100 (0.3 or 1 mg/kg i.p.) were administered 30, 30, 30, 30, 30 and 45 min, respectively, before the training trial. Results with one-way ANOVA were as follows: SL; $F(16, 204) = 3.06$ ($p < .01$), Number of approaches; $F(16, 204) = 2.73$ ($p < .01$), ambulation; $F(16, 204) = 2.15$ ($p < .01$). *$p < .05$ and **$p < .01$ compared to control.

Interaction between Glutamatergic and Sigma Systems

We investigated whether $sigma_1$ receptor agonists, (+)-SKF-10,047, (+)-pentazocine and SA4503, attenuated PCP-induced impairment of latent learning, since $sigma_1$ receptors interact with NMDA mediating neurotransmission and this interaction plays a role in NMDA-dependent learning and memory processes in rodents.

Table 1 shows the effects of SA4503, (+)-SKF-10,047, (+)-pentazocine and NE-100 on exploratory behavior in mice given PCP during the training trial. SA4503 significantly increased the ambulation at the dose of 0.3 mg/kg, although the starting latency and number of approaches did not change significantly. (+)-SKF-10,047 (1 mg/kg) significantly prolonged starting latency, decreased the number of approaches to the water tube and increased the ambulation. NE-100 in combination with SA4503 or (+)-pentazocine did not affect number

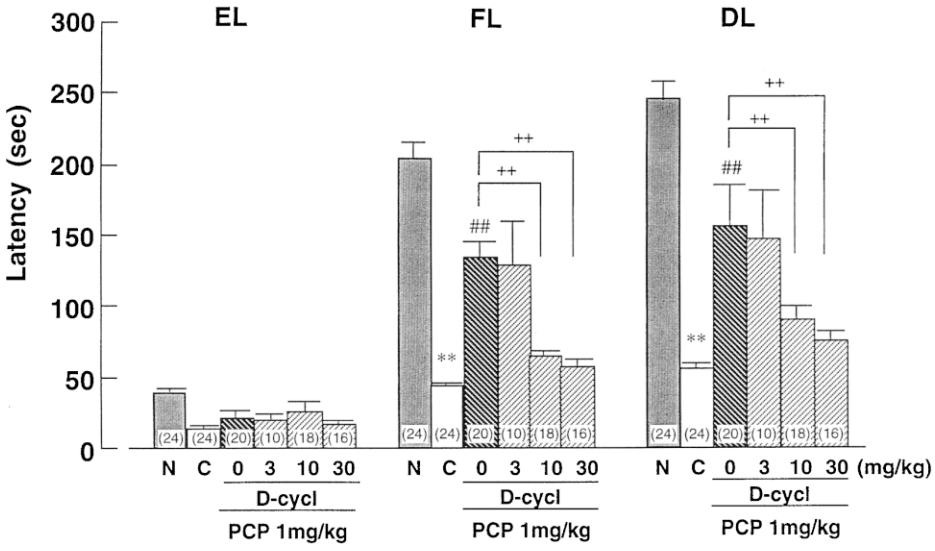

Figure 2. Effect of D-cycloserine on PCP-induced impairment in the water-finding task in the test trial. PCP (1 mg/kg i.p.) and D-cycloserine (D-cycl: 3–30 mg/kg s.c.) were administered 30 min before the training trial. Figure shows latencies in entering the alcove (EL), finding water after first entering the alcove (FL) and drinking water (DL). Each value is the mean ± S.E.M. of the number of animals shown in parentheses. Results with one-way ANOVA were: EL; $F(5,106) = 2.07$ ($p > .05$), FL; $F(5,106) = 23.62$ ($p < .01$), DL; $F(5,106) = 25.21$ ($p < .01$). **$p < .01$ compared to naive mice (N). ##$p < .01$ compared to control (C). ++$p < .01$ compared to PCP alone.

Table 2. Exploratory Behavior in Mice Given Several Compounds during Training for the Water-finding Task

Treatment	Dose (mg/kg)	n(Ns,Nf)	SL (sec)	Number of approaches (counts)	Ambulation (counts)
Vehicle		13(0,0)	9.8 ± 3.6	3.1 ± 0.7	57.2 ± 9.0
D-cycloserine	30	10(0,0)	4.2 ± 0.7	2.8 ± 0.5	106.3 ± 17.8
SA4503	0.3	9(0,1)	3.7 ± 0.9	4.1 ± 0.7	83.4 ± 12.3
(+)-SKF-10,047	0.1	10(0,0)	3.6 ± 0.4	3.2 ± 0.6	83.9 ± 14.0
	3	8(0,2)	4.9 ± 1.0	2.4 ± 0.5	98.8 ± 13.2
NE-100	1	9(0,1)	4.0 ± 0.7	3.4 ± 0.5	104.0 ± 16.2

D-cycloserine (30 mg/kg s.c.), SA4503 (0.3 mg/kg s.c.), (+)-SKF-10,047 (0.1 and 3 mg/kg s.c.), and NE-100 (1 mg/kg i.p.) were administered 30, 30, 30, and 45 min, respectively, before the training trial. Results with one-way ANOVA were as follows: SL; $F(5,43) = 1.78$ ($p > .05$), Number of approaches; $F(5,43) = 0.80$ ($p > .05$), ambulation; $F(5,43) = 1.90$ ($p > .05$). There are no significant differences compared to vehicle (control).

of approaches to the water tube and ambulatory behavior in the open field in mice treated with PCP, compared with those in control. In addition, the number of mice that failed to find the water tube was not significantly different among the groups.

SA4503 and (+)-pentazocine fully counteracted the increase in finding and drinking latencies induced by PCP (Figure 3A and 3B, respectively), whereas (+)-SKF-10,047 did not (Figure 3C). Such ameliorating effects of SA4503 and (+)-pentazocine on the PCP-induced im-

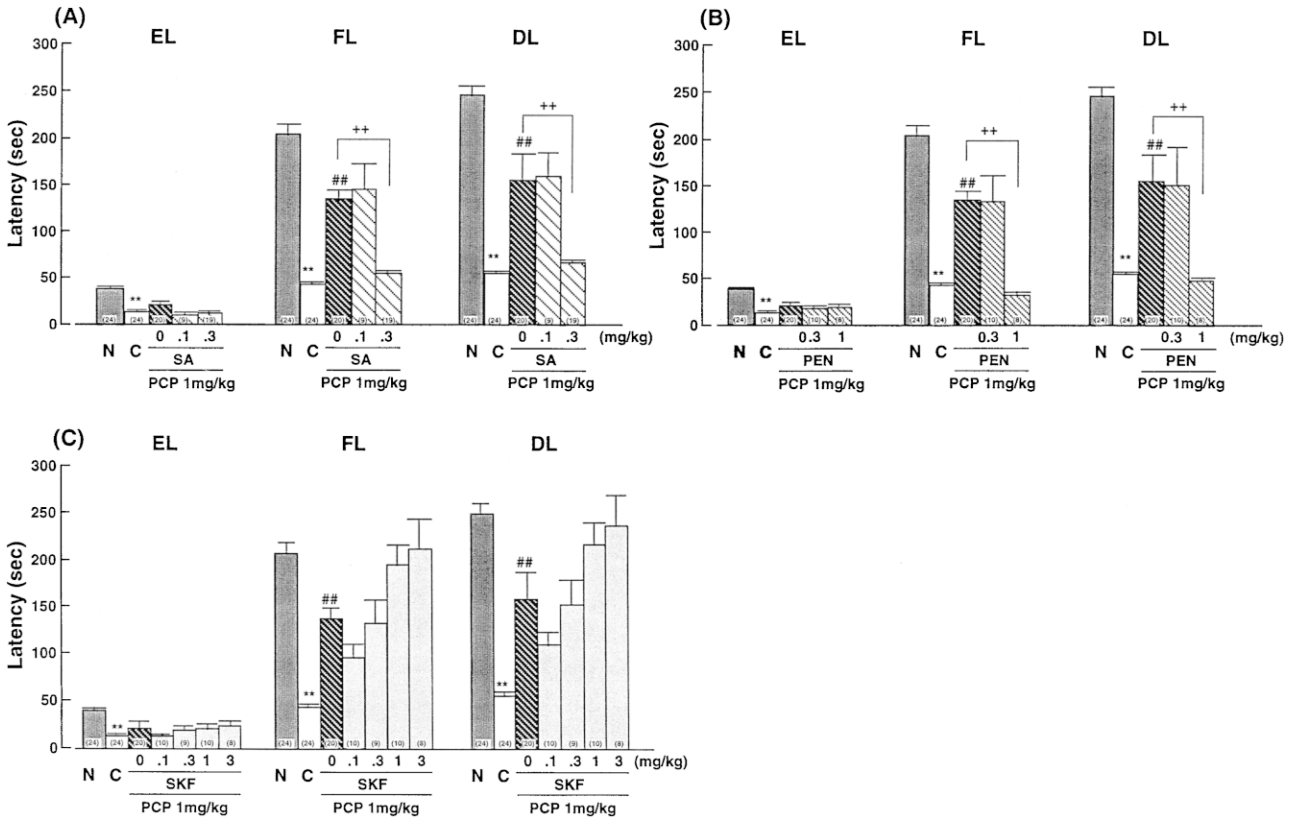

Figure 3. Effects of SA4503 **(A)**, (+)-pentazocine **(B)** and (+)-SKF-10,047 **(C)** on PCP-induced impairment in the water-finding task in the test trial. PCP (1 mg/kg i.p.), SA4503 (SA: 0.1 and 0.3 mg/kg s.c.), (+)-pentazocine (PEN: 0.3 and 1 mg/kg s.c.) and (+)-SKF-10,047 (SKF: 0.1–3 mg/kg s.c.) were administered 30 min before the training trial. Figure shows latencies in entering the alcove (EL), finding water after first entering the alcove (FL) and drinking water (DL). Each value is the mean ± S.E.M. of the number of animals shown in parentheses. Results with one-way ANOVA were: **(A)** EL; $F(4,91) = 6.85$ ($p < .01$), FL; $F(4,91) = 27.69$ ($p < .01$), DL; $F(4,91) = 37.57$ ($p < .01$), **(B)** EL; $F(5,93) = 2.60$ ($p < .05$), FL; $F(5,93) = 18.79$ ($p < .01$), DL; $F(5,93) = 22.29$ ($p < .01$), (C) EL; $F(6,98) = 2.95$ ($p < .05$), FL; $F(6,98) = 15.30$ ($p < .01$), DL; $F(6,98) = 18.50$ ($p < .01$). $^{**}p < .01$ compared to naive mice (N). $\#\#p < .01$ compared to control mice (C). $++p < .01$ compared to PCP alone.

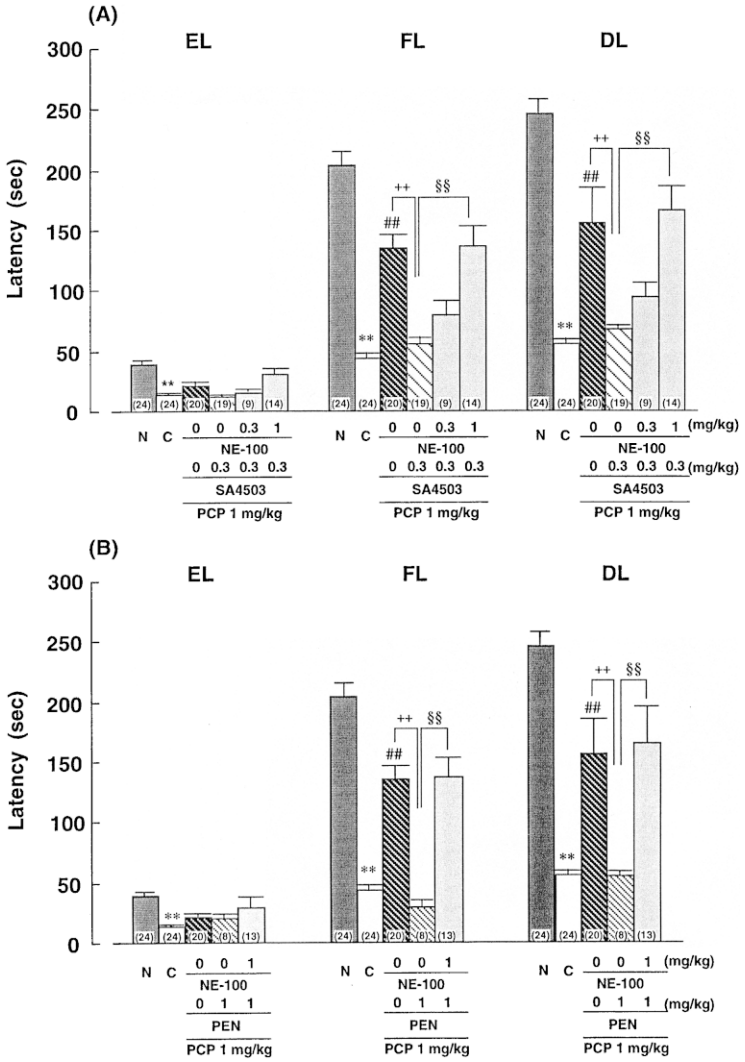

Figure 4. Antagonism by NE-100 of the SA4503 **(A)** and (+)-pentazocine **(B)** effects on PCP-induced impairment in the water-finding task in the test trial. PCP (1 mg/kg i.p.), SA4503 (0.3 mg/kg s.c.), (+)-pentazocine (PEN: 1 mg/kg s.c.) and NE-100 (0.3 or 1 mg/kg i.p.) were administered 30, 30, 30 and 45 min, respectively, before the training trial. Figure shows latencies in entering the alcove (EL), finding water after first entering the alcove (FL) and drinking water (DL). Each value is the mean ± S.E.M. of the number of animals shown in parentheses. Results with one-way ANOVA were: **(A)** EL; $F(5,104) = 4.64$ ($p < .01$), FL; $F(5,104) = 24.57$ ($p < .01$), DL; $F(5,104) = 30.92$ ($p < .01$), **(B)** EL; $F(5,93) = 2.60$ ($p < .05$), FL; $F(5,93) = 18.79$ ($p < .01$), DL; $F(5,93) = 22.29$ ($p < .01$). $^{**}p < .01$ compared to naive mice (N). $^{\#\#}p < .01$ compared to control mice (C). $^{++}p < .01$ compared to PCP alone. $^{\S\S}p < .01$ compared to SA4503 + or (+)-pentazocine + PCP.

pairment of latent learning were antagonized by NE-100, a selective sigma$_1$ receptor antagonist (Figure 4A and 4B, respectively).

In the training trial, (+)-SKF-10,047, SA4503 and NE-100 themselves slightly increased the ambulation, although exploratory behaviors during the training trial were not different among the group (Table 2). SA4503 and NE-100 themselves did not significantly alter the water finding performance in the test trial at the does used, whereas (+)-SKF-10,047 at 3 mg/kg showed the tendency to prolong finding latency, but not drinking latency (Figure 5).

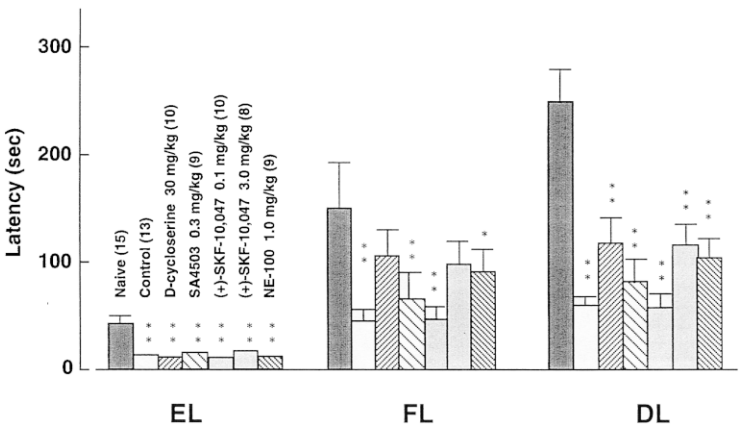

Figure 5. Effects of D-cycloserine, SA4503, (+)-SKF-10,047 and NE-100 alone in the water-finding task in the test trial. D-Cycloserine (30 mg/kg s.c.), SA4503 (0.3 mg/kg s.c.), (+)-SKF-10,047 (0.1 and 3 mg/kg s.c.) and NE-100 (1 mg/kg i.p.) were administered 30, 30, 30 and 45 min, respectively, before the training trial. Figure shows latencies in entering the alcove (EL), finding water after first entering the alcove (FL) and drinking water (DL). Each value is the mean ± S.E.M. of the number of animals shown in parentheses. Results with one-way ANOVA were: EL; $F(6,66) = 11.38$ ($p < .01$), FL; $F(6,66) = 4.72$ ($p < .01$), DL; $F(6,66) = 7.81$ ($p < .01$). $^{*}p < .05$, $^{**}p < .01$, compared to naive mice (N). C: control mice.

Table 3. Effect of Dizocilpine on Exploratory Behavior during Training for the Water-finding Task

Treatment	Dose (mg/kg)	n(Ns,Nf)	SL (sec)	Number of approaches (counts)	Ambulation (counts)
Control + saline		13(0,2)	7.1 ± 2.7	4.0 ± 0.9	56.2 ± 7.2
Dizocilpine + saline		15(0,3)	4.1 ± 0.7	2.2 ± 0.3	98.1 ± 15.4
Dizocilpine + SA4503	0.3	15(0,2)	4.3 ± 1.1	3.1 ± 0.7	97.5 ± 13.4
Dizocilpine + NE-100	1	10(0,0)	10.8 ± 2.6	1.8 ± 0.4	70.3 ± 8.8

Dizocilpine (0.05 mg/kg i.p.), SA4503 (0.3 mg/kg s.c.), and NE-100 (1 mg/kg i.p.) were administered 15, 30, and 45 min, respectively, before the training trial. Results with one-way ANOVA were as follows: SL; $F(3,49) = 2.69$ ($p > .05$), Number of approaches; $F(3,49) = 2.27$ ($p > .05$), ambulation, $F(3,49) = 2.80$ ($p > .05$). There are no significant differences compared to vehicle (control).

Effect of Dizocilpine Given Before the Training Trial on the Water Finding Performance

In the training trial, the exploratory behavior of mice treated with dizocilpine (0.05 mg/kg) is summarized in Table 3. There was no significant effect on number of approaches to the water tube and ambulatory behavior in the open field in mice treated with dizocilpine, compared with those in control.

The results in the test trial, of the water finding performance of mice trained after dizocilpine are shown in Figure 6. Controls showed significantly shorter latencies in finding and drinking the water than naive mice that had no exposure to the test apparatus, indicating the occurrence of latent learning. Dizocilpine (0.05 mg/kg) significantly prolonged the finding and drinking latencies, compared with control, indicating dizocilpine induced the impairment of latent learning in mice.

Effect of SA4503 on the Dizocilpine-induced Impairment of Latent Learning in Mice

Table 3 shows the effect of SA4503 on exploratory behavior in mice given dizocilpine. There was no significant effects on number of approaches to the water tube and ambulatory behavior in the open field in mice treated with SA4503 + dizocilpine and with SA4503 + dizocilpine + NE-100, compared with those in control. Figure 6 depicts the effect of SA4503 in combination with dizocilpine in the test trial. SA4503 (0.3 mg/kg) fully counteracted the increase in finding and drinking latencies induced by dizocilpine. Such ameliorating effect of SA4503 on the dizocilpine-induced impairment

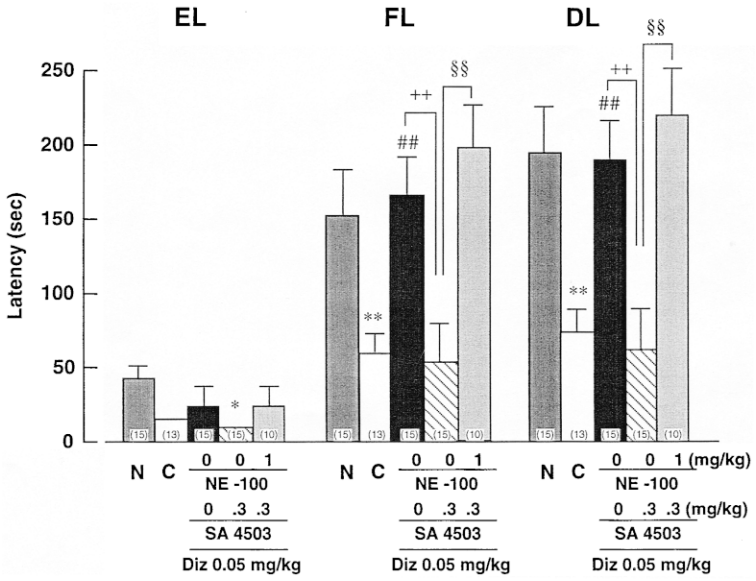

Figure 6. Effect of SA4503 on dizocilpine-induced impairment in the water-finding task in the test trial. Dizocilpine (0.05 mg/kg i.p.), SA4503 (0.3 mg/kg s.c.) and NE-100 (1 mg/kg i.p.) were administered 15, 30 and 45 min, respectively, before the training trial. Figure shows latencies in entering the alcove (EL), finding water after first entering the alcove (FL) and drinking water (DL). Each value is the mean ± S.E.M. of the number of animals shown in parentheses. Results with one-way ANOVA were: EL; $F(4,63) = 2.57$ ($p < .05$), FL; $F(4,63) = 10.82$ ($p < .01$), DL; $F(4,63) = 14.80$ ($p < .01$). $^{*}p < .05$, $^{**}p < .01$ compared to naive mice (N). $^{\#\#}p < .01$ compared to control mice (C). $^{++}p < .01$ compared to dizocilpine alone. $^{\S\S}p < .01$ compared to SA4503 + dizocilpine.

of latent learning was antagonized by NE-100 (1 mg/kg) (Figure 6).

DISCUSSION

The water-finding task provides a measure of memory related mainly to the spatial construction of the test apparatus and to the specific objects in it (Ettenberg et al. 1983; Ichihara et al. 1993; Mamiya et al. 1998; Nabeshima and Ichihara 1993). In this task, there is no need to use any form of motivation to train the animals. Animals are deprived of water only before the test trial to promote recall of the location of the water tube in the alcove of the apparatus in which they have been exposed in the training trial. The end of the water tube is set farther above the floor in the test trial than in training to decrease the probability of it being found by chance. The finding latency of the trained mice is markedly shorter than that of naive mice, indicating finding latency provides a measure of latent learning (attention) in mice. In the present study, vehicle control mice showed a shorter time to find the water tube than naive mice that were not exposed to the apparatus in the training trial did. This result confirmed the results in a previous report (Ichihara et al. 1993). However, PCP treatment significantly prolonged the drinking and finding latencies in the test trial at the dose of 1 mg/kg, indicating an impairment of the latent learning. It is unlikely that the impaired effect of PCP on latent learning is due to motor dysfunction and/or changes in motivational properties to find water, since there are no differences in exploratory behavior and number of approaches to the water tube during the training session of the water finding test between the control and PCP groups.

PCP is known to interact with several neurotransmitter binding sites in the brain, including a PCP binding site within the NMDA receptor ionophore complex (Johnson et al. 1987). The NMDA receptor-mediated glutamatergic neurotransmission has been demonstrated to play an important role in learning and memory. Behavioral studies have shown that NMDA antagonists impair the performance of some kinds of learning tasks. For example, dizocilpine, a non-competitive NMDA receptor antagonist, induces learning and memory impairment in water maze (Heale and Harley 1990; Morris 1989; Morris et al. 1986), radial maze (Butelman 1989; Danysz et al. 1988; Ward et al. 1990), Y-maze (Maurice et al. 1994; Maurice and Privat 1997), and passive avoidance (Yamada et al. 1996) tasks. Further, D-cycloserine, an agonist of glycine binding site coupled with NMDA receptor ionophore complex, improves the dizocilpine-induced learning impairment in the radial maze task in rat (Kawabe et al. 1998). Recently, glutamatergic dysfunction was proposed to contribute to the etiology of some forms of schizophrenia. In the present study, dizocilpine, like PCP, caused an impairment of latent learning in mice without changing the exploratory behavior or motivational properties during the training trail. PCP-induced impairment of latent learning was antagonized by D-cycloserine. From these findings, it may be presumed that NMDA receptor affects latent learning and the impairment of latent learning induced by PCP is suggested to be mediated via PCP binding sites within the NMDA ion-channel complex.

There are many reports that sigma receptor ligands, such as (+)-SKF-10,047, SA4503 and (+)-pentazocine, significantly attenuate via $sigma_1$ receptors, the learning impairment induced by the non-competitive NMDA receptor antagonist, dizocilpine. It has been proposed that $sigma_1$ receptor ligands exert their facilitating actions on the NMDA responses by modulation of the receptor-associated ion channel activity. Interestingly, $sigma_1$ receptor ligands, SA4503 and (+)-pentazocine, attenuated the PCP- or dizocilpine-induced impairment of latent learning in mice. The attenuating effects of SA4503 and (+)-pentazocine on PCP- or dizocilpine-induced impairment of latent learning was antagonized by NE-100, a $sigma_1$ receptor antagonist, suggesting that the attenuating effect of SA4503 and (+)-pentazocine is mediated via $sigma_1$ receptors. It appears that SA4503 has a similar efficiency against the learning impairment induced by dizocilpine. Indeed, selective $sigma_1$ receptor ligands attenuated the dizocilpine-induced impairment of various learning tasks. Maurice et al. (1994) have reported that DTG, (+)-SKF-10,047, (+)-pentazocine and PRE-084 reverse the dizocilpine-induced impairments of learning in a variety of behavioral tests in mice. Results of recent studies (Earley et al. 1995; Ohno and Watanabe 1995), and the present results further support our conclusions, indicating that $sigma_1$ receptor agonists are able to reverse the behavioral effects induced by blockade of the NMDA receptor-mediated neurotransmission. There is functional interaction between $sigma_1$ receptors and NMDA receptor complex (Monnet et al. 1992), showing that $sigma_1$ receptor agonists allow a marked potentiation of several NMDA-evoked responses. The effect of SA4503 on the NMDA-evoked responses has not yet been characterized, but it may be similar to that of other $sigma_1$ receptor agonists as suggested by its efficacy to attenuate the PCP-induced impairment of latent learning.

(+)-SKF-10,047 did not attenuate the PCP-induced impairment of latent learning in mice, rather it potentiated it dose dependently. This result may be explained by the difference of affinities for the PCP binding sites between (+)-SKF10,047, SA4503, and (+)-pentazocine. Indeed, (+)-SKF10,047 has a higher affinity for PCP binding sites (de Costa and He 1994), and SA4503 and (+)-pentazocine are more selective $sigma_1$ receptor ago-

nists (de Costa and He 1994; Matsuno et al. 1996). Thus, it is suggested that (+)-SKF-10,047 at the dose ranges used, especially 3 mg/kg, had PCP-like effects, since (+)-SKF-10,047 (3 mg/kg) itself showed a tendency to prolong finding latency, but not drinking latency, but other $sigma_1$ receptor agonists, SA4503 and (+)-pentazocine, themselves did not show it. However, this point must be considered with caution, as the neuropharmacology of sigma ligands remains to be clarified.

In conclusion, the present study indicates that PCP impairs latent learning, the effect possibly mediated via PCP binding sites within the NMDA receptor ionophore complex. Moreover, the sigma receptor, especially the $sigma_1$ site, could modulate the NMDA-mediated response (i.e., latent learning). Thus, these results suggest that the glutamatergic systems play a role in attention, and $sigma_1$ receptor agonist is a useful therapeutic strategy for deficits in learning and memory that contribute to the symptoms of schizophrenia. This PCP animal model will also contribute to further understanding of the mechanisms of the impairment of latent learning (attention) in schizophrenia.

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