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Short communication

Phencyclidine-induced cognitive deficits in mice are improved by subsequent subchronic administration of clozapine, but not haloperidol

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Abstract

This study was undertaken to examine the effects of subsequent administration of antipsychotic drugs (clozapine and haloperidol) on cognitive deficits in mice after repeated administration of phencyclidine (PCP). In the novel object recognition test, repeated administration of PCP (10 mg/kg) significantly decreased exploratory preference in the retention test session but not in the training test session. PCP-induced deficits were significantly improved by subsequent subchronic (2 weeks) administration of clozapine (5 mg/kg), but not haloperidol (0.1 mg/kg). These findings suggest that PCP-induced cognitive deficits using the novel object recognition test may be a potential animal model of atypical antipsychotic activity.
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1. Introduction

Cognitive deficits in patients with schizophrenia are a core feature of the illness, which predicts vocational and social disabilities for patients (Freedman, 2003; Green et al., 2004). Multiple lines of evidence suggest that a dysfunction in the glutamatergic neurotransmission via the *N*-methyl-D-aspartate (NMDA) receptors might be involved in the pathophysiology of schizophrenia (Javitt and Zukin, 1991; Olney and Farber, 1995; Coyle, 1996; Krystal et al., 1999; Hashimoto et al., 2003, 2004, 2005). The NMDA receptor antagonists such as phencyclidine (PCP) are known to induce schizophrenia-like symptoms including cognitive deficits in healthy subjects (Javitt and Zukin, 1991; Krystal et al., 1999). Therefore, PCP has been used as an animal model of cognitive deficits in schizophrenia (Jentsch and Roth, 1999; Mandillo et al., 2003; Sams-Dodd, 1998). It is also well known that the incidence of extrapyramidal side effects of the atypical antipsychotic drug clozapine is lower than that of the typical antipsychotic drug haloperidol, and that clozapine has more efficacy than haloperidol against cognitive deficits in patients with schizophrenia (Potkin et al., 2001), suggesting that atypical antipsychotic drugs could improve cognitive deficits as compared with typical antipsychotic drugs. In the present study, using the novel object recognition test, we examined the effects of subsequent acute or subchronic treatment with antipsychotic drugs (clozapine and haloperidol) on cognitive deficits in mice after repeated administration of PCP.

2. Methods

2.1. Animals

Male ICR mice (6 weeks old) weighing 25–30 g were purchased from SLC Japan (Hamamatsu, Shizuoka, Japan). Mice were housed in the clear polycarbonate cages (22.5 × 33.8 × 14.0 cm) and in groups of 4 or 5 mice under a controlled 12/12-h light–dark cycle (light from 7:00 AM to 7:00 PM), with room

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temperature at 23±1 °C and humidity at 55±5%. The mice were given free access to water and food pellets for mice. The experimental procedure was approved by the Animal Care and Use Committee of Chiba University Graduate School of Medicine.

2.2. Drug administration

PCP hydrochloride was synthesized in our laboratory. Saline (10 ml/kg) or PCP (10 mg/kg expressed as a hydrochloride salt) were administered subcutaneously (s.c.) for 10 days (once daily on days 1–5, 8–12), and no treatment was on days 6, 7, 13 and 14. In a single and acute experiment, 3 days (days 15) after a final administration of saline or PCP, vehicle (10 ml/kg; 0.8% acetic acid), clozapine (5 mg/kg; Novartis Pharmaceuticals, Ltd., Basel, Switzerland)) or haloperidol (0.1 mg/kg; Wako Pure Chemicals Ltd., Tokyo, Japan) were administered intraperitoneally (i.p.). In a separate experiment, 3 days (days 15) after a final administration of saline or PCP, vehicle (10 ml/kg; 0.8% acetic acid), clozapine (5 mg/kg) or haloperidol (0.1 mg/kg) were administered intraperitoneally (i.p.) for consecutive 2 weeks (once daily on days 15–28). The dose of clozapine and haloperidol was selected since these dose were effective in the latent inhibition models of adult offspring of poly I:C treated dams (Zuckerman et al., 2003).

2.3. Novel object recognition test

In the experiment of acute treatment, 1 h after a final administration of vehicle, clozapine or haloperidol, novel object recognition test was performed as previously reported (Tang et al., 1999, 2001). In the experiment of subchronic treatment, novel object recognition test was performed 1 day after a final administration of vehicle, clozapine or haloperidol. The apparatus for this task consisted of a black open field box (50.8×50.8×25.4 cm). Before the test, mice were habituated in the box for 3 days. During a training session, two objects (various objects differing in their shape and color but similar in size) were placed in the box 35.5 cm apart (symmetrically) and each animal was allowed to explore in the box for 5 min. The animals were considered to be exploring the object when the head of the animal was facing the object within an inch from the object or any part of the body, except for the tail, was touching the object. The time that mice spent exploring each object was recorded. After training, mice were immediately returned to their homecages, and the box and objects were cleaned with 75% ethanol to avoid any possible instinctive odorant cues. Retention tests were carried out at 1-day intervals following the respective training. During the retention test, each mouse was placed back into the same box, in which one of the objects used during training was replaced by a novel one. The mice were then allowed to freely explore for 5 min and the time spent exploring each object was recorded. Throughout the experiments, the objects were used in a counter-balanced manner in terms of their physical complexity. A preference index, a ratio of the amount of time spent exploring any one of the two objects (training session) or the novel one (retention session) over the total time spent exploring respective to both objects, was used to measure memory performance.

2.4. Statistical analysis

Data are expressed as mean±S.E.M. Statistical analysis was performed by using Student *t*-test or one-way analysis of variance (ANOVA) and post hoc Bonferroni test. *P* values less than 0.05 were considered statistically significant.

3. Results

In the novel object recognition test, repeated administration of PCP (10 mg/kg/day for 10 days) caused significant cognitive deficits 3 days (days 15) and 6 weeks (days 57) after a final administration of PCP. In the training session, there was no

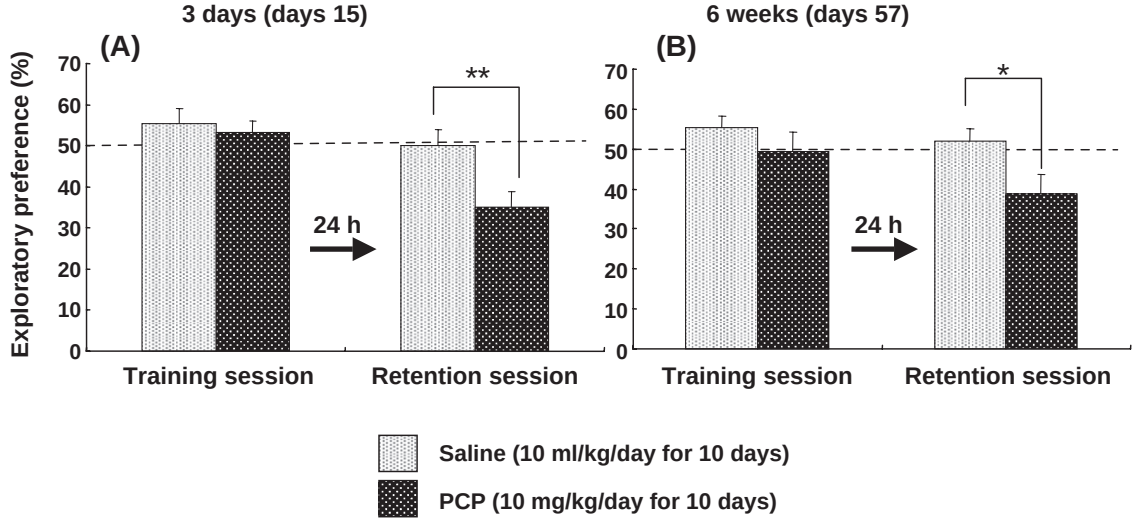

Fig. 1. Repeated administration of PCP caused cognitive deficits in mice. Saline (10 ml/kg, s.c.) or PCP (10 mg/kg, s.c.) were administered for 10 days (once daily on days 1–5, 8–12). In the short-term experiment (3 days after the last administration), the novel object recognition test was performed on days 15 and 16. In the long-term experiment (6 weeks after the last administration), the novel object recognition test was performed on days 57 and 58. The exploratory preference (%) on the *Y* axis is referring to the preference toward the novel object, thus meaning the ability to discriminate between novel object and no-novel object. Values are the mean±S.E.M ($n=7$). *$P<0.05$, **$P<0.01$ as compared with saline-treated group.

difference in the exploratory preference of mice in the PCP-treated and saline-treated control groups (Fig. 1). In the retention session, the exploratory preference of PCP-treated mice was significantly lower than that of mice in the saline-treated group at 3 days ($t=3.14$, $P=0.009$) and 6 weeks ($t=2.20$, $P=0.048$) after a final administration of PCP (Fig. 1). During the training session, there were no significant differences between the two groups in the total amount of time spent exploring two objects.

In the retention session, a single administration of clozapine (5 mg/kg, 1 h) or haloperidol (0.1 mg/kg, 1 h) did not alter reduction of the exploratory preference in mice after repeated administration of PCP (Fig. 2A). In contrast, PCP-induced deficits were significantly improved after subsequent subchronic (2 weeks) administration of clozapine (5 mg/kg/day), but not haloperidol (0.1 mg/kg/day). In the training session, the exploratory preference of four groups was not different (F (3,44)= 1.52, $P=0.224$) (Fig. 2B). However, in the retention session, ANOVA analysis revealed that the exploratory preference of mice in the four groups was significantly different (F (3,44)= 9.44, $P<0.001$) (Fig. 2B). A post hoc Bonferroni test indicated that the exploratory preference of the PCP-treated group was significantly increased after subchronic administration of clozapine ($P<0.001$), but not haloperidol ($P=1.00$) (Fig. 2B).

4. Discussion

The major findings of the present study are that repeated administration of PCP (10 mg/kg/day for 10 days) caused cognitive deficits in mice for a long time (more than 6 weeks after a final administration of PCP), and that PCP-induced cognitive deficits could be improved by subsequent subchronic administration of clozapine, but not haloperidol. In the novel object recognition test, no significant differences in total amount of time spent exploring two objects or in exploratory preference were found between two groups during the training session, suggesting that levels of motivation, curiosity, and interest in exploring novel objects were the same in the two groups. Repeated administration of PCP significantly decreased the exploratory preference in the retention session but not in the training session. In the

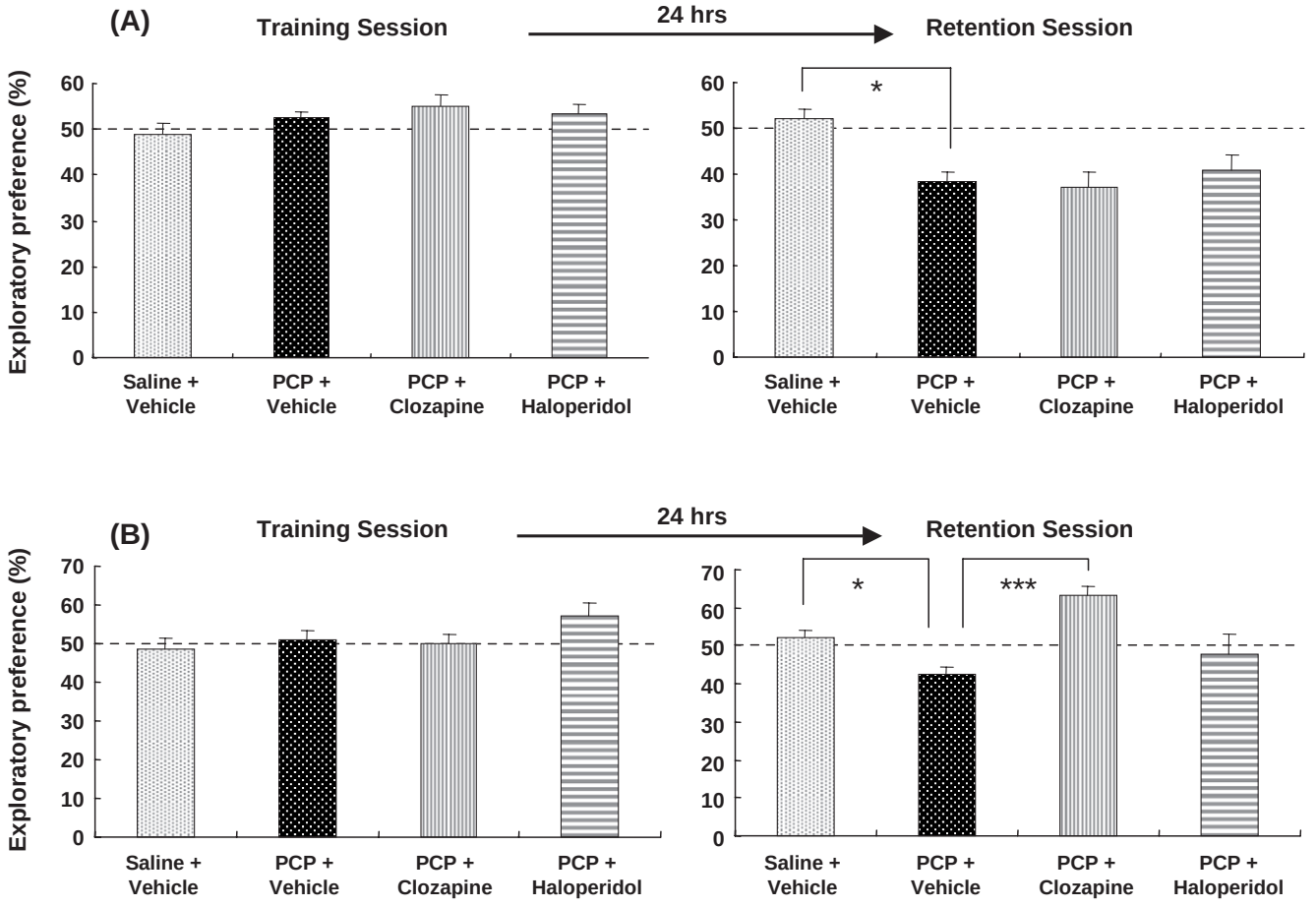

Fig. 2. Effects of clozapine and haloperidol on PCP-induced cognitive deficits in mice. Saline (10 ml/kg) or PCP (10 mg/kg) were administered s.c. for 10 days (once daily on days 1–5, 8–12). (A) Three days (days 15) after the last administration of saline or PCP, vehicle (10 ml/kg; 0.8% acetic acid), clozapine (5 mg/kg) or haloperidol (0.1 mg/kg) were administered i.p. into mice. The novel object recognition test was performed 1 h after administration. Values are the mean±S.E.M ($n=6$–13). *$P<0.05$ as compared with saline-treated group. (B) Three days (days 15) after the last administration of saline or PCP, vehicle (10 ml/kg; 0.8% acetic acid), clozapine (5 mg/kg) or haloperidol (0.1 mg/kg) were administered i.p. into mice for consecutive 2 weeks (once daily on days 15–28). On days 29 and 30, the novel object recognition test was performed. Values are the mean±S.E.M ($n=9$–14). *$P<0.05$ as compared with saline-treated group. ***$P<0.001$ as compared with PCP-treated group.

retention session, the exploratory preference (approximately 40%) of the PCP-treated group was significantly lower than that (approximately 50%) of the saline-treated group, suggesting that the behavior of the PCP-treated mice may not have been due to memory impairment. In contrast, in the 1-h retention session, the exploratory preference (approximately 50%) of the PCP-treated group was significantly lower than that (approximately 60%) of the saline-treated group (data not shown), suggesting that these acute deficits may, in part, be associated with retention memory deficits. Furthermore, it has been reported that repeated administration of PCP caused social interaction deficits in animals (Mandillo et al., 2003; Sams-Dodd, 1998). In addition, negative symptoms such as social withdrawal are related to cognitive deficits in schizophrenic patients (Zakzanis, 1998). Considering these findings, it is likely that our model of PCP-induced cognitive deficits, using the novel object recognition test, may show negative symptoms such as social withdrawal, which are related to cognitive deficits. Interestingly, we found that PCP-induced cognitive deficits could be improved by subsequent subchronic administration of clozapine, but not haloperidol. Therefore, reversal of PCP-induced cognitive deficits, using the novel object recognition test, may be a potential animal model of atypical antipsychotic activity in relation to amelioration of cognitive deficits in schizophrenia.

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