Effect of PCP on performance in novel object recognition

Eight days after the last treatment with PCP (10 mg/kg, for 14 days), the novel object recognition test was performed. During habituation, no behavioral change was observed between PCP- and saline-treated mice. In the training session, PCP-treated and saline-treated mice spent equal amounts of time exploring either of the two objects (Fig. 3b,c), and thus there was no biased exploratory preference in either group of animals. In addition, total time spent in the exploration of objects in the training session did not differ between PCP- and saline-treated mice (Fig. 3d,e). These observations suggest that repeated PCP treatment has no effect on motivation, curiosity, or motor function.

When retention session was performed 24 h after the training session, the level of exploratory preference for the novel objects in the PCP-treated mice was significantly decreased compared to that in the saline-treated mice ($p<0.01$, Fig. 3b,c). The total exploration time did not differ between the two groups in the retention session (Fig. 3d,e). These results suggest that repeated PCP treatment for 14 days induces recognition memory impairment 8 days after the withdrawal of PCP.

Effects of single and repeated administrations of aripiprazole and haloperidol on PCP-induced cognitive impairment in novel object recognition

We examined whether PCP-induced cognitive impairment was reversed by aripiprazole, an atypical antipsychotic, or haloperidol, a typical antipsychotic, treatment. After the cessation of repeated PCP treatment (10 mg/kg s.c., for 14 days), mice were subjected to the novel object recognition test. Aripiprazole (0.01–1.0 mg/kg, p.o.) or haloperidol (0.3–1.0 mg/kg, p.o.) was acutely administered 1 h before the training session. A one-way ANOVA revealed that single treatment with aripiprazole dose-dependently improved cognitive impairment in PCP-treated mice [$F(5,56)=3.474$, $p<0.01$, Fig. 3b]. In contrast, single treatment with haloperidol had no effect on the cognitive impairment induced by repeated PCP treatment (Fig. 3c). Neither aripiprazole nor haloperidol affected the level of exploratory preference for the novel object in the training session (Fig. 3b,c). However, single treatment with aripiprazole and haloperidol decreased total exploration time in the training session of PCP-treated mice [one-way ANOVA: $F(6,77)=5.009$, $p<0.01$, Fig. 3d; $F(3,39)=4.665$, $p<0.01$, Fig. 3e]. Single treatment with aripiprazole (0.3 and 1.0 mg/kg) or haloperidol (1.0 mg/kg) significantly decreased the total exploration time in the training session of PCP-treated mice ($p<0.05$ and $p<0.01$, respectively, Fig. 3d; $p<0.05$, Fig. 3e). In the saline-treated mice, neither aripiprazole nor haloperidol affected the level of exploratory preference for the novel object in the training session (data not shown). However, single treatment with aripiprazole and haloperidol decreased total exploration time in the training session of saline-treated mice [one-way ANOVA: $F(5,66)=6.532$, $p<0.01$ for aripiprazole; $F(2,30)=22.346$, $p<0.01$ for haloperidol].

Next, we examined the effect of repeated treatment with antipsychotics on PCP-induced cognitive impairment. Aripiprazole (0.01–0.1 mg/kg) or haloperidol (0.3 and 1.0 mg/kg) was repeatedly administered p.o. for 7 days to mice that had been previously treated with PCP for 14 days. As shown in Fig. 4, repeated treatment with aripiprazole dose-dependently improved cognitive impairment in PCP-treated mice [$F(4,43)=9.166$, $p<0.01$], and a significant change was observed with doses of 0.03 and 0.1 mg/kg ($p<0.05$ and $p<0.01$, respectively, Fig. 4b). In contrast, repeated treatment with haloperidol failed to improve PCP-induced cognitive impairment (Fig. 4c). Repeated treatment with aripiprazole and haloperidol affected neither the level of exploratory preference for the novel object in the training session nor the total exploration time in either the training or retention sessions for PCP-treated mice (Fig. 4). In the saline-treated mice, repeated treatment with aripiprazole or haloperidol alone showed no effect on performance in the novel object recognition test (data not shown).

Effects of dopamine D_1 and D_2 receptor antagonists on ameliorative effect of aripiprazole against PCP-induced cognitive impairment

We have previously demonstrated that repeated PCP treatment in mice induces the dysfunction of dopamine neurotransmission in the prefrontal cortex which is necessary for the recognition memory (Mouri et al. 2007b; Nagai et al. 2007). Therefore, we investigated whether activation of dopamine receptors was involved in the ameliorating effect of aripiprazole on memory impairment in PCP-treated mice. SCH23390 (0.05 mg/kg i.p.), a dopamine D_1 receptor antagonist, or raclopride (0.3 mg/kg i.p.), a dopamine D_2 receptor antagonist, was co-administered with aripiprazole for 7 days, and the training session of the novel object recognition test was performed 1 day after the last treatment.

SCH23390 significantly blocked the ameliorating effect of aripiprazole on the impairment of exploratory preference for a novel object in PCP-treated mice ($p<0.01$, Fig. 5b), although it had no effect on PCP-induced impairment of memory retention (Fig. 5b). Treatment with SCH23390 did not affect the total exploration time in either the training or retention sessions (Fig. 5d). In contrast, treatment with raclopride had no effect on exploratory preference or total exploration time in the training and retention sessions (Fig. 5c,e).

Effect of serotonin 5-HT_{1A} receptor antagonist on ameliorative effect of aripiprazole against PCP-induced cognitive impairment

It has been reported that aripiprazole also has partial agonistic activity for serotonin 5-HT_{1A} receptors in parallel to its actions at dopamine D_2 receptors (Jordan et al. 2002); therefore, we examined whether 5-HT_{1A} receptors were involved in the ameliorative effect of aripiprazole on memory impairment in PCP-treated mice. The 5-HT_{1A} receptor antagonist WAY100635 (0.6 mg/kg, i.p.) was co-administered with aripiprazole for 7 days, and the training session of the novel object recognition test was performed 1 day after the last treatment.

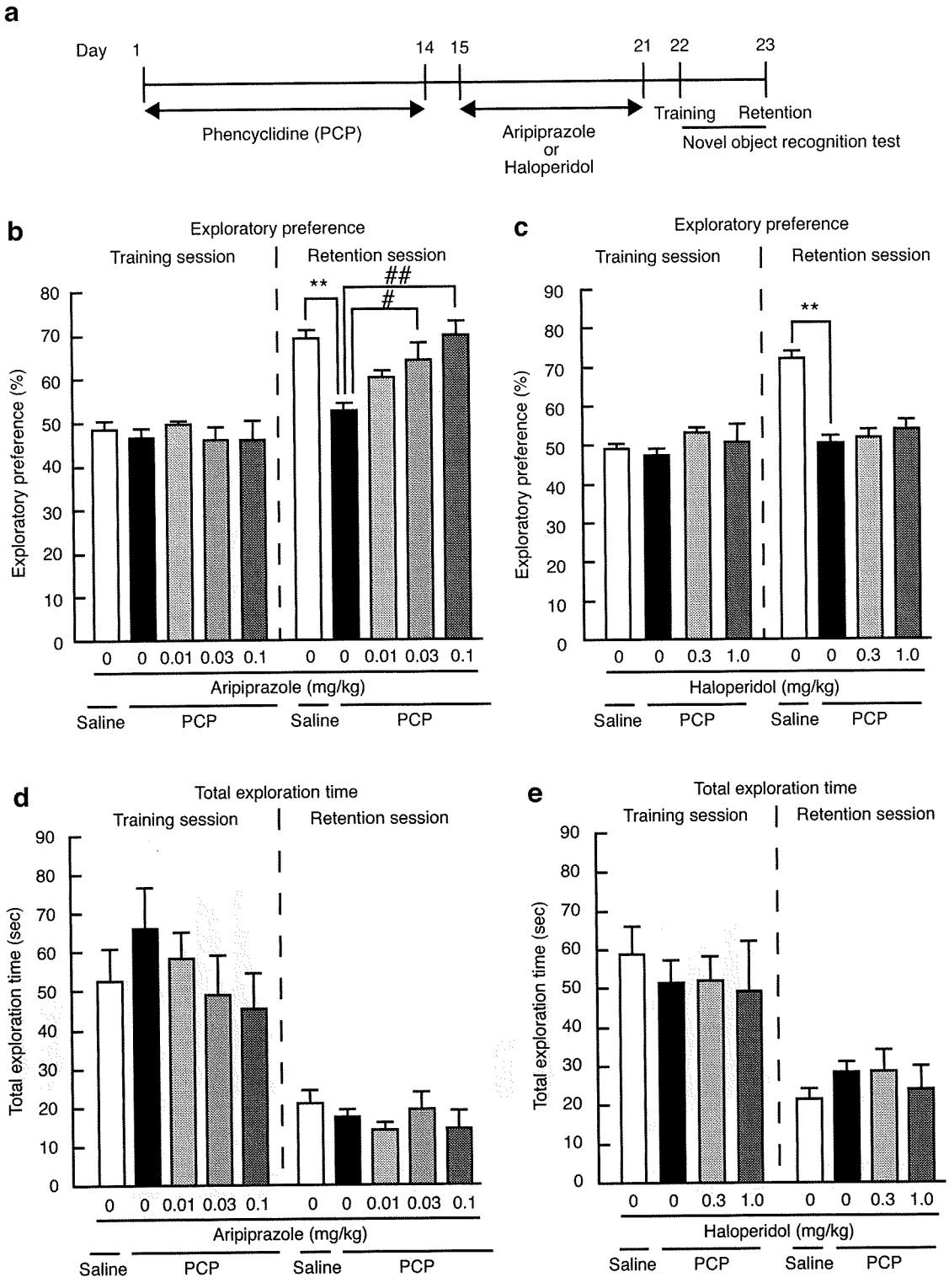
In the training session, treatment with WAY100635 alone did not affect the exploratory preference for objects in PCP-treated mice (Fig. 6b). In the retention session, WAY100635 blocked the ameliorating effect of aripiprazole on the impairment of exploratory preference for a novel object in PCP-treated mice ($p<0.01$, Fig. 6b), although it had no effect on PCP-induced impairment of memory retention (Fig. 6b). The antagonistic effect of WAY100635 on aripiprazole-induced improvement of exploratory preference in PCP-treated mice was not associated with changes in total exploration time (Fig. 6c).

Discussion

Cognitive deficits, including memory impairment, are regarded as a core feature in schizophrenia (Tamminga 2006). Recognition memory is a fundamental facet of the ability to remember and an integral component of the class of memory lost in amnesia (Aggleton and Brown 1999). The ability to discriminate familiar from novel stimuli is supported by this form of memory. To assess the effect of a novel atypical antipsychotic, aripiprazole, on cognitive dysfunction, we used a novel object recognition task, which is similar to visual recognition tests widely used in subhuman primates (Ennaceur and Delacour 1988). In the present study, an object preference index, the ratio of the amount of time spent exploring any one of the two objects (training session) or the novel object (retention session) over the total time spent exploring both object, was used to measure cognitive function. However, it is possible that the ratio misleads the results when based on a low amount a little second on average for the whole group of mice. Therefore, we also run a paired comparisons test for each group comparing the time spent on a novel with that spent on a familiar object. The saline-treated mice spent an equal time exploring the two objects (object A and object B) in the training session (Supplemental Fig. 1a), but a significantly greater time exploring the novel object (object C) versus the familiar object (object A) in the retention session (Supplemental Fig. 1b), showing that they were able to discriminate the novel object during the retention session. PCP + vehicle-treated mice also spent an equal time exploring the two objects in training session (Supplemental Fig. 1a). However, PCP + vehicle-treated mice spent equivalent times exploring the novel and the familiar objects in retention session (Supplemental Fig. 1b), whereas PCP + single aripiprazole-treated mice spent greater time exploring the novel (Supplemental Fig. 1b). These observations agree with the results using an object preference index ratio. Taken together, it is unlikely that the ratio misleads the results in this study.

In the present study, repeated PCP treatment for 14 days induced recognition memory impairment 8 days after the withdrawal of PCP, and PCP-induced cognitive impairment was ameliorated by aripiprazole, but not haloperidol. The results are consistent with a previous report that PCP-induced cognitive deficits were improved by atypical antipsychotics, such as clozapine and perospiron, but not a typical antipsychotic, haloperidol, in a novel object recognition test (Hagiwara et al. 2008; Hashimoto et al. 2005). It is possible that the doses of haloperidol (0.3–1.0 mg/kg) used in the present study is probably too high in terms of occupancy compared to that produced by aripiprazole (0.01–1.0 mg/kg) and the relative affinities for D_2 receptors (Hirose and Kikuchi 2005). Haloperidol at the dose of 0.3 mg/kg was used as the maximal dose which did not cause locomotor suppression in this study, but it had no effect on PCP-induced memory impairment. In addition, it has been demonstrated that more low doses of haloperidol (0.05–0.1 mg/kg) do not improve PCP-induced memory impairment in the novel object recognition test (Grayson et al. 2007; Hashimoto et al. 2005). Taken together, these findings suggest that aripiprazole, but not haloperidol, may be useful for the treatment of cognitive dysfunction in schizophrenia.

Fig. 4 Effects of repeated administrations of aripiprazole and haloperidol on PCP-induced cognitive impairment in novel object recognition. **a** Experimental schedule for the novel object recognition test. Aripiprazole (0.01–0.1 mg/kg, p.o.), haloperidol (0.3–1.0 mg/kg, p.o.), or vehicle (0.1% CMC) was administered for 7 days to mice that had previously been treated with saline or PCP (10 mg/kg, s.c.) for 14 days. The novel-object recognition test was performed 24 h after the last treatment with aripiprazole or saline. **b** and **d** Effect of aripiprazole on PCP-induced cognitive impairment. **c** and **e** Effect of haloperidol on PCP-induced cognitive impairment. **b** and **c** Exploratory preference. **d** and **e** Total exploration time. Values indicate the mean±SE (n=8–15). Analysis of variance: $F(4,43)=0.851$, $p=0.50$ for (**b**) training; $F(4,43)=9.166$, $p<0.01$ for (**b**) retention; $F(3,42)=1.049$, $p=0.38$ for (**c**) training; $F(3,42)=25.898$, $p<0.01$ for (**c**) retention; $F(4,43)=1.157$, $p=0.34$ for (**d**) training; $F(4,43)=1.029$, $p=0.40$ for (**d**) retention; $F(3,42)=0.305$, $p=0.82$ for (**e**) training; $F(3,42)=0.915$, $p=0.44$ for (**e**) retention. $^{**}p<0.01$ compared with saline + vehicle group. $\#p<0.05$ and $\#\#p<0.01$ compared with PCP + vehicle group



It has been reported that aripiprazole acts as a dopamine D_2 receptor antagonist in the state of excessive dopamine neurotransmission and as a dopamine D_2 receptor agonist in the state of low dopaminergic neurotransmission (Burris et al. 2002; Kikuchi et al. 1995; Inoue et al. 1996). Single treatment with aripiprazole (1.0 mg/kg) ameliorated PCP-induced impairment of recognition memory, although the treatment significantly decreased total exploration time in

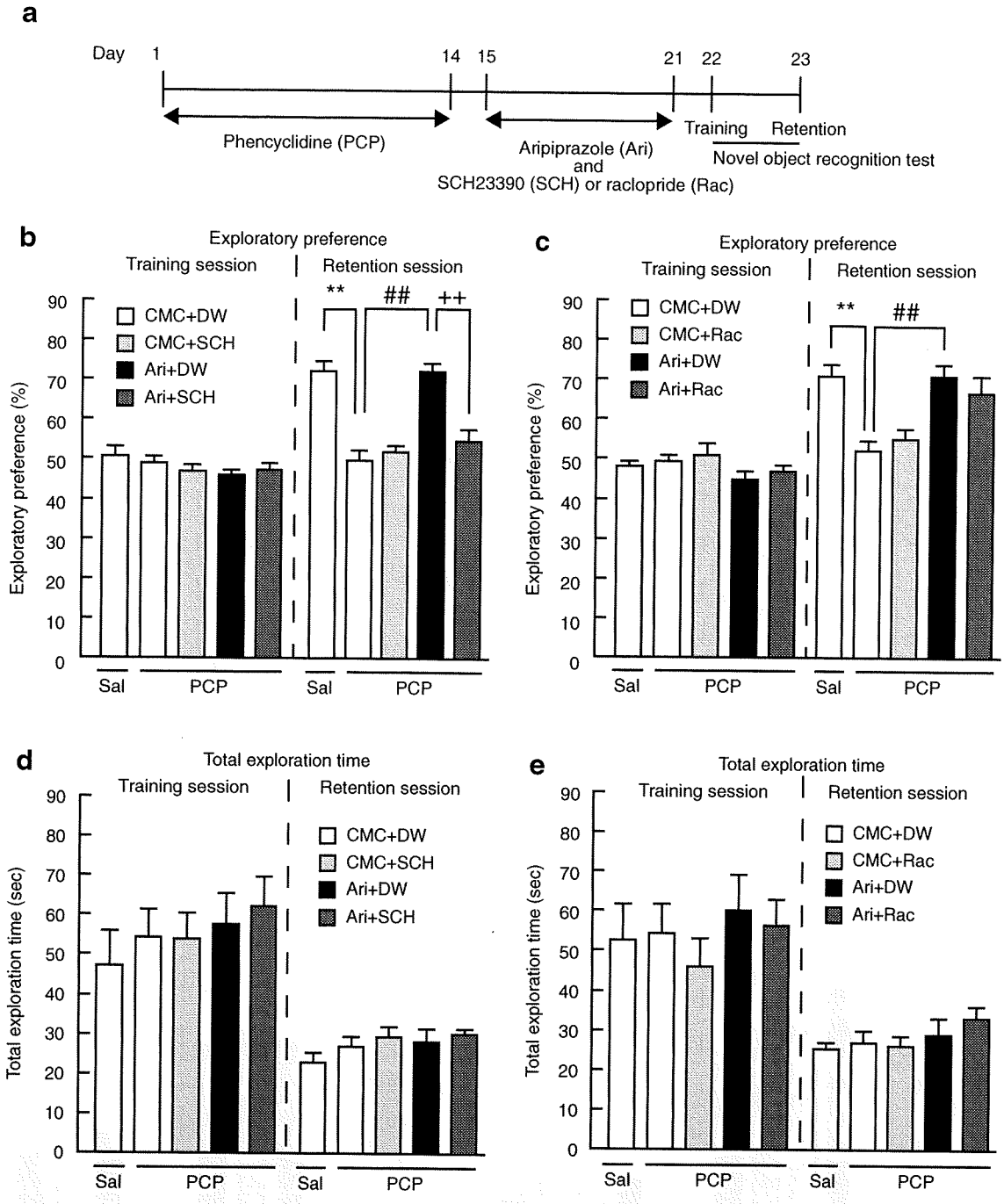


Fig. 5 Effect of dopamine D_1 and D_2 receptor antagonists on ameliorative effect of aripiprazole against PCP-induced cognitive impairment. **a** Experimental schedule for the novel object recognition test using dopamine D_1 and D_2 receptor antagonists. Aripiprazole (Ari, 0.1 mg/kg, p.o.) or vehicle (0.1% CMC) was administered for 7 days to mice that had previously been treated with saline (Sal) or PCP (10 mg/kg, s.c.) for 14 days. SCH23390 (SCH, 0.05 mg/kg, i.p.), Raclopride (Rac, 0.3 mg/kg, i.p.) or distilled water (DW) was administered 30 min after aripiprazole treatment for 7 days. The novel-object recognition test was performed 24 h after the last treatment with Ari. **b** and **d** Effect of SCH on ameliorative effect of Ari against PCP-induced cognitive impairment. **c** and **e** Effect of Rac on ameliorative effect of Ari against PCP-induced cognitive impairment. **b** and **c** Exploratory preference. **d** and **e** Total exploration time. Values indicate the mean±SE (n=9–11). Analysis of variance: $F(4,50)=0.951$, $p=0.44$ for (**b**) training; $F(4,50)=20.732$, $p<0.01$ for (**b**) retention; $F(4,42)=1.212$, $p=0.32$ for (**c**) training; $F(4,42)=8.520$, $p<0.01$ for (**c**) retention; $F(4,50)=0.527$, $p=0.72$ for (**d**) training; $F(4,50)=1.261$, $p=0.30$ for (**d**) retention. $F(4,42)=0.426$, $p=0.79$ for (**e**) training; $F(4,42)=1.210$, $p=0.32$ for (**e**) retention. $^{**}p<0.01$ compared with Sal + CMC + DW group. $^{\#\#}p<0.01$ compared with PCP + CMC + DW group. $^{++}p<0.01$ compared with PCP + Ari + DW group

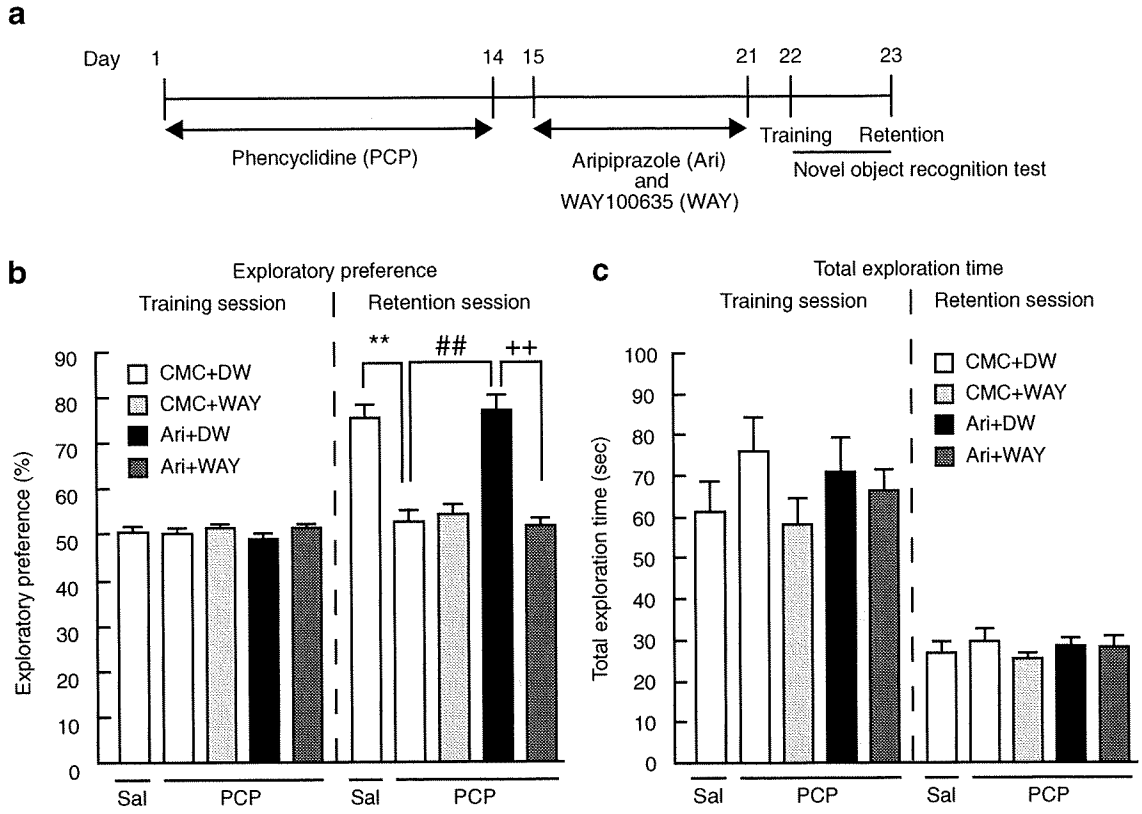


Fig. 6 Effect of serotonin 5-HT_{1A} receptor antagonist on ameliorative effect of aripiprazole against PCP-induced cognitive impairment. **a** Experimental schedule for the novel object recognition test using a serotonin 5-HT_{1A} receptor antagonist. Aripiprazole (Ari, 0.1 mg/kg, p.o.) or vehicle (0.1% CMC) was administered for 7 days to mice that had previously been treated with saline (Sal) or PCP (10 mg/kg, s.c.) for 14 days. WAY100635 (WAY, 0.6 mg/kg, i.p.) or distilled water (DW) was administered 30 min after Ari treatment for 7 days. The novel-object recognition test was performed 24 h after the last treatment with Ari. **b** Exploratory preference. **c** Total exploration time. Values indicate the mean±SE (n=9–10). Analysis of variance: $F(4,44)=0.921$, $p=0.46$ for (**b**) training; $F(4,44)=25.562$, $p<0.01$ for (**b**) retention; $F(4,44)=0.915$, $p=0.46$ for (**c**) training; $F(4,44)=0.327$, $p=0.86$ for (**c**) retention. $^{**}p<0.01$ compared with Sal + CMC + DW group. $\#\#p<0.01$ compared with PCP + CMC + DW group. $++p<0.01$ compared with PCP + Ari + DW group

the training session. Therefore, aripiprazole at 0.1 mg/kg was used in the repeated treatment as the maximal dose which did not cause locomotor suppression in mice.

Accumulating evidence suggests that the dopaminergic system in the prefrontal cortex is involved in cognitive function. For instance, disruption of dopamine transmission in the prefrontal cortex by infusions of dopamine D_1 receptor antagonists or by excitotoxic lesions impairs the performance of object retrieval detour tasks, as well as delayed response tasks in nonhuman primates (Sawaguchi and Goldman-Rakic 1991; Dias et al. 1996a, b). A recent study with functional magnetic resonance imaging showed that dysfunction in the prefrontal cortex of schizophrenic patients is related to cognitive impairment (Tan et al. 2007). Accordingly, cognitive impairment in schizophrenia may be associated with deficits in dopamine transmission in the prefrontal cortex. In the present study, the ameliorative effect of aripiprazole on PCP-induced cognitive impairment was prevented by a dopamine D_1 receptor antagonist, but not a dopamine D_2 receptor antagonist. Our previous study has demonstrated that stimulation with dopamine D_1 receptors is necessary for long-term retention of recognition memory in the prefrontal cortex (Kamei et al. 2006; Nagai et al. 2007). Taken together, these findings indicated that dopamine D_1 receptor in the prefrontal cortex may play a critical role in the ameliorative effect of aripiprazole on PCP-induced cognitive impairment.

Although aripiprazole has high affinity for dopamine D_2 receptors, a dopamine D_2 receptor antagonist had no effect on the ameliorative effect of aripiprazole on PCP-induced cognitive impairment. One possible reason for this discrepancy is that, the ability of dopamine D_1 receptor stimulation to improve cognition is due to a particular cellular localization in cortical networks: It has been demonstrated that dopamine D_1 receptors preferentially localize to non-pyramidal neurons, while dopamine D_2 receptors localize to both nonpyramidal and pyramidal cells in the prefrontal cortex of rats (Vincent et al. 1995). The other possible reason is that the ameliorative effect of aripiprazole on PCP-induced cognitive impairment may be involved in

receptors other than the dopamine D_2 receptor, since aripiprazole interacts with not only dopamine D_2 receptor, but also a large number of biogenic amine receptors (Shapiro et al. 2003). However, we cannot exclude the possibility that a part of ameliorative effect of aripiprazole on PCP-induced cognitive impairment is through dopamine D_2 receptor. Further studies are needed by using other dopamine D_2 antagonists or dopamine D_2 receptor knockout mice.

Accumulating evidence has suggested that serotonin 5-HT_{1A} receptors are an important target for cognitive dysfunction in schizophrenia (Bantick et al. 2001; Meltzer 1999). The density of 5-HT_{1A} receptor binding is altered in the hippocampus and cerebral cortex of the postmortem brain of schizophrenic patients (Burnet et al. 1996; Gurevich and Joyce 1997; Joyce et al. 1993; Lopez-Figueroa et al. 2004). Adjunctive treatment with tandospirone, a selective 5-HT_{1A} receptor agonist, is associated with improvements in some types of memory function as well as the cognitive performance of schizophrenic patients (Sumiyoshi et al. 2001a, b). Preclinical studies on the action of aripiprazole at 5-HT_{1A} receptors have shown partial agonist activity in vitro and in vivo (Jordan et al. 2002; Shapiro et al. 2003; Stark et al. 2007). In the present study, 5-HT_{1A} receptor antagonist blocked the ameliorating effect of aripiprazole on cognitive impairment in PCP-treated mice. Therefore, these results supported that atypical antipsychotic drugs, such as aripiprazole, clozapine, ziprasidone, and quetiapine, which have 5-HT_{1A} receptor agonist activity, are useful for cognitive impairment in schizophrenia (Jordan et al. 2002; Newman-Tancredi et al. 2001; Rollema et al. 2000; Sprouse et al. 1999)

The mechanisms by which aripiprazole ameliorates PCP-induced cognitive dysfunction through serotonin 5-HT_{1A} and dopamine D_1 receptors remain to be determined; however, it is known that the activation of 5-HT_{1A} receptors in the prefrontal cortex enhances the activity of dopaminergic neurons in the ventral tegmental area and mesocortical dopamine release (Díaz-Mataix et al. 2005). Aripiprazole increases the release of dopamine in the prefrontal cortex of rats and mice through the activation of 5-HT_{1A} receptors (Bortolozzi et al. 2007; Li et al. 2004; Zocchi et al. 2005). Recently, we have also observed that microinjection of 5-HT_{1A} receptor antagonist into the prefrontal cortex blocked the ameliorating effect of aripiprazole on cognitive impairment in PCP-treated mice (unpublished data). Accordingly, it is likely that stimulation of 5-HT_{1A} receptors in the prefrontal cortex induces dopamine D_1 receptor activation through the mesocortical dopaminergic pathway, which is involved in the ameliorating effect of aripiprazole on PCP-induced cognitive dysfunction.

Since aripiprazole has the 5-HT_{2A} receptor antagonistic activity displayed by atypical antipsychotics, such as clozapine, olanzapine, and risperidone (McQuade et al. 2002), involvement of 5-HT_{2A} receptors in the ameliorating effect of aripiprazole remains to be determined. However, it has been reported that 5-HT_{2A} receptor blockade increases dopamine release in the prefrontal cortex by atypical antipsychotics, and the increase of dopamine release is partly or totally antagonized by 5-HT_{1A} antagonist and by a defect of the 5-HT_{1A} receptor gene (Ichikawa et al. 2001; Díaz-Mataix et al. 2005). Therefore, it is likely that atypical antipsychotics through 5-HT_{2A} blockade, regardless of intrinsic 5-HT_{1A} affinity, may promote the ability of 5-HT_{1A} receptor stimulation to increase dopamine release.

In conclusion, we demonstrated that repeated PCP treatment impaired the recognition memory of novel objects. Single treatment with aripiprazole (1.0 mg/kg) ameliorated PCP-induced impairment of recognition memory, although it significantly decreased the total exploration time in the training session. Repeated treatment with aripiprazole at doses of 0.03 and 0.1 mg/kg for 7 days showed a significant ameliorating effect on PCP-induced impairment of recognition memory without affecting the total exploration time in training and retention sessions. In contrast, both single and repeated treatment with haloperidol (0.3 and 1.0 mg/kg) failed to reverse PCP-induced cognitive impairment. The ameliorating effect of aripiprazole on recognition memory in PCP-treated mice was blocked by dopamine D_1 and serotonin 5-HT_{1A} receptor antagonists; however, dopamine D_2 receptor antagonist had no effect on the ameliorating effect of aripiprazole. These results suggest that the ameliorative effect of aripiprazole on PCP-induced cognitive impairment is associated with dopamine D_1 and serotonin 5-HT_{1A} receptors.

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ORIGINAL INVESTIGATION

Therapeutic potential of nicotine for methamphetamine-induced impairment of sensorimotor gating: involvement of pallidotegmental neurons

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Abstract
Introduction We have previously found that a disruption to prepulse inhibiton (PPI) induced by methamphetamine (METH) is associated with impaired functioning of pallidotegmental neurons, which play a crucial role in PPI of the startle reflex, through the activation of gamma-aminobutyric acid type B receptors in pedunculopontine tegmental neurons in mice.
Objectives Here, we examined the effect of nicotine on METH-induced impairment of PPI of the startle reflex focusing on dysfunctional pallidotegmental neurons and the neural system.
Results Nicotine (0.15–0.5 mg/kg) ameliorated the deficit in PPI induced by acute METH, and the ameliorating effect of nicotine was antagonized by nicotinic receptor antagonists such as methyllycaconitine and dihydro-β-erythroidine. The acute METH-induced disruption of PPI was accompanied by suppression of c-Fos expression in the lateral globus pallidus (LGP) as well as its induction in the caudal pontine reticular nucleus (PnC) in mice subjected to the PPI test. Nicotine-induced amelioration of PPI deficits in METH-treated mice was accompanied by a reversal of the changes in c-Fos expression in both the LGP and PnC to the basal level.
Conclusions Nicotine is effective in ameliorating the impairment of PPI caused by METH, which may be associated with normalization of the pallidotegmental neurons.

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Keywords Nicotine · Methamphetamine · Schizophrenia · Prepulse inhibition · Memory

Abbreviations

Ach	Acetylcholine
ADHD	Attention deficit hyperactivity disorders
DHβE	Dihydro-β-erythroidine
DRN	Dorsal raphe nucleus
GABA	gamma-aminobutyric acid
LGP	Lateral globus pallidus
METH	Methamphetamine
nAChRs	Nicotinic acetylcholine receptors
PFA	Paraformaldehyde
PnC	Caudal pontine reticular nucleus
PPI	Prepulse inhibiton
PPTg	Pedunculopontine tegmental neurons

Introduction

Prepulse inhibition (PPI) of the startle reflex is viewed as a measure of a process called "sensorimotor gating." Deficits in PPI are observed in patients suffering from certain psychiatric disorders such as schizophrenia (Swerdlow et al. 1994; Castellanos et al. 1996). Over the past 20 years, several studies have shown a primary neural map of brain substrates that control PPI in rodents and humans. Many papers have shown that there are two distinct but interacting neuronal circuits in PPI of the startle reflex, PPI-mediating, and regulating circuits (Fendt et al. 2001; Swerdlow et al. 2001). The acoustic startle response is mediated by the primary mammalian acoustic startle circuit that includes serial connections linking the auditory nerve, the cochlear root neurons, the caudal pontine reticular nucleus (PnC), and the spinal motor neuron (Hoffman and Searle 1968; Fendt et al. 2001; Swerdlow et al. 2001). It is well known that PnC is a critical part of the fast auditory pathway (Fendt et al. 2001; Swerdlow et al. 2001). The PPI-regulating circuit is also the serial connection composed of the hippocampus, prefrontal cortex, and nucleus accumbens (Swerdlow et al. 2001), and recent studies indicate a significant role of the ventral hippocampus–medial prefrontal cortex circuit in PPI (Shoemaker et al. 2005). Additionally, prepulse effects on the startle response may be mediated via the pedunculopontine tegmental neurons (PPTg), which are regulated by descending serial and parallel projections from the forebrain (Swerdlow et al. 2001).

Knowledge of the PPI-regulatory circuit will be an important step towards understanding the neural basis of some of the fundamental underpinnings of cognition (Swerdlow et al. 2001). Despite these studies previously mentioned, however, the neuronal mechanisms and circuits that are involved in PPI of the startle reflex have not yet been completely elucidated (Fendt et al. 2001). We have previously demonstrated that neural circuits containing pallidotegmental gamma-aminobutyric acid (GABA) neurons from the lateral globus pallidus (LGP) to PPTg, play a crucial role in PPI of the acoustic startle reflex in mice (Takahashi et al. 2007). Furthermore, single and repeated treatment with methamphetamine (METH) or MK-801 dose-dependently impaired PPI of the startle reflex (Arai et al. 2008). c-Fos immunohistochemistry revealed that the LGP was activated while the PnC was not after the PPI test in saline-treated control mice. In contrast, the LGP was not activated, whereas the PnC was in the PPI-disrupted mice treated with METH or MK-801. These results suggest that impaired functioning of the pallidotegmental neurons is involved in the disruption of PPI caused by METH and MK-801 in mice (Arai et al. 2008).

Recent studies have presented evidence that nicotine administration or cigarette smoking transiently corrects or ameliorates some of the psychophysiological abnormalities such as impairment of auditory sensory gating found in schizophrenic patients (Adler et al. 1992, 1993). Nicotine has been reported to normalize deficient auditory sensory gating in both schizophrenics and their family members (Adler et al. 1992), and nicotine blocks apomorphine-induced disruption of PPI of the acoustic startle via α7 nAchRs in rats (Suemaru et al. 2004). These studies have led researchers to hypothesize that nicotine has therapeutic effects on the attentional abnormalities in schizophrenia (Adler et al. 1992). However, it remains to be determined how nicotine ameliorates deficits in auditory sensory gating and cognitive dysfunction in schizophrenia.

In the present study, we investigated the effect of nicotine on deficits in PPI in METH-treated mice focusing on dysfunctional pallidotegmental neurons and the neural system.

Materials and methods

Animals

Male ICR mice (Nihon SLC Co., Shizuoka, Japan), 7 weeks old at the beginning of the experiments, were used. The animals were housed in plastic cages (5–6 mice per cage) and kept in a regulated environment (23±1°C, 50±5% humidity) with a 12-h light–dark cycle (lights on at 9:00 h). Food (Labo MR Stock, Nihon Nosan Kogyou Inc., Kanagawa, Japan) and tap water were available ad libitum. The animals were used for the experiments after 7 days of acclimatization to laboratory conditions. All behavioral experiments were carried out between 10:00 and 17:00 h. All animal care and use were in accordance with the National Institutes of Health Guide for the Care and Use of

Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Kanazawa University.

Drugs

METH hydrochloride (Dainippon Sumitomo Pharma Co. Ltd., Osaka, Japan), nicotine (Sigma-Aldrich, St. Louis, MO, USA), methyllycaconitine (Sigma-Aldrich), and dihydro-β-erythroidine (DHβE, Sigma-Aldrich) were dissolved in physiological saline and administered at a volume of 0.1 ml/10 g body weight. The doses examined in the present study were calculated as salt weights.

PPI test

A standard startle box applicable to mice and rats (San Diego Instruments, San Diego, CA) was used. The startle chamber consisted of a Plexiglas tube for mice (105 mm, 38 mm ID, 50 mm OD) placed in a sound-attenuated external box in which animals were individually placed. The tube was mounted on a plastic frame under which a piezoelectric accelerometer was mounted, which recorded and transduced the motion of the tube. Animals were randomly divided into nonstimulated and PPI groups and subjected to the behavioral test only once. Mice were placed into the chamber in the PPI test cage and then habituated to the experimental environment for 10 min with 65 dB of background white noise. The PPI test has three continuous sessions: (1) five startle trials; (2) ten startle trials, ten no-stimulus trials, and 40 PPI trials; and (3) five startle trials. The intertrial interval was between 10 and 20 s, and the total duration of the three sessions was 17 min. The startle trial consisted of a single 120-dB white noise burst lasting 40 ms. The PPI trials consisted of a prepulse (20 ms burst of white noise with any intensity of 69, 73, 77, or 81 dB) followed, 100 ms later, by the startle stimulus (120 dB, 40-ms white noise). Each of the four prepulse trials (69, 73, 77, or 81 dB) was presented ten times. During the no-stimulus trial, no stimulus was presented but the movement of the animal was scored. Sixty different trials were presented pseudorandomly, ensuring that each trial was presented ten times and that no two consecutive trials were identical. The resulting movement of the animal in the external box was measured during 100 ms after startle stimulus onset (sampling frequency 1 kHz), rectified, amplified, and fed into a computer, which calculated the maximal response over the 100-ms period. Basal startle amplitude was determined as the mean amplitude of the ten startle trials. PPI was calculated according to the formula: $100 \times [1 - (PPx/P120)]\%$ in which PPx was the mean of the ten PPI trials (PP69, PP73, PP75, or PP80), and P120 was the basal startle amplitude (Ellenbroek et al. 2002; Takahashi et al. 2007).

Nonexposed control mice were placed into the chamber in the PPI test cage and habituated to the experimental environment for 10 min with 65 dB of background white nose as was the experimental group. They were kept in the chamber for any additional 17 min without any startle or PPI trials.

Mice were given saline or METH (3 mg/kg, sc) 15 min before the PPI test. Nicotine (0.1 or 0.5 mg/kg, sc) was administered 15 min before the saline or METH. Methyllycaconitine, a selective α7 subunit-containing nAChR (α7 nAChR) antagonist, and DHβE, a α4β2 subunit-containing nAChR (α4β2 nAChR) antagonist, were given 15 min before nicotine treatment.

Fos immunohistochemistry

c-Fos immunostaining was performed as described previously (Takahashi et al. 2007; Arai et al. 2008). As Fos expression was shown to occur from 1 to 4 h after a single short stimulation, animals were deeply anesthetized with sodium pentobarbital (50 mg/kg) 2 h after the PPI test. Alternatively, mice were treated with saline or METH (3 mg/kg, sc) and killed 2.5 h after treatment without the PPI test. The animals were transcardially perfused with ice-cold phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in PBS. The brains were removed, post-fixed in the same fixative for 2 h, and then cryoprotected in 30% sucrose in PBS. Frozen serial coronal sections (20 mm) of the entire brain were made and incubated with 10% goat serum and 0.1% Triton X-100 in 0.1-M phosphate buffer and then incubated with rabbit anti-c-Fos antibody (1:200; sc-253, Santa Cruz Biotechnology, CA) for 24 h at 41°C. They were washed with phosphate buffer and incubated with biotinylated goat anti-rabbit antibody (1:200; BA-1000, Vector Laboratories, Burlingame) at room temperature for 1 h. The sections were washed and processed with avidin-biotinylated horseradish peroxidase complex (Vector ABC kit, Vector Laboratories) and the reaction was visualized using diaminobenzidine.

Quantitative analysis of c-Fos immunohistochemistry

To quantify the number of Fos-positive cells in the brain, we used a fluorescence microscope with a cooled CDD digital camera system (Axio Imager A1/AxioCam MRc5; Carl Zeiss, Jena, Germany) to scan the sections and calculated the cell numbers from the digitized images using image-analyzing software Win ROOF (ver. 5.6, Mitani Co., Fukui, Japan). After analyzing effects of various drugs treatments on PPI, four or five mice in each treatment group that showed typical PPI responses (the values startle amplitude and PPI at 69–81 dB were

within the range of mean ±SE) were used for c-Fos experiment. We selected three different sections from each animal and defined the region of interest (ROI), 500×500 pixels (168×168 μm), using the software in both the right and left hemispheres of the sections according to a mouse brain atlas (Franklin and Paxinos 1997). To avoid double counting errors, we chose a counting protocol in the software, which does not calculate cell numbers at the border of a ROI. The counting of Fos-positive cells was repeated three times with differential ROI in one section. This procedure resulted in a total of nine determinations of the number of Fos-positive cells within a specified area for each brain. The average of the resulting nine determinations of the c-Fos-positive cell numbers was used as a measure of each animal for statistical analysis (Takahashi et al. 2007; Arai et al. 2008). c-Fos-positive cells were counted by an individual blind to the treatment conditions. In the drug treatment test, the areas selected were LGP and PnC.

Statistical analysis

All data were expressed as the mean ±SE. Statistical significance was determined by Student's *t* test for two group comparisons and one-way analysis of variance (ANOVA) for multigroup comparisons in experiments counting c-Fos-positive cell counts and by a repeated measure ANOVA for PPI response measurements. Student Newman–Keuls test was used for post hoc comparisons when *F* values were significant ($p<0.05$).

Results

Effect of nicotine on METH-induced impairment of PPI

METH at a dose of 3 mg/kg significantly reduced PPI compared with saline-treated control group (Fig. 1b; $F(1,32)=31.2$, $p<0.0001$). METH at a 3 mg/kg increased startle amplitude (160% of control), but the effect was not statistically significant (Fig. 1a; $p=0.0713$ by *t* test). Nicotine (0.15–0.5 mg/kg, sc) had little effect on startle amplitude in saline-treated control group (Fig. 1a; $F(2,30)=1.60$, $p=0.2197$) or METH-treated group (Fig. 1a, $F(2,32)=3.30$, $p=0.0497$), but it reversed the impairment of PPI induced by METH. For the influence of nicotine on METH-induced deficits in PPI, there were significant effects of drug treatment (Fig. 1b, $F(5,62)=14.9$, $p<0.0001$ by repeated one-way ANOVA), prepulse intensity ($F(3,186)=59.2$, $p<0.0001$ by repeated one-way ANOVA), and their interaction ($F(15,186)=2.26$, $p=0.0059$ by repeated one-way ANOVA).

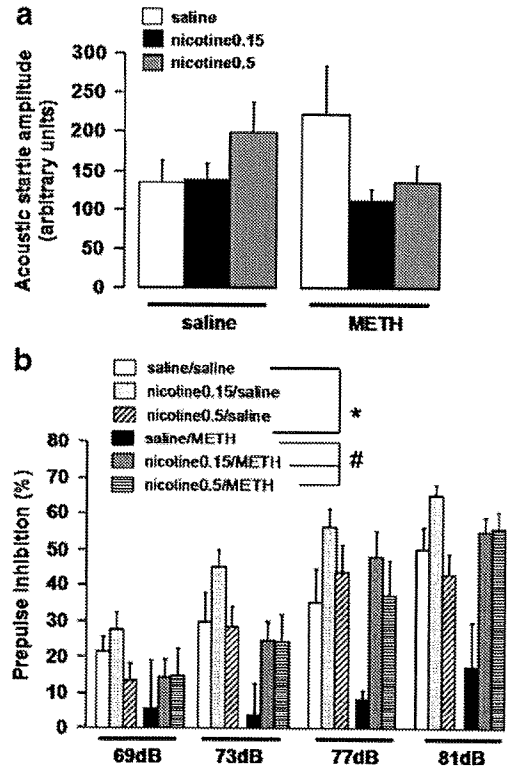

Fig. 1 Effect of nicotine on acute METH-induced impairment of PPI in mice. Mice were treated with nicotine (0.15–0.5 mg/kg, sc) 15 min before receiving METH (3 mg/kg, sc). **a** Acoustic startle amplitude as measured in trials without a prepulse. **b** PPI (%) at four different prepulse intensities (69, 73, 77, and 81 dB). Values are the mean±SE (saline/saline, $n=18$; saline/METH: $n=16$; nicotine 0.15/saline, $n=9$; nicotine 0.15/METH, $n=11$; nicotine 0.5/saline, $n=6$; nicotine 0.5/METH, $n=8$). *$p<0.05$ vs. saline/saline. #$p<0.05$ vs. saline/METH

Effect of nicotinic receptor antagonists on recovery of METH-induced deficits in PPI by nicotine

Firstly, we examined the effects of methyllycaconitine, an α7 nAchR antagonist, and DHβE, an α4β2 nAchR antagonist, alone on PPI of the startle reflex in mice. Neither antagonists at doses of 1–2 mg/kg had significant effects on the startle amplitude (Fig. 2a; methyllycaconitine, $F(2,22)=0.21$, $p=0.8092$; DHβE, $F(2,19)=1.39$, $p=0.2724$) or PPI (Fig. 2b; methyllycaconitine, drug treatment, $F(2,22)=0.33$, $p=0.7245$; prepulse intensity, $F(3,66)=21.0$, $p<0.0001$; interaction, $F(6,66)=1.39$, $p=0.2331$; DHβE, drug treatment, $F(2,19)=0.22$, $p=0.8020$; prepulse intensity, $F(3,57)=17.4$, $p<0.0001$; interaction, $F(6,57)=0.12$, $p=0.9940$).

Next, we examined the effect of nicotinic receptor antagonists on recovery of METH-induced deficits in PPI by nicotine (0.15 mg/kg). There was no difference in the startle amplitude between saline and METH-treated group (Fig. 3, $p=0.3377$ by *t* test). METH at a dose of 3 mg/kg significantly reduced PPI compared with saline-treated control group as well as the result in Fig. 1b (Fig. 3, $F(1,29)=20.6$, $p=0.0001$). Prior treatment with methylly-

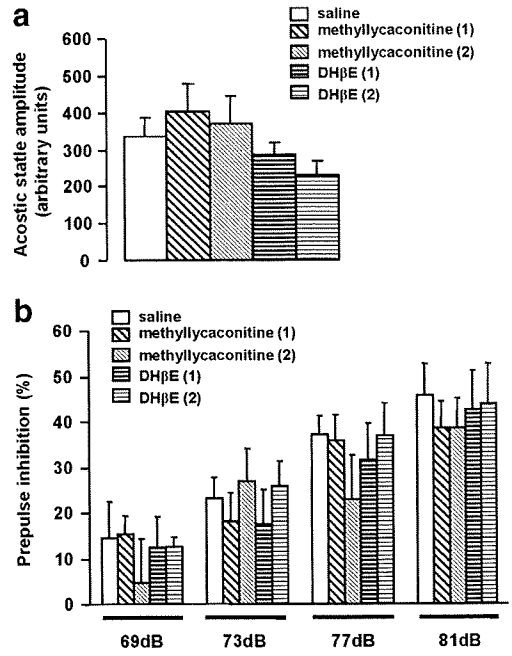

Fig. 2 Effects of a selective α7 subunit-containing nAChR antagonist, methyllycaconitine, and α4β2 subunit-containing nAChR antagonist, DHβE, on acoustic startle amplitude (**a**) and PPI (**b**) in mice. Mice were treated with methyllycaconitine (1-2 mg/kg, sc) or DHβE (1–2 mg/kg) 45 min before the PPI test. Values are the mean±SE (A, saline, n=7; methyllycaconitine 1 mg/kg, n=8; methyllycaconitine 2 mg/kg, n=10; DHβE 1 mg/kg, n=8; DHβE 2 mg/kg, n=7)

caconitine significantly increased the startle amplitude in METH with nicotine group (Fig. 3a, $F(3,45)=4.01$, $p=0.013$ by one-way ANOVA) and dose-dependently blocked the reversal of the METH-induced disruption to PPI by nicotine (Fig. 3a, drug treatment, $F(3,45)=7.71$, $p=0.0003$; prepulse intensity, $F(3,135)=63.3$, $p<0.0001$; interaction, $F(9,135)=1.45$, $p=0.1750$).

Likewise, the reversal of METH-induced disruption to PPI by nicotine was dose-dependently eliminated by DHβE (Fig. 3b, drug treatment, $F(3,46)=9.08$, $p=0.0001$; prepulse intensity, $F(3,138)=60.6$, $p<0.0001$; interaction, $F(9,138)=1.88$, $p=0.0598$) without any effects on startle amplitude in METH with nicotine group ($F(3,46)=2.62$, $p=0.0619$ by one-way ANOVA).

Effects of nicotine on the METH-induced changes in c-Fos expression in the LGP and PnC of mice subjected to the PPI test

To study the effects of drug treatment on pallidotegmental neural activation after the PPI test, we examined c-Fos expression immunohistochemically in the LGP and PnC of mice treated with saline and METH (3 mg/kg, sc). Representative photomicrographs of c-Fos staining in the LGP and PnC after the PPI test are shown in Figs. 4 and 5, respectively. Quantitative analysis of the staining indicated a significant difference in the number of c-Fos-positive cells in the LGP (Fig. 4, $F(4,16)=44.8$, $p<0.0001$ by one-way ANOVA). There was a significant increase in the number of c-Fos-positive cells in saline-treated mice subjected to the PPI test (n=4) compared with nonexposed control mice (n=5; $p=0.0001$). The number of c-Fos-positive cells in the LGP of mice treated with METH (n=4) was significantly less than that in saline-treated control mice subjected to the PPI test (Fig. 4, $p<0.0001$).

To study the effects of nicotine (0.15 mg/kg, sc) on METH-induced changes in neural activity associated with the PPI deficits, we examined c-Fos expression immunohistochemically in the LGP of mice after the PPI test. Pretreatment with nicotine significantly increased the number of c-Fos-positive cells in the LGP compared with the METH-treated group (n=4; $p<0.0001$), although nicotine had no effect in saline-treated control mice (Fig. 4).

A significant change in c-Fos expression was also observed in the PnC (Fig. 5, $F(4,15)=11.7$, $p=0.0002$ by one-way ANOVA). There was no difference in the number of c-Fos-positive cells between nonexposed control mice (n=4) and saline-treated mice subjected to the PPI test (n=4). The number of c-Fos-positive cells in the PnC of mice treated with METH (n=4) was significantly increased compared with that in saline-treated mice ($p=0.0003$). Although nicotine itself had no effect on c-Fos expression in the PnC, the METH-induced increase in the number of c-Fos-positive cells in PnC was suppressed by pretreatment with nicotine (Fig. 5, $F(4,15)=11.7$, $p=0.0002$ by one-way ANOVA).

Discussion

Deficits of PPI in psychosis and schizophrenia can be mimicked in rodents by treatment with psychostimulants such as METH (Arai et al. 2008; Dai et al. 2004). Acute METH-induced PPI impairment in mice was accompanied by an increase in startle amplitude. Amphetamine is also reported to increase startle response (Davis 1988). Thus, the alterations of startle response may account, at least in part, for the METH-induced disruption of PPI. Our findings showed that nicotine ameliorated the disruption to PPI induced by METH via both α7 and α4β2 nicotinic receptors (Figs. 1, 2 and 3). Nicotine blocked a dopamine agonist-induced disruption of PPI of the acoustic startle response via central α7 nicotinic receptors in rats (Suemaru et al. 2004). A α7 nicotinic receptor agonist reversed PPI deficits in isolation-reared rats suggesting the activation of α7 nicotinic receptors to be of benefit in reestablishing an efficient gating function (Cilia et al. 2002). Stimulation of heteromeric nicotine acetylcholine receptors containing both α and β subunits, and possibly of the α4/β2 type, affects

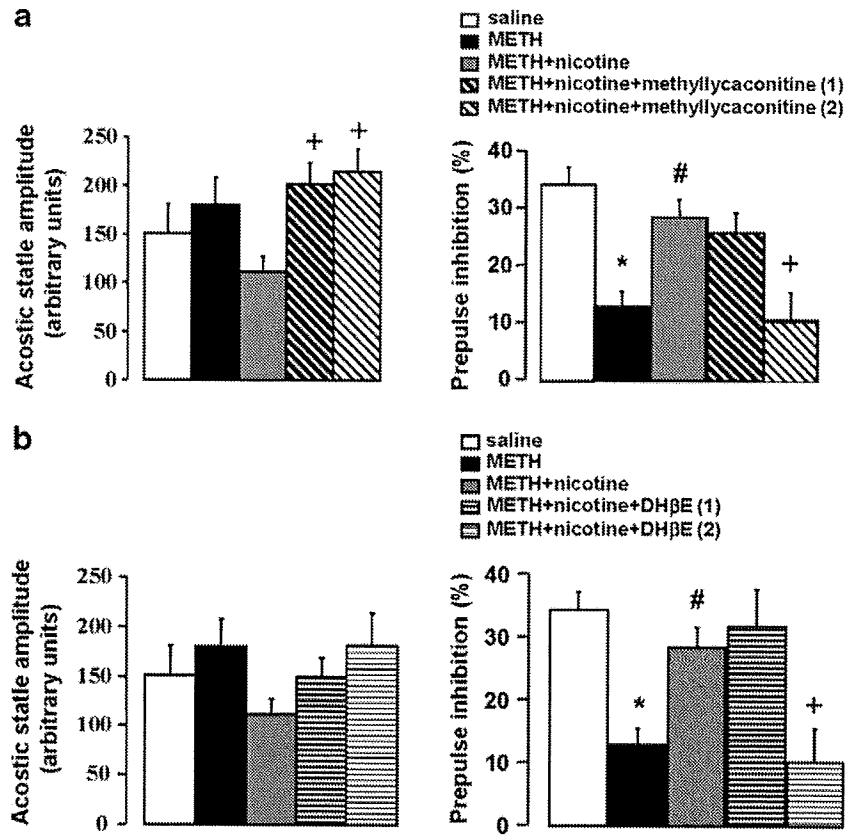

Fig. 3 Effects of a selective α7 subunit-containing nAChR antagonist, methyllycaconitine, (**a**) and α4β2 subunit-containing nAChR antagonist, DHβE, (**b**) on the ameliorating effects of a nicotine agonist on the METH-induced PPI deficit in mice. Mice were treated with methyllycaconitine (1–2 mg/kg, sc) or DHβE (1–2 mg/kg) 15 min before receiving nicotine (0.15 mg/kg, sc). Results of PPI test are shown as average of various PPI test from 69–81 dB. Values are the mean±SE (A, saline, $n=14$; METH, $n=17$; METH with nicotine, $n=16$; METH with nicotine and methyllycaconitine 1 mg/kg, $n=7$; METH with nicotine and methyllycaconitine 2 mg/kg, $n=9$; B, saline, $n=14$; METH, $n=17$; METH with nicotine, $n=16$; METH with nicotine and DHβE 1 mg/kg, $n=9$; METH with nicotine and DHβE 2 mg/kg, $n=8$). *$p<0.05$ vs. saline-treated control group. #$p<0.05$ vs. METH-treated group. +$p<0.05$ vs. METH with nicotine group

sensorimotor gating (Schreiber et al. 2002). Our findings are consistent with these previous reports and support that nicotine has a beneficial effect on PPI deficits. Alternatively, α6-containing nicotinic receptors may play a role since both methyllycaconitine (Klink et al. 2001) and DHβE (Exley et al. 2008) have high affinity for these receptors.

As shown in Fig. 1, nicotine at a dose of 0.15 mg/kg treatment apparently increased PPI in saline-treated mice although such potentiation by nicotine was not observed at a higher dose (0.5 mg/kg). It is possible that nicotine may have biphasic effects on PPI. In fact, previous studies have shown that nicotine increases (Acri et al. 1995) or decreases (Schreiber et al. 2002) sensorimotor gating by disparities in the dose, prepulse intensity, age, species, and strain. In contrast, our data showed that both low and high doses of nicotine ameliorated PPI impairment in METH-treated mice. Thus, the effect of nicotine on PPI may depend on the subject's condition (saline or METH-treated mice).

We have previously demonstrated that GABAergic neurons in the LGP, which project directly to the PPTg, are activated by a prepulse stimulus regardless of startle pulse stimuli, and that the pallidotegmental GABAergic neurons act as an interface between the brainstem PPI-mediating and the forebrain PPI-regulating circuits (Takahashi et al. 2007). In the present study, we demonstrated that the disruption of PPI caused by METH was accompanied by impairment of the LGP and hyperactivation of the PnC, which manifested as changes of c-Fos expression in the LGP and PnC after the PPI test, and it is reasonable to assume that METH disrupts PPI of the startle reflex in mice by inhibiting the activation of pallidotegmental GABAergic neurons evoked by a prepulse stimulus (Arai et al. 2008). Previously, as a control experiment, we measured the effects of METH (3 mg/kg, sc) on c-Fos expression in the LGP and PnC of mice that were not subjected to the PPI test. Single METH treatment had no effect on c-Fos expression in the LGP and PnC (Arai et al. 2008, supplementary Fig. 1). From this control experiment, it is obvious that the effects of METH on c-Fos expression in mice that were subjected to the PPI test are markedly different from those found in mice that were not subjected to the test (Arai et al. 2008). In this study, the ameliorating effect of nicotine on PPI was associated with the normalization of PnC hyperactivation, and nicotine increased the number of c-Fos-positive cells in the LGP of METH-treated mice subjected to the PPI test. Thus, nicotine may modulate pallidotegmental GABAergic neurons resulting in inhibition of the activation in the PnC induced by METH. However, it is clear that further studies are required to clarify the neurobiological mechanisms underlying the deficit in PPI induced by METH as well as the ameliorating effect of nicotine on the disruption.

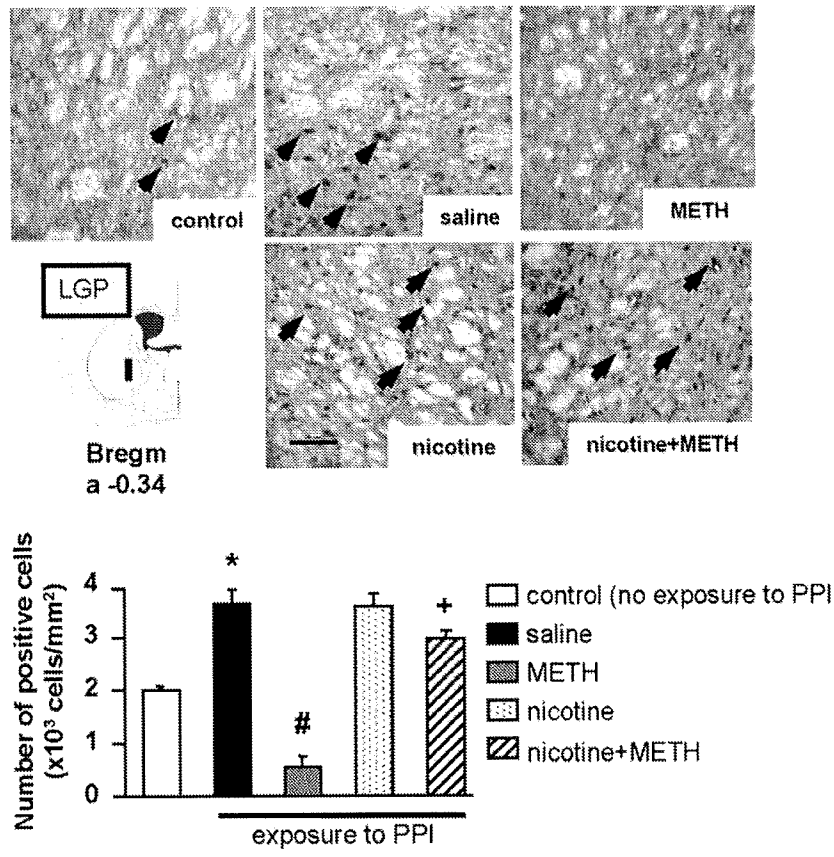

Fig. 4 Effect of nicotine on METH-induced changes in c-Fos expression in LGP after the PPI test. Mice were restricted to the PPI test cage but not subjected to the PPI test (nonexposed control group). Alternatively, mice were subjected to the PPI test after pretreatment with saline, METH (3 mg/kg, sc), nicotine (0.15 mg/kg, sc), or both METH and nicotine. Values are the mean±SE (control, $n=5$; other groups, $n=4$). *$p<0.05$ vs. control group. #$p<0.05$ vs. saline-treated group. +$p<0.05$ vs. METH-treated group

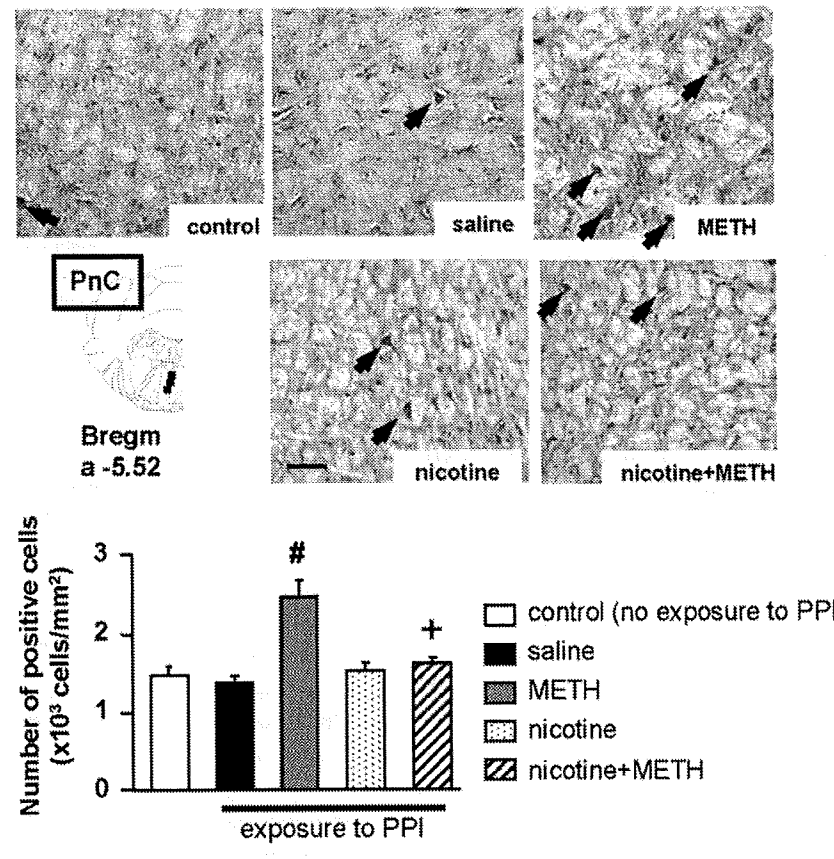

Fig. 5 Effect of nicotine on METH-induced changes in c-Fos expression in the PnC after the PPI test. Mice were restricted to the PPI test cage but not subjected to the PPI test (nonexposed group). Alternatively, mice were subjected to the PPI test after pretreatment with saline, METH (3 mg/kg, sc) nicotine (0.15 mg/kg, sc), or both METH and nicotine. Values are the mean±SE ($n=4$). #$p<0.05$ vs. saline-treated group. +$p<0.05$ vs. METH-treated group. #$p<0.05$ vs. saline-treated group. +$p<0.05$ vs. METH-treated group

The PnC is a critical part of the primary acoustic startle pathway (Fendt et al. 2001). The microinfusion of a nonspecific AchR agonist, carbachol, into the PnC attenuated the startle response and enhanced PPI while the injection of scopolamine reduced PPI (Fendt and Koch 1999). Acetylcholine (Ach) is one of the transmitters of projections from the PPTg to the PnC (Bosch and Schmid 2006) and exerts an inhibitory effect on the tone-evoked activity of acoustically responsive PnC neurons (Koch and Schnitzler 1997; Kungel et al. 1994). Notably, our data showed that nicotine treatment alone had little effect on c-Fos expression in the PnC of saline-treated group after the PPI test (Fig. 5). Although the effect of nicotine on auditory gating appears highly variable, our results suggest that systemic treatment with nicotine at a dose of 0.15–0.5 mg/kg is not directly associated with neuronal activation in the PnC.

Nicotine alters the release of several neurotransmitters, including dopamine, noraderenaline, GABA, glutamate (Dani and De Biasi 2002), and serotonin (Mihailescu et al. 2001, 2002). Interestingly, in the globus pallidus, nicotine can elicit the release of GABA by acting either directly on GABA terminals or indirectly through interaction with a neuronal component such as dopamine, suggesting that nicotine can modulate GABAergic neurons (Kayadjanian et al. 1994). Nicotine activates the neuronal population of the PPTg by directly targeting nicotinic receptors that may be located in noncholinergic neurons including GABAergic and glutamatergic neurons (Lanca et al. 2000). Nicotine stimulation of dorsal raphe nucleus (DRN) alters the activity precisely of the area where the PnC we studied is located, though the release of serotonin (Mihailescu et al. 2002), and nicotine, injected locally into DRN inhibits the activity of pedunculopontine cholinergic neurons through stimulation of DRN serotoninergic neurons, indicating a suppressible effect of nicotine in the PPTg neurons (Mihailescu et al. 2001). Systematic treatment with nicotine may therefore directly or indirectly activate GABAergic neurons in both the LGP and PPTg by altering the release of these neurotransmitters, recovering the function of pallidotegmental GABAergic neurons in METH-treated mice.

Further studies we should carry out are the following: (1) The release of neurotransmitters including Ach, dopamine, GABA, and serotonin in PPTg and PnC after nicotine administration will be examined to make the LGP–PPTg–PnC connection clear; (2) We should look at Fos expression in PPI-regulated and PPI-mediated regions such as superior colliculus (SC), inferior colliculus (IC), and DRN because the PPTg–PnC pathway is modulated by various input–output organization including SC (Kobayashi and Isa 2002; Yeomans et al. 2006), IC, (Yeomans et al. 2006) and DRN (Mihailescu et al. 2001, 2002). PPTg activates SC via nicotinic receptors, which facilitates PPI by activating the fast cascades.

In conclusion, we demonstrated that nicotine ameliorated the impairment of PPI induced by acute treatment with METH through $\alpha 7$ and $\alpha 4\beta 2$ nicotinic receptors and reversed the changes in c-Fos expression in both the LGP and PnC to the basal level. In patients suffering from schizophrenia and other psychiatric disorders, PPI is disrupted (Swerdlow et al. 1994; Castellanos et al. 1996). Nicotine receptors may therefore constitute a putative target in the treatment of neuropsychiatric disorders with sensorimotor gating deficits.

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精神科領域の薬剤開発
――抗精神病薬の過去と未来

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第一世代（定型）抗精神病薬の開発とドパミン仮説

統合失調症の薬物治療は、1952年にフェノチアジン系薬物のハロペリドールが合成されました。

その後、数多くの類似化合物が合成され、統合失調症の薬物療法は急速に発展していきました。

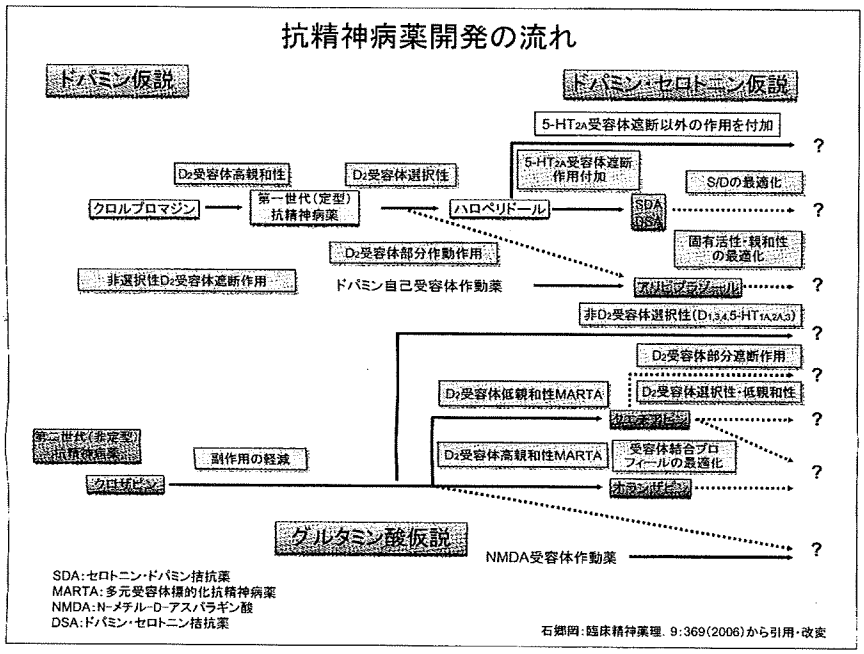
63年に抗精神病薬は、脳内ドパミン作動性神経系を遮断することが発見され、さらに抗精神病薬の臨床力価と脳内ドパミンD_2受容体遮断作用が相関することや、覚醒剤などのドパミン作動薬が、統合失調症の陽性症状様の幻覚・妄想状態を善起することが見出され、統合失調症では脳内ドパミン神経伝達系が異常亢進しているという「ドパミン仮説」が提唱されました。

ドパミンD_2受容体遮断作用を主作用とするクロルプロマジンや、ハロペリドールなどの第一世代（定型）抗精神病薬は、全ての脳内ドパミン作動性神経系を抑制してしまうため、中脳辺縁系に関わるドパミンの過活動を抑制することにより、幻覚・妄想などの陽性症状には効果が認められるものの、黒質線条体系ドパミン作動性神経や漏斗下垂体系ドパミン作動性神経の抑制によって、それぞれ錐体外路症状や、高プロラクチン血症などの副作用を惹起してしまう問題がありました。また、感情鈍麻や自発性欠如などの陰性症状や、認知障害に対する効果も不十分でした。

第二世代（非定型）抗精神病薬の開発

クロルプロマジンと類似した化学構造を有するクロザピンは、61年に合成され、69年にオーストリアで最初に承認されました。その後、多くの欧米諸国で承認され、優れた陽性症状改善作用に加え、陰性症状改善作用も有し、錐体外路症状を起こさず、プロラクチンも上昇させなかったため、大いに注目されました。

しかし、75年、フィンランドにおいて、無顆粒球症が報告されたため、各国で販売や開発が中止され、日本でも開発が中止されました。その後、



石郷岡：臨床精神薬理．9：369（2006）から引用・改変

クロザピンの細かい薬理学的プロフィールが解明され、ドパミンD_2受容体遮断作用に加え、セロトニン5・HT2受容体にも遮断作用を有することが、臨床的に優れた効果を発揮するのではないかと、一部で考えられました。

こうした背景のもと、第一世代（定型）抗精神病薬やクロザピンによる薬物治療上の問題点を克服するために、①セロトニン受容体に作用する化合物②クロザピンをプロトタイプとした化合物③ドパミン受容体を部分的に刺激する化合物――の三つの大きな流れで、新規抗精神病薬の開発が進んでいきました。

新規抗精神病薬の開発の主流は、セロトニン受容体に作用する化合物でロトニン5・HT2受容体遮断作用を有することに着目し、セロトニン5・HT2受容体遮断薬であるリタンセリンを合成しました。リタンセリン単剤では有効性が認められなかったものの、第一世代（定型）の抗精神病薬と併用すると、錐体外路症状は軽減され、陰性症状は改善されることが見出されました。

そこで、ドパミンD_2受容体遮断作用に加え、セロトニン5・HT2受容体遮断作用を有する薬物（セロトニン・ドパミンアンタゴニスト：SDA）として、84年にリスペリドンが開発されました。

こうして第二世代（非定型）抗精神病薬の示す非定型性は、ドパミンD_2受容体遮断作用に比べ、セロトニン受容体遮断作用が相対的に強いことによると考えられ、ドパミン・セロトニン仮説が提唱されました。

セロトニン受容体のサブタイプの神経化学的研究から、セロトニン5・HT2A受容体遮断作用が、黒質線条体系ドパミン作動性神経終末部におけるドパミン遊離量を増加させることにより、錐体外路症状を軽減し、前頭前皮質や海馬でのドパミン遊量を増加させることで、陰性症状や認知障害を改善すると推察されるようになりました。

リスペリドンの登場は、統合失調症の薬物治療に大きな変革をもたらし、国産初のSDAとして、ペロスピロンが開発されました（01年2月上市）。ペロスピロンは、セロトニン5・HT1A受容体に対する親和性も高く、不安、抑うつ、神経症などの症状にも適しています。

5・HT2／D_2受容体に高親和性の薬剤が登場

08年4月に、国内2番目のSDAとして上市されたブロナンセリンは、陽性症状と陰性症状の改善作用に加え、体重増加や耐糖能異常、錐体外路症状などの副作用発現頻度が低く、認知障害の改善効果も期待されています。

本薬物は、セロトニン5・HT2A受容体に対しても高い親和性を示しますが、ドパミンD_2受容体遮断作用の方が強いため、ドパミン・セロトニンアンタゴニスト（DSA）と称されています。

そのほか、現在開発中のSDAとしては、ペロスピロンの後継としてルラシドン（SM・13496）、四環系構造を有するアセナピン（ORG・5222）、5・HT2A／D_2親和性比が高く、様々なドパミン、セロトニン受容体サブタイプにも強力に作用するジプラシドン（CP・88059）：米国では01年に承認）などがあります。

クロザピンの臨床効果を期待しながら、クロザピン特有の副作用を持たない薬物として、オランザピン（日本：01年6月上市）や、クエチアピン（日本：01年2月上市）が開発されました。

両薬物の化学構造はクロザピンと類似しており、ドパミンD_2受容体およびセロトニン5・HT2受容体に対し、高い親和性を有しますが、他の多くの受容体に対しても作用することから、多元受容体標的化抗精神病薬（MARTA）と称されています。オランザピンは、ドパミン作動性神経伝達が過活動状態の場合には抗抗的に作用して陽性症状を、ドパミン作動性神経伝達が低下している場合には、刺激的に作用して陰性症状を改善することから、ドパミン・システムスタビライザー（DSS）と称されています。

国内外の臨床試験において、陽性症状、陰性症状に対して改善作用が認められ、錐体外路症状、内分泌代謝異常、体重増加などの有害事象が少なく、有効性と認容性に優れていることが確認されています。アリピプラゾールの成功を機に、新たなドパミン受容体部分作動薬の開発に期待が高まり、ビフェプルノックス（DU・127090）が現在開発中です。

新たな病態仮説と仮説にとらわれない治療薬の開発

近年、グルタミン酸作動性神経系、ニューロペプチド関連因子や一酸化窒素（nACh）作動性神経系などを介する、新たな薬理学的特徴を持つ薬物が開発されています。

グルタミン酸作動性神経系に作用する治療薬の開発は、精神異常発現薬のフェンシクリジンなどのNMDA受容体拮抗薬が、統合失調症様の精神障害を惹起することから注目されました（グルタミン酸仮説）。NMDA受容体を活性化するグリシン結合部位作動薬であるグリシン、D・セリンやD・シクロセリンなどが新たな治療薬として開発されています。

前二者は脳内移行性が悪く、治療には高用量を要するなどの問題があり、後者は脳移行性が比較的高いものの、部分作動薬であるために、用量設定が難しいとされています。

シナプス間隙における、グリシン濃度を上昇させるための戦略として、脳内移行性が高いグリシントランスポーター阻害薬のN・メチルグリシン（サルコシン）などの開発が進められています。

ほかに、AMPA受容体作動薬、代謝型グルタミン酸受容体作動薬が治療薬の候補として開発されています。オサネタント（SR・142801）、タルネタント（SB・223412）などの選択的タキキニンNK3受容体拮抗薬は、中脳被蓋野におけるドパミン作動性神経系の過活動を正常化することで、抗精神病作用を発揮する可能性があることから、注目されています。

アルツハイマー病治療薬で、ACh受容体作動薬のガランタミンは、リスペリドンとの併用により、統合失調症における認知障害を改善したことが報告されています。

ヒトゲノム研究の進展を背景に、統合失調症の感受性遺伝子が複数見出され、将来的に病態の解明や、有効な治療法・予防法の開発に結びつく可能性のある成果が報告されるようになってきています。また、疫学的研究において、統合失調症に関連する環境因子（妊娠母体のインフルエンザ感染、低体重出生など）が見出されています。

現在では、遺伝因子と環境因子が相互作用することにより、脳の発達を障害した結果、統合失調症の発症に至るという神経発達障害仮説が提唱されています。こうした遺伝的、環境的解析がさらに発展すれば、現在の対症療法的治療とは異なった、原因に基づいた根本的治療法の開発に結びつくと期待されます。

一方で、治療薬の作用部位などをコードする遺伝子を解析し、臨床経過や臨床症状の重症度、投薬内容との関連を検討して、テーラーメイド医療の実現に向けた研究もさかんになってきています。近い将来、統合失調症の薬物治療は新たな局面を迎えるものと考え、今後の新規治療薬の開発に大いに注目していきたいと思います。

特集 精神神経疾患の分子的理解と創薬のアプローチ

統合失調症における発症脆弱性遺伝子と創薬

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KEY WORDS

・統合失調症
・遺伝子
・遺伝子多型
・モデル動物

SUMMARY

統合失調症は重篤な精神障害を呈する精神疾患であり，複数の遺伝子と環境因子が発症に関与している．これまでに統合失調症の発症に関与すると考えられる十数種類の脆弱性遺伝子が同定され，統合失調症の病態解明に向けた研究が盛んに行われている．一方，既存の抗精神病薬とは異なる作用メカニズムを有する新規治療薬の開発も行われており，臨床研究において有効性を示す数種類の候補化合物がすでに報告されている．本稿では，統合失調症の発症に関与していることが示唆されている発症脆弱性遺伝子と新規抗精神病薬の開発状況について概説する．

はじめに

統合失調症は重篤な精神障害を呈する精神疾患であり，生涯発病率は1%と高い．統合失調症の主症状は陽性症状（妄想・幻覚，連合弛緩，緊張病症状），陰性症状（感情の平板化，思考の貧困，意欲の低下）および認知障害（注意集中困難，記憶の問題，遂行機能障害）に分類される．これらさまざまな精神症状は長期にわたって学業面でも就労面でも患者の社会的機能を低下させ，患者の約10%は自殺既遂に至る．疫学的研究によって統合失調症に遺伝的因子が強く関与することが実証されている[1]．また，連鎖解析および関連解析に代表される遺伝学的研究によれば，統合失調症は単一遺伝子の異常というよりは複数の遺伝子が関与して起こる複合遺伝疾患であるとの見解が強い．一方，一卵性双生児であっても約半数は統合失調症不一致例が存在するため，統合失調症は遺伝因子と出生の季節や場所，社会経済状況，母体感染などの環境因子が相互に組み合わさって発症に至る複雑精神疾患であると考えられている[1]．

本総説では，統合失調症病態仮説と発症に関与していることが示唆されている遺伝子（発症脆弱性遺伝子）について概説する（**表1**）．さらに，臨床試験が行われている新規抗精神病薬の候補化合物についても述べる．

1．統合失調症病態仮説

1）ドパミン仮説

統合失調症では中脳―辺縁系ドパミン作動性神経系の機能の亢進が陽性症状を，中脳―皮質系ドパミン作動性神経系の機能低下が陰性症状や認知障害の発現に関与していると考えられている．臨床において使用されている定型および非定型抗精神病薬のほとんどがドパミンD2受容体に作用することから，ドパミンD2受容体と統合失調症との関連性について検討されている．遺伝学的研究において，Taq A2およびCys311Serの多型が統合失

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表 1. 統合失調症の発症脆弱性遺伝子

遺伝子名	染色体領域	脳内発現変化	機能
COMT	22q11	増加もしくは減少	カテコールアミン分解
DARPP-32	17q12	減少	ドパミン作動性神経系の細胞内情報伝達
PPP3CC	8p21	減少	カルシウム依存性セリン/スレオニンフォスファターゼ
BDNF	11p13	減少	神経栄養因子
NRG1	8p12-21	上昇	NMDA 受容体機能調節，神経発達，神経伝達，シナプス可塑性
DTNBP1	6p22	減少	ドパミンおよびグルタミン酸作動性神経伝達
G72	13q34	増加	DAAO 活性化
PRODH	22q11	変化なし	プロリン分解
RGS4	1p21-22	減少	G 蛋白質共役型受容体シグナル抑制
DISC1	1p42	変化なし	神経突起の伸長，神経細胞の移動
CHRNA7	15q13-14	減少	コリン作動性神経伝達
AKT1	14q22-32	減少	PI3K シグナル伝達

（著者作成）

調症と有意に関連することが示されている[2]．また，健常者において線条体ドパミン D2 受容体結合に影響を与える C957T の機能的多型について，統合失調症患者との関連性が見出されている[3]．ドパミン D2 受容体以外についても関連研究がなされ，D3 受容体では Ser9Gly の多型は統合失調と有意な関連を示すことが報告されている[4]．一方，D1 および D4 受容体では有意な関連は認められない[5]．

2）グルタミン酸仮説

グルタミン酸受容体には 3 種類のイオンチャネル型受容体（NMDA 受容体，AMPA 受容体とカイニン酸受容体）と代謝型受容体が存在する．phencyclidine（PCP）あるいはケタミンなどの NMDA 受容体拮抗薬は統合失調症の陽性症状と陰性症状に類似した症状を惹起することから，統合失調症にグルタミン酸作動性神経系の機能低下が関与しているという仮説である．実際，統合失調患者の線条体ではグルタミン酸作動性神経のマーカーである神経型グルタミン酸トランスポーターの excitatory amino acid transporter（EAAT）-3 および vesicular glutamate transporter-1 の発現が健常者にくらべて減少していることが示されている[6]．遺伝学的研究では，*EAAT-2* 遺伝子（*SLC1A2*）と *EAAT-4* 遺伝子（*SLC1A6*）に統合失調症との関連が認められている[7][8]．また，NMDA 受容体に関する遺伝学的研究では，NMDA 受容体 NR1 サブユニット遺伝子（*GRIN1*）および *NR2B* サブユニット遺伝子（*GRIN2B*）の多型は統合失調症と有意な関連を示すことが報告されている[9][10]．

3）神経発達障害仮説

統合失調症の神経発達障害仮説は，胎児期および新生児期における何らかのイベントが初期の脳発達に異常をもたらし，生じた神経回路の形成不全や神経機能の変化が統合失調症の発症を引き起こすというものである．MRI（magnetic resonance imaging）研究から，統合失調症における脳の構造異常が実証されており[11]，初回エピソードの統合失調症患者において認められる著しい所見は脳室の拡大である．全脳容積は脳室拡張に伴い縮小するが，その縮小率はわずかである．その他に海馬の萎縮が統合失調症患者では観察されている．実際，妊婦の母体感染，周産期イベントあるいは分娩時の合併症は出生時の統合失調症の発症リスクを増大させる[12]．また，小児の中枢神経系の感染または出生時の低酸素状態は精神発達障害をきたすリスクを 5 倍に増加させることが報告されている[12]．

2．発症脆弱性遺伝子と統合失調症の病態

1）Catechol-O-methyl transferase

口蓋心顔面症候群（vero-cardio-facial syndrome：VCFS）は染色体 22q11 領域の欠損により顔面や心臓の奇形が認められる遺伝疾患である．VCFS 患者の約