been previously located was used as an index of spatial reference memory.

Prepulse Inhibition of acoustic startle response

The rats were tested for their acoustic startle responses (ASR) in a startle chamber (SR-LAB, San Diego Instruments, CA, USA). The sessions consisted of five trial types: 1) pulse alone, a 40-millisecond broadband burst; 100 milliseconds preceding the pulse, a 20-millisecond prepulse (PP) that was either 2) 4 dB (PP74), 3) 8 dB (PP78), or 4) 16 dB (PP86) over the background (70 dB), and 5) background only (no stimulus). The amount of prepulse inhibition (PPI) is expressed as the percentage decrease in the amplitude of the startle response caused by presentation of the prepulse (%PPI).

To examine the effects of clozapine on PPI deficits in irradiated rats, vehicle (0.8% acetic acids; 1 ml/kg for 3 weeks) or clozapine (5 mg/kg/day for 3 weeks) were i.p. administered into control and irradiated rats (control/vehicle = 6, control/clozapine = 6, irradiated/vehicle = 6, irradiated/clozapine = 6). After the chronic (3 weeks) administration of vehicle or clozapine, PPI of acoustic startle response was examined as described above.

Statistical analysis

Data are expressed as means±standard errors of the means (SEM). The data from two experimental groups were compared by unpaired t-test, except for PPI analysis, which was performed by a two-way (irradiation and prepulse intensity) analysis of variance (ANOVA). The level of significance was set at $p<0.05$.

Results

Three months after fractionated ionizing irradiation, the total numbers of BrdU-positive cells in both the subventricular (SVZ: **Figure 1B**) and subgranular (SGZ: **Figure 1D**) zones of irradiated rats were significantly lower than those (SVZ: **Figure 1A**, SGZ: **Figure 1C**) of control (sham-irradiated) rats (SVZ: **Figure 1E**, SGZ: **Figure 1F**). These findings are consistent with those of previous reports [9,10,12]. In contrast, the cumulative numbers of granule cells in the granule layer were not different between the two groups (**Figure 1G**).

As shown in **Figure 2A**, the nocturnal spontaneous locomotion of irradiated rats was significantly ($t = 2.34$, $df = 38.1$, $p = 0.025$) lower than that of control rats. Furthermore, locomotor activity after administration of methamphetamine (2.0 mg/kg, i.p.) to irradiated rats was significantly ($t = -2.26$, $df = 32$, $p = 0.031$) higher than that of control (sham-irradiated) rats (**Figure 2B**). HPLC analysis revealed that levels of dopamine and its major metabolite DOPAC, and dopamine turnover (DOPAC/dopamine ratio) in the frontal cortex and striatum of irradiated rats were not different from those of sham-control rats (**Figure 3**). In contrast, locomotor activity after administration of the NMDA receptor antagonist dizocilpine ((+)-MK-801, 0.03 mg/kg, i.p.) to irradiated rats was not different from that of sham-control rats (**Figure 2C**). Furthermore, levels of amino acids (glutamate, glycine, glutamine, D-serine, L-serine) related with the NMDA receptor neurotransmission in the frontal cortex, hippocampus, and striatum, and cerebellum of irradiated rats were not different from those of sham-control rats (**Figure 4**).

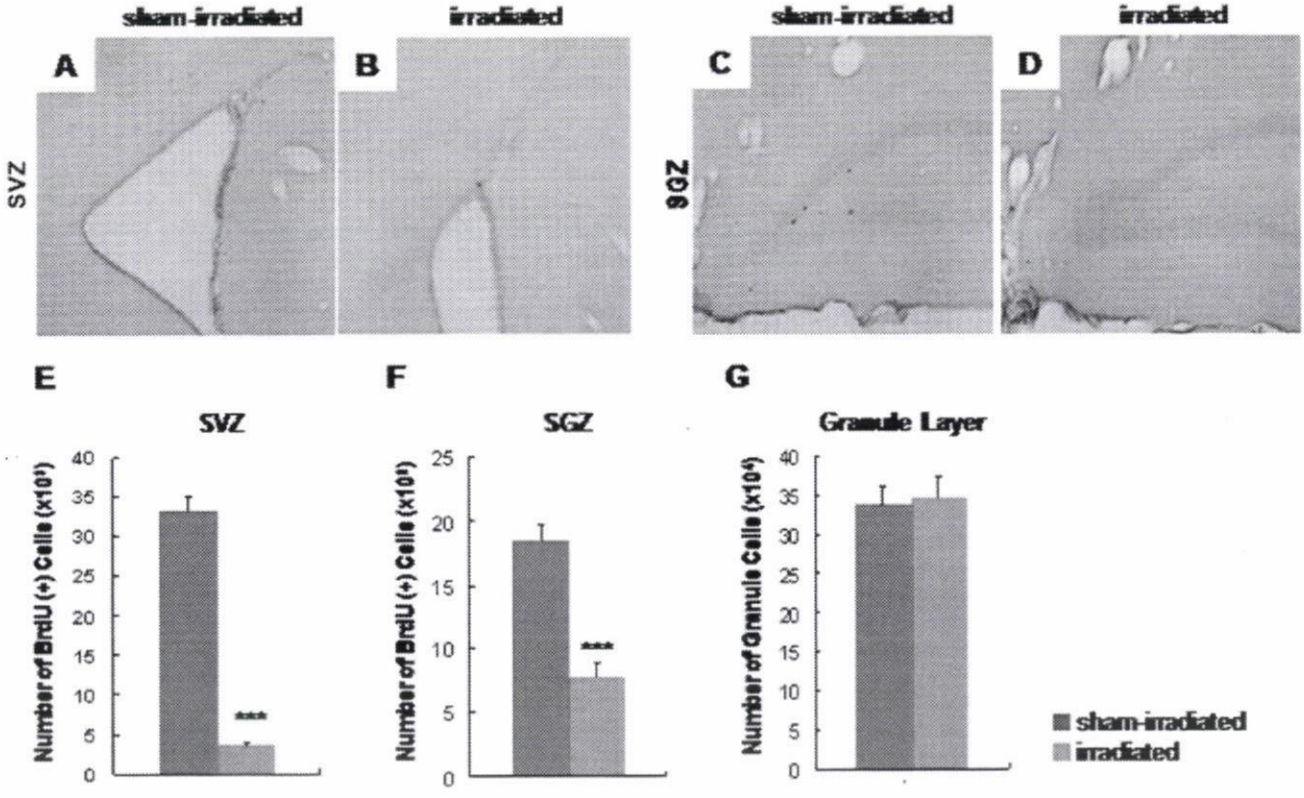

Figure 1. Decreased neurogenesis in the irradiated adult rats. The BrdU-positive cells in both SVZ (B and E) and SGZ (D and F) of the irradiated rats (n = 8) were significantly fewer than those (SVZ, A and E; SGZ, C and F) of control (sham-irradiated) rats (n = 8). Data are given as means±SEM. ***$p<0.001$ as compared with controls. (G) The total numbers of granule cells in the dentate gyrus in irradiated rats (n = 6) and control (sham-irradiated) rats (n = 6) were not different.
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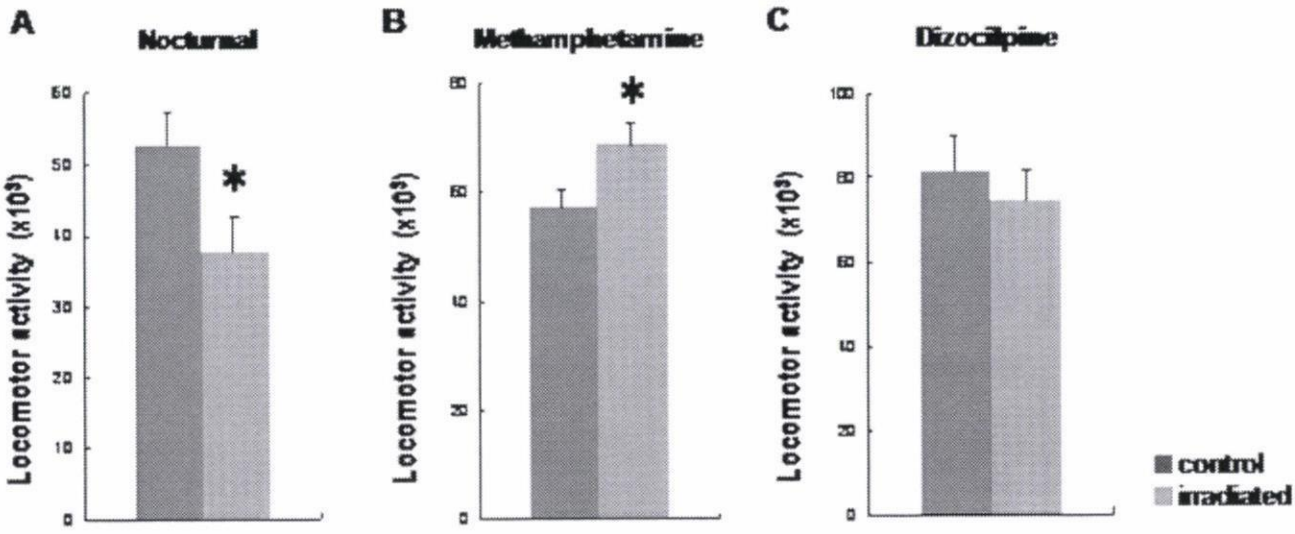

Figure 2. Spontaneous locomotion and response to methamphetamine and dizocilpine in irradiated adult rats. (A) Nocturnal spontaneous locomotion (21:00–3:00) in the irradiated rats (n = 17) was significantly lower than that of control (sham-irradiated) rats (n = 17). (B) Horizontal locomotor activity during the 120-min period after administration of the psychostimulant drug methamphetamine (2 mg/kg, i.p.) in irradiated rats (n = 17) was significantly higher than that of control rats (n = 17). (C) Horizontal locomotor activity during the 120-min period after administration of dizocilpine (0.03 mg/kg, i.p.) in the irradiated rats (n = 18) was not different from that of control rats (n = 18). Data are given as means ± SEM.
doi:10.1371/journal.pone.0002283.g002

In the sensorimotor gating test, two-way ANOVA revealed a significant effect [F (1, 42) = 47.1, $p<0.001$] of irradiation exposure on prepulse inhibition (PPI) (**Figure 5A**), while acoustic response amplitude in the two groups was not different (**Figure 5B**). PPI deficits in irradiated rats were shown at each level of prepulse intensity (72, 76, and 84 dB) (**Figure 5A**). In the social interaction test, the time spent in social behavior in the irradiated rats was significantly ($t = 3.73$, $df = 10$, $p = 0.004$) lower than that that in the control rats (**Figure 6**). In the eight-arm radial maze test, the number of working memory errors in the irradiated rats was significantly ($t = -3.63$, $df = 27.3$, $p = 0.001$) higher than that of control rats (**Figure 7A**). In contrast, the two groups' times in the probe test of a Morris water maze as an index of spatial reference memory were not different (**Figure 7B**).

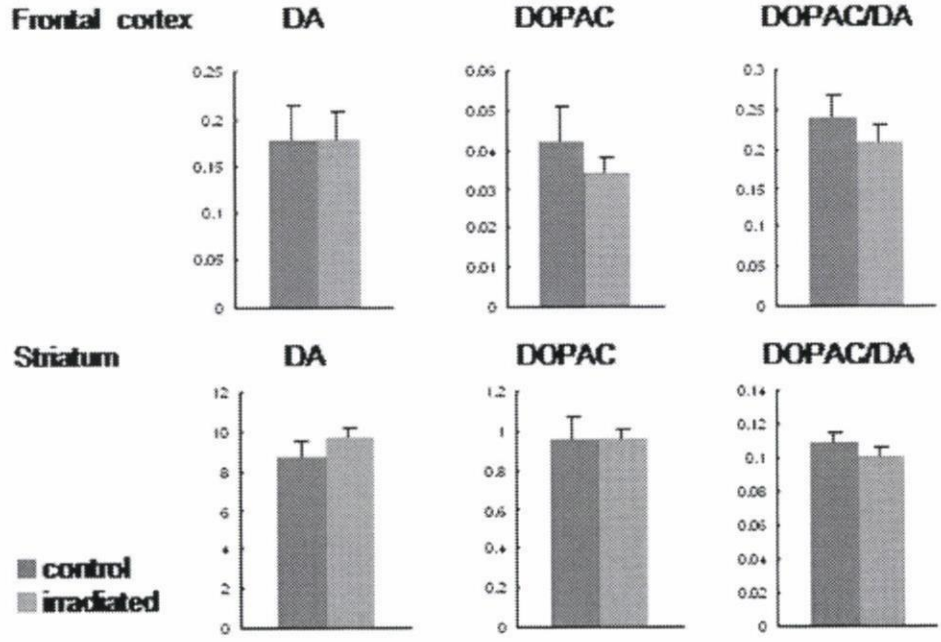

Figure 3. Dopamine and its major metabolite DOPAC levels in the frontal cortex and striatum of rat brain. Levels of dopamine and its major metabolite DOPAC, and dopamine turnover (DOPAC/dopamine ratio) in the frontal cortex and striatum were determined by HPLC analysis. There are no differences between irradiated rats (n = 6) and sham-control rats (n = 6).
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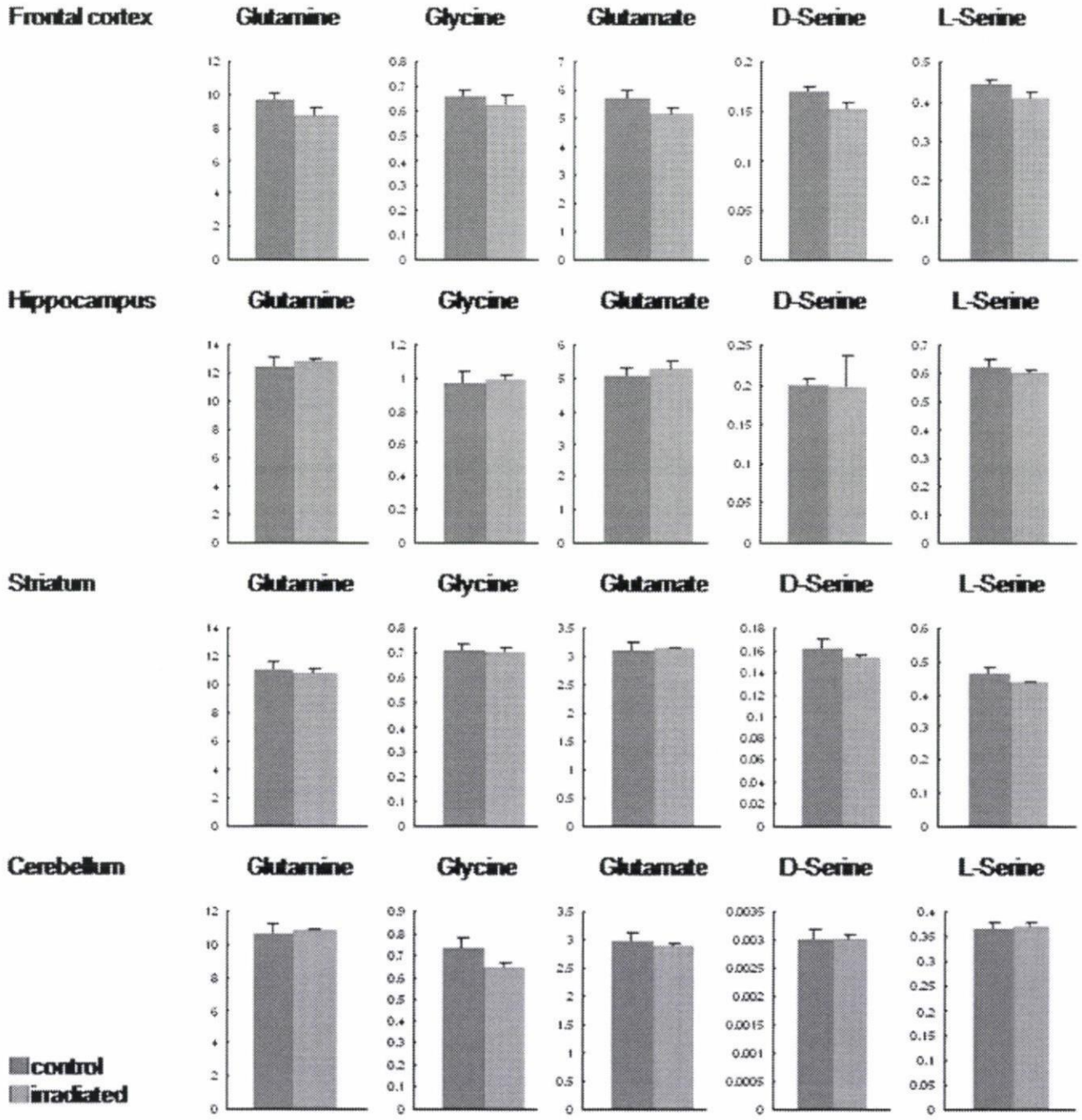

Figure 4. Levels of amino acids in the brain. Levels of amino acids (glutamate, glycine, glutamine, D-serine, L-serine) related with the NMDA receptor neurotransmission in the frontal cortex, hippocampus, and striatum, and cerebellum were determined by HPLC analysis. There are no differences between irradiated rats (n = 6) and sham-control rats (n = 6).
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We examined whether the antipsychotic drug clozapine could improve the reduction of neurogenesis and PPI deficits in irradiated rats. Subsequent chronic administration of clozapine (5 mg/kg/day for 3 weeks) did not alter the reduction of neurogenesis in the irradiated (data not shown). Furthermore, subsequent chronic administration of clozapine (5 mg/kg/day for 3 weeks) did not alter PPI in control rats (**Figure 8A**). However, we found that chronic administration of clozapine (5 mg/kg/day for 3 weeks) slightly improved PPI deficits in irradiated rats although a statistical analysis was not significant (**Figure 8B**).

Discussion

The major findings of the present study are that fractionated ionizing irradiation to the adult male rat brain causes schizophrenia-relevant abnormal behaviors (e.g., methamphetamine-induced

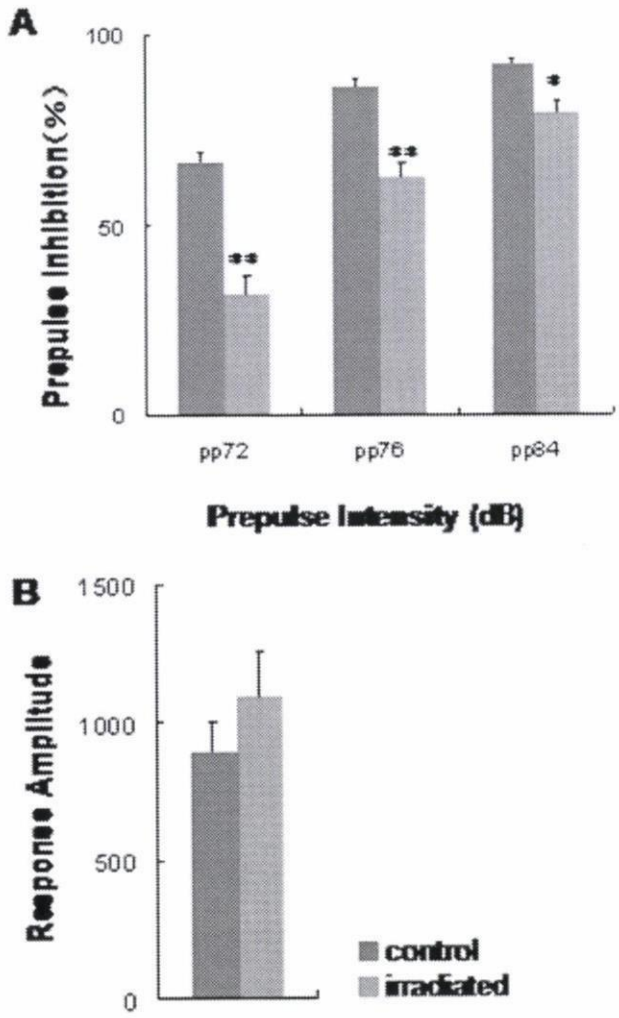

Figure 5. Sensorimotor gating deficits in the irradiated adult rats. (A) Auditory sensorimotor gating test: The irradiated rats (n = 23) show significant PPI deficits as compared with control (sham-irradiated) rats (n = 21). (B) Amplitude (in arbitrary units) of acoustic startle responses to the 120 dB auditory stimuli without prepulse in both groups was not different. Data are given as means±SEM. *p<0.05, **p<0.01 as compared with control (sham-irradiated) rats.
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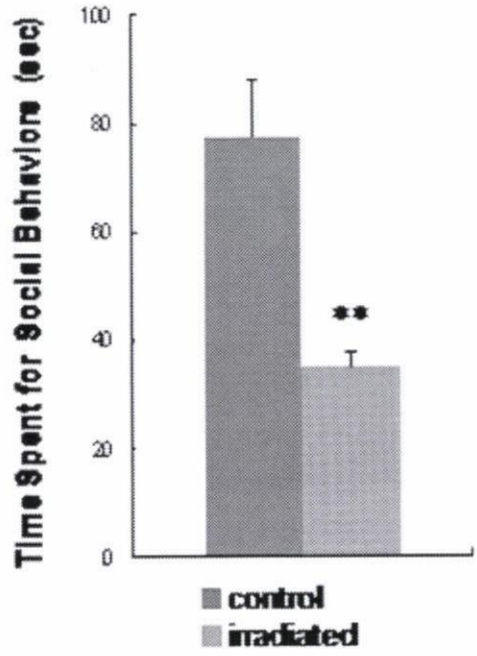

Figure 6. Social withdrawal in the irradiated adult rats. Social interaction test: Total time (sec) spent in social behaviors for 10 min in the irradiated rats (n = 6) was significantly lower than that of control rats (n = 6). Data are given as means±SEM. **p<0.01 as compared with control (sham-irradiated) rats.
doi:10.1371/journal.pone.0002283.g006

hyperactivity, sensory motor gating deficits, social interaction deficits, and working memory deficits) at three months after the irradiation. To the best of our knowledge, this is the first report demonstrating an animal model of schizophrenia by irradiation at adulthood. Although the irradiated adult rats may show essential features (positive and negative symptoms as well as cognitive deficits) relevant to schizophrenia, the pathophysiological mechanism underlying these behavioral changes remains unclear. A recent study using postmortem brain samples demonstrated that proliferation of hippocampal neural stem sells was significantly reduced in patients with schizophrenia, but not unipolar depression [16], suggesting that reduced neural stem cell proliferation may contribute to the pathogenesis of schizophrenia. Moreover, it has been reported that the reduction of cell proliferation in the SGZ after repeated administration of the NMDA receptor antagonist phencyclidine (PCP) may occur in tandem with PCP-induced behavioral changes in rats [22]. In this regard, it is likely that reduction of adult neurogenesis by irradiation may be involved in the schizophrenia-like behavioral abnormalities in rats. Recently, the association between neurogenesis dysfunction and schizophrenia has been also demonstrated [23].

Monje et al. [11] observed that irradiation of the brains of adult rats produced neural progenitor cell dysfunction within the neurogenic zones of the hippocampus, regions plausibly implicated in cognitive deficits. Furthermore, it has been suggested that irradiation-induced cognitive deficits in animals may be associated with a decrease in hippocampal proliferation and a decrease in adult neurogenesis [9–15]. In the eight-arm radial maze test, irradiated rats showed a deficit in working memory, which is also shown in schizophrenic patients [24]. It has been suggested that adult neurogenesis may serve an important role in hippocampal-dependent memory processes [25,26]. First, exposure to an enriched environment or increased physical activity leads to increased hippocampal neurogenesis and improved spatial memory [26–28]. Second, the comprehensive loss of hippocampal-dependent memory function in old age is related to decreased neurogenesis [29]. Taken together, it seems that cognitive impairment in irradiated rats may be due to reduction of hippocampal neurogenesis.

In this study, we found that methamphetamine-induced hyperactivity was significantly enhanced in the irradiated rats, suggesting hyperdopaminergic activity. The precise mechanisms underlying the hyperdopaminergic states in irradiated rats could not be explained, as we found no alteration of dopamine or its major metabolite DOPAC in the irradiated rat brains. Since mesolimbic dopaminergic neurons innervate the SGZ of the dentate gyrus [30], the dopaminergic activities of these neurons may be involved in the regulation of hippocampal neurogenesis. Furthermore, we previously reported that cell destruction of

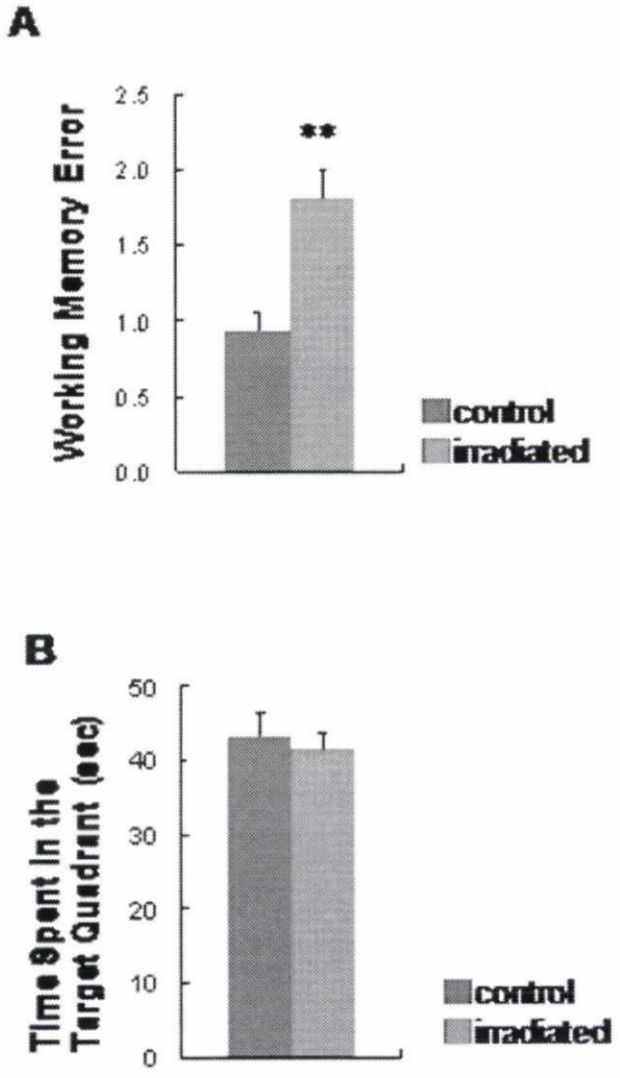

Figure 7. Cognitive impairments in the irradiated adult rats. (A) Spatial working memory in the eight-arm radial maze with 30-sec delay. Total number of revisits to arms from which pellets had already been taken, (i.e., working memory error) is represented as mean±SEM. Irradiated rats (n = 17) showed a higher number of working memory errors than control (sham-irradiated) rats (n = 15). (B): In the probe trials of the Morris water maze, spatial reference memory was intact in the irradiated rats (n = 12). Data are given as means±SEM. **p<0.01 as compared with control (sham-irradiated) rats (n = 12).
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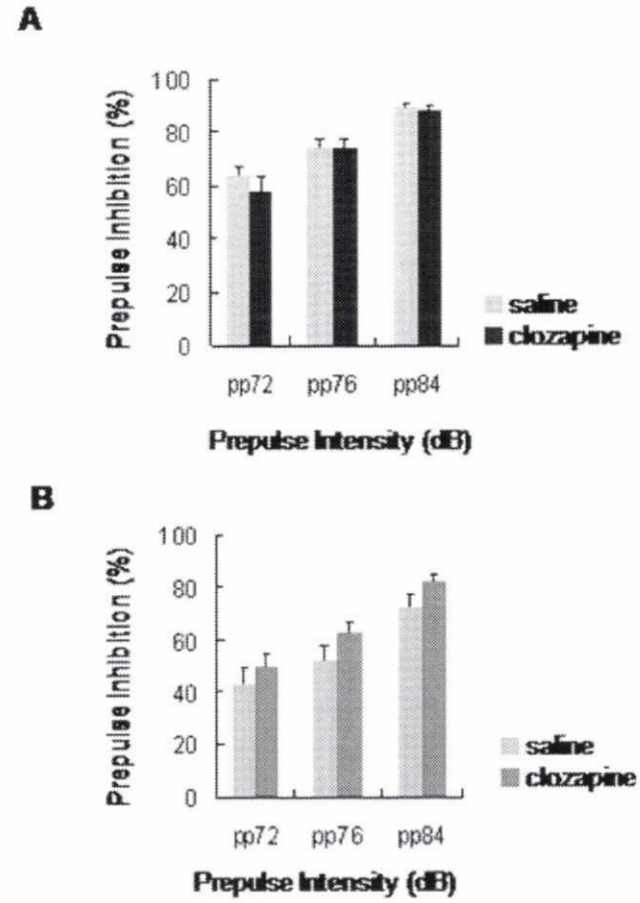

Figure 8. Effects of chronic clozapine administration on PPI deficits. (A) Control (sham-irradiated) rats: Chronic administration of clozapine (5 mg/kg/day for 3 weeks, i.p.) did not alter PPI deficits in the control rats (clozapine: n = 6; vehicle: n = 6). (B) Irradiated rats: Chronic administration of clozapine (5 mg/kg/day for 3 weeks, i.p.) significantly did not alter PPI deficits in the irradiated rats (clozapine: n = 6; vehicle: n = 6). Data are given as means±SEM.
doi:10.1371/journal.pone.0002283.g008

dentate granules by intrahippocampal injection of colchicine enhanced methamphetamine-induced hyperactivity in rats [31], suggesting that dentate granule cells may regulate methamphetamine-induced behavioral changes. Taken together, the evidence suggests that the decrease in hippocampal neurogenesis by irradiation may, in part, be implicated in the hyperdopaminergic activity of irradiated rats although the cumulative numbers of granule cells in the granule layer were not altered in irradiated rats.

Accumulating evidence suggests that hypofunction of the NMDA receptors may play a role in the pathophysiology of schizophrenia [32–34]. However, we did not find any alteration in dizocilpine-induced hyperactivity and levels of amino acids related to NMDA receptor neurotransmission in irradiated rat brains. Therefore, it is unlikely that alteration in the NMDA receptors is involved in the behavioral abnormalities in irradiated rats, although further studies are necessary.

The idea that antipsychotic drugs may increase neurogenesis in the rat hippocampus has not been consistently supported [17]. In this study, we found that chronic administration of clozapine (5 mg/kg/day for 3 weeks) did not alter the reduction of neurogenesis in the irradiated and control rats. In addition, we found that chronic administration of clozapine (5 mg/kg/day for 3 weeks) significantly did not improve PPI deficits in irradiated rats although a slight improvement by clozapine was shown. Therefore, it is likely that the inefficiency of clozapine treatment on PPI deficits in irradiated rats may be dependent upon the reduction of adult neurogenesis, although a further study will be necessary.

The total numbers of BrdU-positive cells in both SVZ and SGZ were significantly lower than those of sham-irradiated rats three months after fractionated irradiation. The static BrdU-positive cell count may reflect neurogenesis and/or survival of the recent born cells. Monje et al. [10] have demonstrated that normal number of neural progenitors was surviving two months after radiation exposure although proliferative activity was reduced. They also

have shown that the neural stem/precursor cells isolated from irradiated hippocampi failed to expand only 2–3 passages. These findings suggest that the reduction of proliferating cells in the present study might be due to ablated neurogenesis without reduction of cell survival. Actually, it is reported that the decline of proliferating cells lasted at 15 months after irradiation [35].

In the present study, we have regarded the neurogenesis dysfunction as a possible mechanism underlying the radiation induced abnormal behaviors associated with schizophrenia, based on the findings suggesting the link between neurogenesis dysfunction and schizophrenia. However, it has been reported that irradiation also induces apoptosis [9], neuroinflammation [11], and loss of oligodendrocyte precursor [35]. Further detailed investigation is required to discriminate the involvement of these factors on irradiation induced abnormal behavior relevant to schizophrenia. Further studies on the optimization of radiation dose, phenotypic alteration by the exposure age, and sex differences are also needed.

In conclusion, the present findings suggest that irradiation in adulthood might provide a new animal model of schizophrenia. Further understanding the molecular and cellular mechanisms underlying the behavioral abnormalities in irradiated rats would contribute to the pathophysiology of schizophrenia.

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Author Contributions

Conceived and designed the experiments: KH NM YI KSuzuki. Performed the experiments: KH KN YI KSuzuki TW NS IT HM TM TU SM TS SS SY KT GS KSato. Analyzed the data: KH YI NT. Contributed reagents/materials/analysis tools: KH YI. Wrote the paper: KH YI.

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ORIGINAL INVESTIGATION

Aripiprazole ameliorates phencyclidine-induced impairment of recognition memory through dopamine D_1 and serotonin 5-HT_{1A} receptors

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Abstract
Rationale Cognitive deficits, including memory impairment, are regarded as a core feature of schizophrenia. Aripiprazole, an atypical antipsychotic drug, has been shown to improve disruption of prepulse inhibition and social interaction in an animal model of schizophrenia induced by phencyclidine (PCP); however, the effects of aripiprazole on recognition memory remain to be investigated.

Objectives In this study, we examined the effect of aripiprazole on cognitive impairment in mice treated with PCP repeatedly.
Materials and methods Mice were repeatedly administered PCP at a dose of 10mg/kg for 14days, and their cognitive function was assessed using a novel-object recognition task. We investigated the therapeutic effects of aripiprazole (0.01–1.0mg/kg) and haloperidol (0.3 and 1.0mg/kg) on cognitive impairment in mice treated with PCP repeatedly.
Results Single (1.0mg/kg) and repeated (0.03 and 0.1mg/kg, for 7days) treatment with aripiprazole ameliorated PCP-induced impairment of recognition memory, although single treatment significantly decreased the total exploration time during the training session. In contrast, both single and repeated treatment with haloperidol (0.3 and 1.0mg/kg) failed to attenuate PCP-induced cognitive impairment. The ameliorating effect of aripiprazole on recognition memory in PCP-treated mice was blocked by co-treatment with a dopamine D_1 receptor antagonist, SCH23390, and a serotonin 5-HT_{1A} receptor antagonist, WAY100635; however, co-treatment with a D_2 receptor antagonist raclopride had no effect on the ameliorating effect of aripiprazole.
Conclusions These results suggest that the ameliorative effect of aripiprazole on PCP-induced memory impairment is associated with dopamine D_1 and serotonin 5-HT_{1A} receptors.

Keywords Aripiprazole · Dopamine D_1 receptor · Memory · Phencyclidine · Serotonin 5-HT_{1A} receptor

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Introduction

Schizophrenia is a devastating psychiatric disorder that impairs mental and social functioning and affects approx-

imately 1% of the population worldwide (Rössler et al. 2005). Typical symptoms can be separated into positive symptoms (e.g., hallucinations, delusions, and thought disorder), negative symptoms (e.g., deficits in social interaction, emotional expression, and motivation), and cognitive dysfunction (e.g., impaired attention/information processing, problem-solving, processing speed, verbal and visual learning, and memory and working memory) (Nuechterlein et al. 2004; Pearlson 2000). Pharmacological treatment of schizophrenia is available. First-generation (typical) antipsychotics alleviate psychotic symptoms, but lead to severe motor side effects through the blockade of dopamine D_2 receptors (Kapur et al. 2000). Second-generation (atypical) antipsychotics have improved tolerability and milder motor side effects than typical antipsychotics but induce weight gain and metabolic disturbances (Newcomer 2005). Despite appropriate treatment with either typical antipsychotics or atypical antipsychotics, schizophrenic patients continue to exhibit pronounced cognitive impairment (Keefe et al. 2007; Mishara and Goldberg 2004; Woodward et al. 2005).

Aripiprazole, 7-(4-[4-(2,3-dichlorophenyl)-1-piperazinyl] butyloxy)-3,4-dihydro- carbostycil, is a novel atypical antipsychotic drug that differs from other typical and atypical antipsychotics, improving both positive and negative symptoms of psychosis without producing extrapyramidal side effects or increases in serum prolactin (DeLeon et al. 2004; Tamminga 2002). It has been demonstrated that aripiprazole has high affinity for a large number of monoaminergic receptors, including dopamine D_2, serotonin 5-HT_{1A}, and 5-HT_{2A} receptors (Green 2004; Shapiro et al. 2003) and acts as a partial dopamine D_2 receptor agonist (Kikuchi et al. 1995), a partial 5-HT_{1A} receptor agonist (Jordan et al. 2002), and as an 5-HT_{2A} receptor antagonist (McQuade et al. 2002). These pharmacological properties may play a role in the therapeutic effects of aripiprazole. Although aripiprazole has been reported to enhance cognitive function in schizophrenia (Rivas-Vasquez 2003), the mechanism of the improving effect of aripiprazole on cognitive impairment is unclear.

Phencyclidine [1-(1-phenylcyclohexyl) piperidine hydrochloride (PCP)], a noncompetitive *N*-metyl-D-aspartate receptor antagonist, has been shown to induce schizophrenia-like psychosis, presenting as positive symptoms, negative symptoms, and cognitive deficits in humans (Javitt and Zukin 1991), which persist several weeks after withdrawal of chronic PCP use (Allen and Young 1978; Lerner and Burns 1986; Rainey and Crowder 1975). To investigate the pathophysiology of schizophrenia, an animal model of schizophrenia was established using PCP (Mouri et al. 2007a). We have previously demonstrated that repeated treatment with PCP (10mg/kg/day s.c. for 14days) induces several schizophrenia-like behavioral abnormalities, such as increased immobility in a forced swimming test (Murai et al. 2007; Noda et al. 1995, 1997, 2000), social deficits in a social interaction test (Qiao et al. 2001), impairment of latent learning in a water finding test (Mouri et al. 2007b), and associative learning impairment in cued and contextual fear conditioning tests (Enomoto et al. 2005) in mice. Moreover, it has been reported that PCP induces the disruption of sensorimotor gating in a prepulse inhibition test (Bakshi et al. 1994) and recognition memory in a novel object recognition test (Hashimoto et al. 2005); therefore, PCP-treated mice might be a useful animal model of schizophrenia.

There are a few reports suggesting the effectiveness of aripiprazole on cognitive dysfunction and negative symptoms in PCP-treated animals. For example, aripiprazole improves PCP-induced disruption of prepulse inhibition (Fejgin et al. 2007) and social interaction (Bruins Slot et al. 2005) in mice and rats, respectively; however, the effects of aripiprazole on recognition memory remain to be investigated. In this study, we examined whether aripiprazole improves PCP-induced cognitive impairment in a novel object recognition test in mice.

Materials and methods

Animals

Male ICR mice (7weeks old) were obtained from Nihon SLC (Shizuoka, Japan). The animals were housed in plastic cages and kept in a regulated environment (23 ± 1°C, 50 ± 5% humidity), with a 12/12h light–dark cycle (lights on at 09:00hours). Food (CE2; Clea Japan, Tokyo, Japan) and tap water were available ad libitum. 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 Nagoya University.

Drugs

PCP hydrochloride was synthesized by the authors according to the method of Maddox et al. (1965) and was checked for purity. Aripiprazole was provided by Otsuka Pharmaceutical (Tokyo, Japan). SCH23390 hydrochloride, *S*-(−)-raclopride (+)-tartrate, haloperidol and WAY100635 were purchased from Sigma-Aldrich (St. Louis, MO, USA). The dose of each drug refers to previous reports (Noda et al. 1995; Bruins Slot et al. 2005; Kamei et al. 2006; Ito et al. 2007b).

PCP was dissolved in saline. SCH23390, raclopride, and WAY100635 was dissolved in distilled water. Aripiprazole and haloperidol were suspended in saline containing 0.1% carboxymethylcellulose (CMC) sodium salt. All drugs were administered in a volume of 0.1ml/10g body weight.

Measurement of locomotor activity

Each mouse was placed in a standard transparent rectangular rodent cage (25 × 30 × 18 high cm). Locomotor activity was then measured for 1h, using an infrared sensor (NS-AS01; Neuroscience, Tokyo, Japan) placed over the cage (Ito et al. 2007a).

Novel object recognition test

The novel object recognition test was performed according to previously reported methods (Nagai et al. 2003; Tang et al. 1999). The experimental apparatus consisted of a Plexiglas open-field box (30 × 30 × 35 high cm), the floor of which was covered in sawdust. The apparatus was located in a sound-attenuated room and illuminated with a 20-W bulb.

The procedure for the novel object recognition test consisted of three different sessions: habituation, training, and retention. Each mouse was individually habituated to the box, with 10min of exploration in the absence of objects each day for three consecutive days (habituation session, days1–3). In the training session, two different novel objects were symmetrically fixed to the floor of the box, 8cm from the walls, and each animal was allowed to explore the box for 10min (day4). The objects were a golf ball, wooden cylinders, and square pyramids, which were different in shape and color but similar in size. An animal was considered to be exploring the object when its head was facing the object or it was touching or sniffing the object. The time spent exploring each object was recorded. After training, mice were immediately returned to their home cages. In the retention sessions, the animals were placed back into the same box 24h (day5) after the training session, but with one of the familiar objects used during training replaced by a novel object. The animals were then allowed to explore freely for 5min, and the time spent exploring each object was recorded. Throughout the experiments, the objects were balanced in terms of their physical complexity and emotional neutrality. A 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 objects, was used to measure cognitive function.

Drug treatment

For effect of single treatment on locomotor activity, 0.1% CMC, aripiprazole (0.1−1.0mg/kg) or haloperidol (0.3−1.0mg/kg) was orally (p.o.) administered 1h before the experiment. The number of animals included in each drug treatment was as follows: CMC (n = 12), 0.1mg/kg aripiprazole (n = 12), 0.3mg/kg aripiprazole (n = 12), 1.0mg/kg aripiprazole (n = 11) for Fig. 1b,c; CMC (n = 10), 0.3mg/kg haloperidol (n = 10), 1.0mg/kg haloperidol (n = 10) for Fig. 1d,e.

For effect of repeated treatment on locomotor activity, 0.1% CMC, aripiprazole (0.01−0.1mg/kg), or haloperidol (0.3−1.0mg/kg) was p.o. administered for 7days; the experiment was performed 24h after last treatment. Locomotor activity was recorded for 1h. The number of animals included in each drug treatment was as follows: CMC (n = 10), 0.01mg/kg aripiprazole (n = 10), 0.03mg/kg aripiprazole (n = 10), 0.1mg/kg aripiprazole (n = 10) for Fig. 2b,c; CMC (n = 10), 0.3mg/kg haloperidol (n = 10), 1.0mg/kg haloperidol (n = 10) for Fig. 2d,e.

We have previously demonstrated that repeated treatment with PCP (10mg/kg/day s.c. for 14days) induces several schizophrenia-like behavioral and neurochemical abnormalities in mice (Murai et al. 2007; Noda et al. 1995, 1997, 2000; Qiao et al. 2001; Mouri et al. 2007b; Enomoto et al. 2005). Therefore, in typical experimental conditions, mice were given the same regimen of PCP (10mg/kg, s.c., for 14days, days1–14). Five days after the last treatment with PCP, the novel object recognition test was performed, including habituation (i.e., days19–21), training (i.e., day22), and retention (i.e., day23) sessions.

To study the single effects of antipsychotics, aripiprazole (0.01−1.0mg/kg) or haloperidol (0.3−1.0mg/kg) was administered p.o. (i.e., day22) to mice that had been previously treated with PCP for 14days (day1–14). One hour after treatment with antipsychotics, the training session using the novel object recognition test was conducted. The number of animals included in each drug treatment was as follows: saline + CMC (n = 16), PCP + CMC (n = 14), PCP + 0.01mg/kg aripiprazole (n = 10), PCP + 0.03mg/kg aripiprazole (n = 9), PCP + 0.1mg/kg aripiprazole (n = 8), PCP + 0.3mg/kg aripiprazole (n = 10), PCP + 1.0mg/kg aripiprazole (n = 11) for Fig. 3b,d; saline + CMC (n = 14), PCP + CMC (n = 13), PCP + 0.3mg/kg haloperidol (n = 7), PCP + 1.0mg/kg haloperidol (n = 9) for Fig. 3c,e.

To study the subchronic effects of antipsychotics, aripiprazole (0.01–0.1mg/kg) or haloperidol (0.3–1.0mg/kg) was administered p.o. once a day for seven consecutive days (i.e., days15–21) to mice that had been previously treated with PCP for 14days (days1–14). During habituation session of the novel object recognition test (i.e., days19–21), mice were administered antipsychotics after the habituation session. One day after the last treatment with antipsychotics (i.e., day22), the training session of the novel object recognition test was conducted. The number of animals included in each drug treatment was as follows: saline + CMC (n = 11), PCP + CMC (n = 10), PCP + 0.01mg/kg aripiprazole (n = 11), PCP + 0.03mg/kg aripiprazole (n = 8), PCP + 0.1mg/kg aripiprazole (n = 8) for Fig. 4b,d; saline + CMC (n = 15), PCP + CMC (n = 14), PCP + 0.3mg/kg haloperidol (n = 8), PCP + 1.0mg/kg haloperidol (n = 9) for Fig. 4c,e.

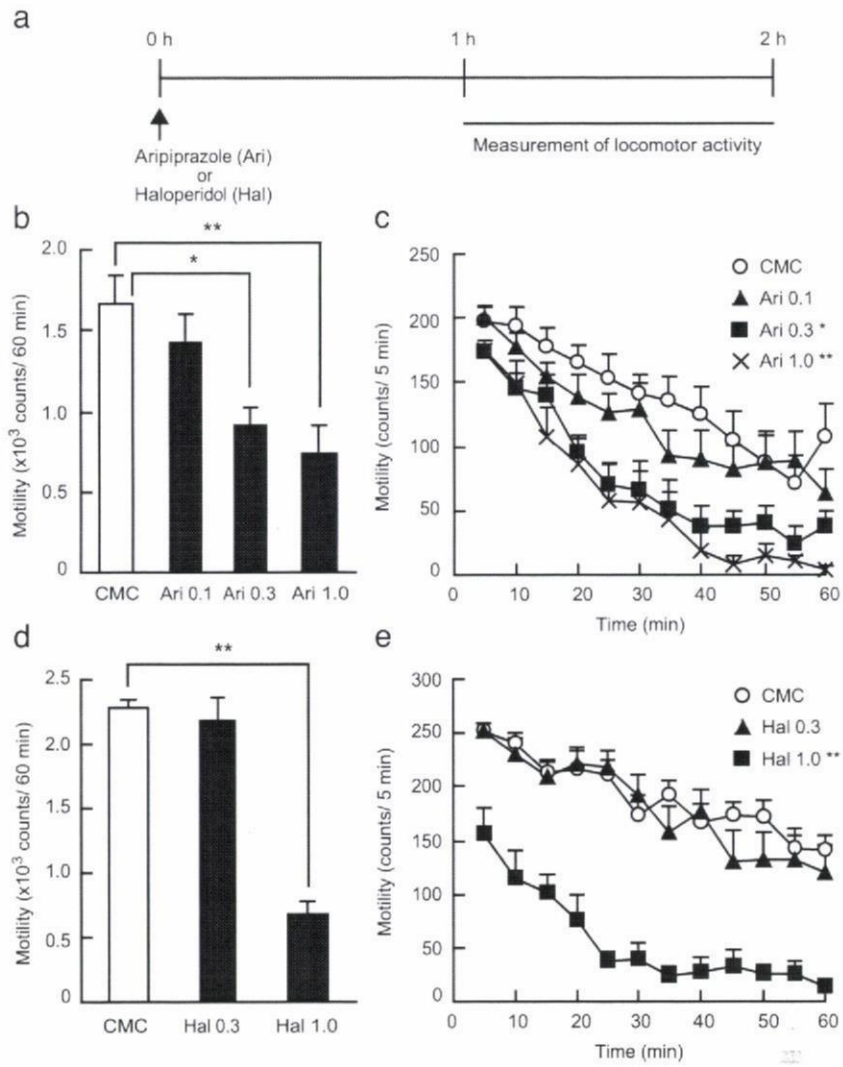


Fig. 1 Effects of single administration of aripiprazole and haloperidol on locomotor activity. **a** Experimental schedule for the measurement of locomotor activity. Mice were administered aripiprazole (*Ari*, 0.1–1.0 mg/kg, p.o.), haloperidol (*Hal*, 0.3–1.0 mg/kg, p.o.) or vehicle (0.1% *CMC*) 1 h before the measurement of locomotor activity. **b** and **c** Effect of single administration of aripiprazole on locomotor activity. **d** and **e** Effect of single administration of haloperidol on locomotor activity. **b** and **d** Total locomotor activity for 1 h. **c** and **e**: Time course of changes in locomotor activity. Values indicate the mean±SE (n=11–12). Analysis of variance: group, $F(3,43)=7.323$, $p<0.01$ for (**c**); time, $F(11,473)=58.971$, $p<0.01$ for (**c**); group × time, $F(33,473)=1.168$, $p=0.24$ for (**c**); group, $F(2,27)=46.806$, $p<0.01$ for (**e**); time, $F(11,297)=24.709$, $p<0.01$ for (**e**); group × time, $F(22,297)=1.370$, $p=0.13$ for (**e**). $^{*}p<0.05$ and $^{**}p<0.01$ compared with CMC group

To examine the role of dopamine D_1, D_2, and serotonin 5-HT_{1A} receptors in ameliorating the effect of aripiprazole on PCP-induced cognitive impairment, SCH23390 (0.03mg/kg), a dopamine D_1 receptor antagonist, raclopride (0.3mg/kg), a dopamine D_2 receptor antagonist, or WAY100635 (0.6mg/kg), a serotonin 5-HT_{1A} receptor antagonist, was administered intraperitoneally (i.p.) 30min after treatment with aripiprazole (0.1mg/kg, p.o.) for 7days because brain concentration of aripiprazole is the maximum at 2–3h after the oral administration, and declined $t_{1/2}$ of 1.8–2.0h in rats (Shimokawa et al. 2005). One day after the last treatment with aripiprazole and dopamine or serotonin receptor antagonists, the novel object recognition test was performed. The number of animals included in each drug treatment was as follows: saline + CMC + DW (n = 11), PCP + CMC + DW (n = 11), PCP + CMC + SCH23390 (n = 11), PCP + aripiprazole + DW (n = 11), PCP + aripiprazole + SCH23390 (n = 11) for Fig. 5b,d; saline + CMC + DW (n = 9), PCP + CMC + DW (n = 9), PCP + CMC + raclopride (n = 10), PCP + aripiprazole + DW (n = 9), PCP + aripiprazole + raclopride (n = 10) for Fig. 5c,e; saline + CMC + DW (n = 10), PCP + CMC + DW (n = 10), PCP + CMC + WAY100635 (n = 9), PCP + aripiprazole + DW (n = 10), PCP + aripiprazole + WAY100635 (n = 10) for Fig. 6.

Statistical analysis

All data were expressed as the mean±SEM. Statistical significance was determined using analysis of variance (ANOVA) with repeated measures (Figs. 1c,e, and 2c,e) or one-way (Figs. 1b,d, 2b,d, and 3–6), followed by the Bonferroni/Dunn test when F ratios were significant ($p<0.05$).

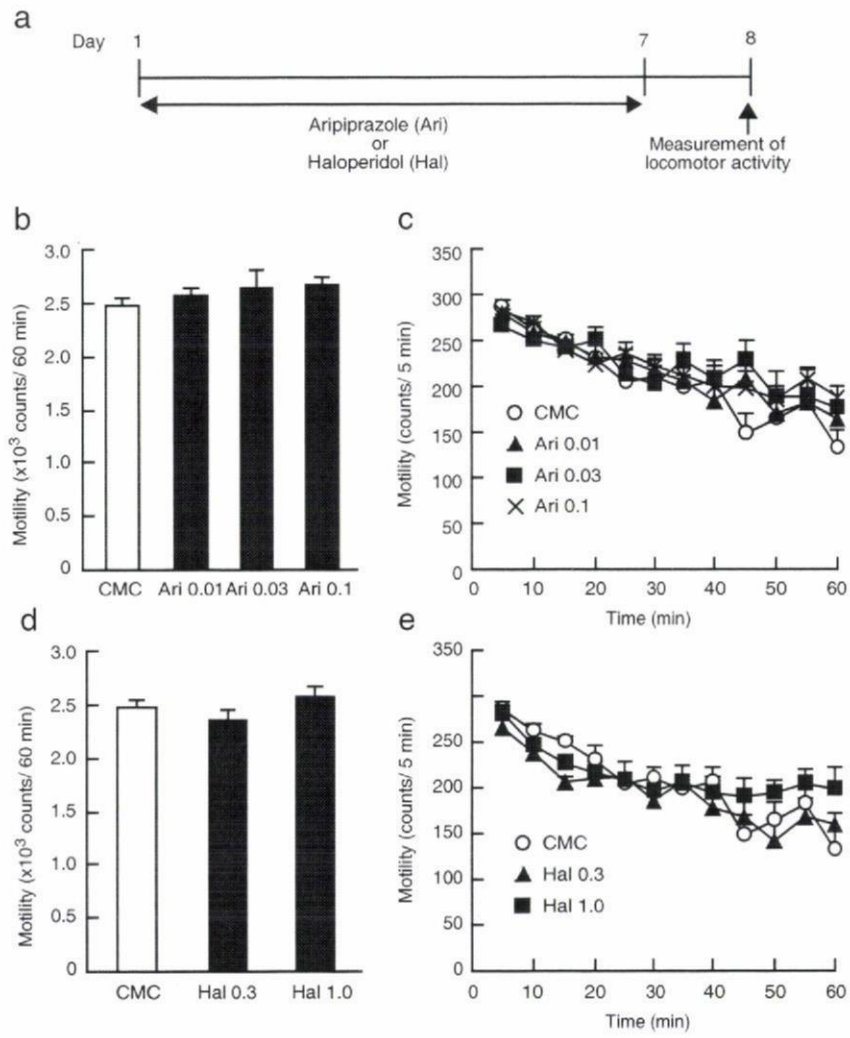


Fig. 2 Effects of repeated administration of aripiprazole and haloperidol on locomotor activity. **a** Experimental schedule for the measurement of locomotor activity. Mice were administered aripiprazole (*Ari*, 0.01–0.1 mg/kg, p.o.), haloperidol (*Hal*, 0.3–1.0 mg/kg, p.o.) or vehicle (0.1% *CMC*) for 7 days. Locomotor activity was measured 24 h after the last treatment. **b** and **c** Effect of repeated administration of aripiprazole on locomotor activity. **d** and **e** Effect of repeated administration of haloperidol on locomotor activity. **b** and **d** Total locomotor activity for 1 h. **c** and **e** Time course of changes in locomotor activity. Values indicate the mean±SE (n=10). Analysis of variance: group, $F(3,35)=0.743$, $p=0.53$ for (**c**); time, $F(11,385)=24.376$, $p<0.01$ for (**c**); group × time, $F(33,375)=1.099$, $p=0.33$ for (**c**); group, $F(2,27)=1.290$, $p=0.29$ for (**e**); time, $F(11,297)=18.444$, $p<0.01$ for (**e**); group × time, $F(22,297)=1.318$, $p=0.16$ for (**e**)

Results

Effects of administration of aripiprazole and haloperidol on locomotor activity

To explore the dose of aripiprazole and haloperidol which did not cause sedation in mice, we measured locomotor activity after oral administration of aripiprazole. Figure 1b and c shows the effect of a single administration of aripiprazole on locomotor activity in mice. Treatment with aripiprazole decreased total locomotor activity in a dose-dependent manner [$F(3,43)=7.323$, $p<0.01$, Fig. 1b]. The time course of changes in locomotor activity revealed that aripiprazole at doses of 0.3 and 1.0 mg/kg caused marked locomotor suppression 1 h after treatment [effect of group: $F(3,43)=7.323$, $p<0.01$; effect of time: $F(11,473)=58.971$, $p<0.01$; effect of interaction between group and time: $F(33,473)=1.168$, $p=0.24$ by two-way ANOVA with repeated measures, Fig. 1c]. Single treatment with haloperidol also decreased total locomotor activity in a dose-dependent manner [$F(2,27)=46.806$, $p<0.01$, Fig. 1d]. The time course of changes in locomotor activity revealed that haloperidol at the dose of 1.0 mg/kg caused marked locomotor suppression 1 h after treatment [effect of group: $F(2,27)=46.806$, $p<0.01$; effect of time: $F(11,297)=24.709$, $p<0.01$; effect of interaction between group and time: group × time, $F(22,297)=1.370$, $p=0.13$ by two-way ANOVA with repeated measures, Fig. 1e].

Effects of repeated administration of aripiprazole and haloperidol on locomotor activity were also examined. Mice were administered 0.1% CMC, aripiprazole (0.01–0.1 mg/kg, p.o.) or haloperidol (0.3–1.0 mg/kg, p.o.) was administered for 7 days. Locomotor activity was recorded 24 h after the last treatment. In contrast to the single treatment, repeated treatment with aripiprazole (0.01–0.1 mg/kg) and haloperidol (0.3–1.0 mg/kg) had no effect on the locomotor activity (Fig. 2).

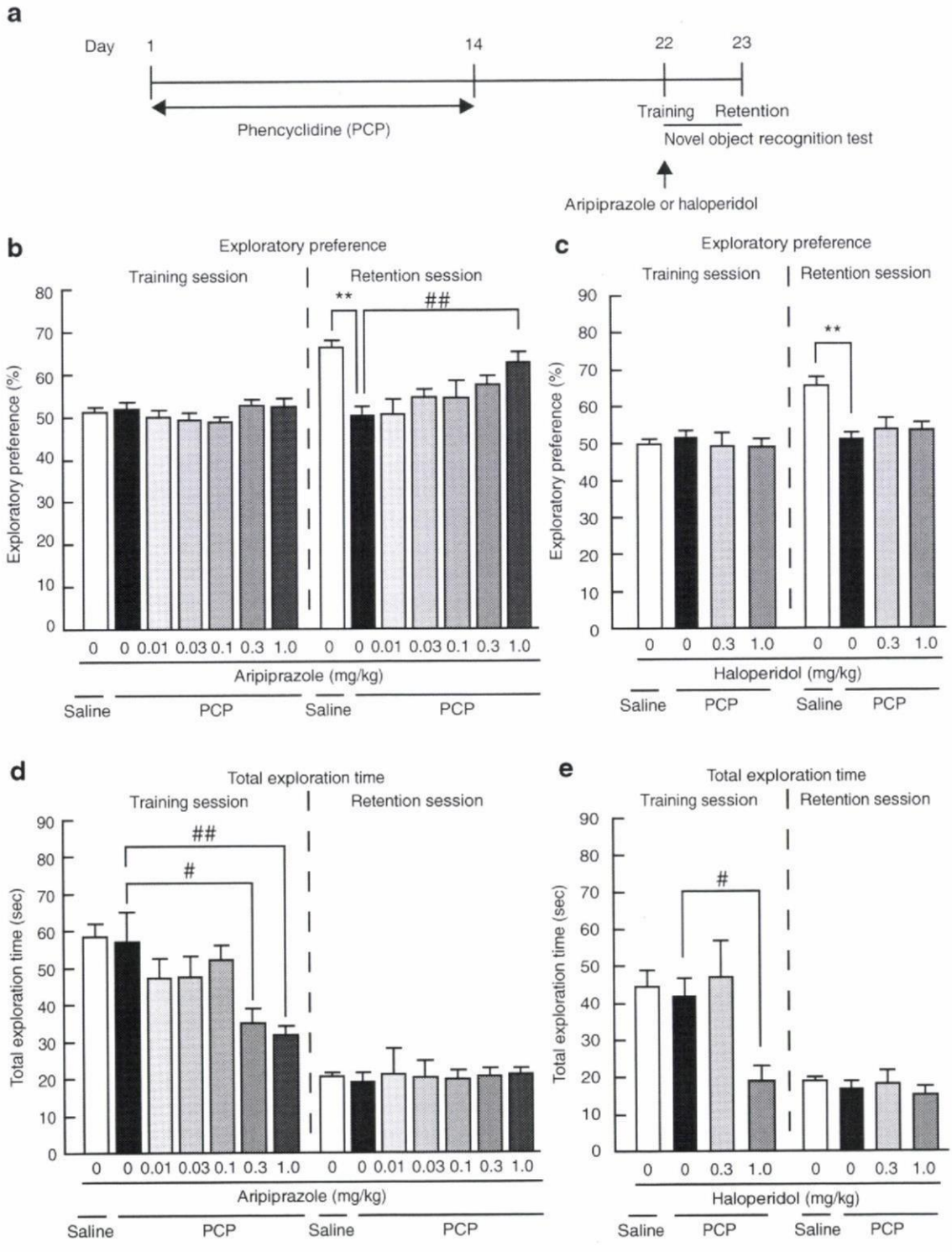


Fig. 3 Effects of single administrations of aripiprazole and haloperidol on PCP-induced cognitive impairment in novel object recognition. **a** Experimental schedule for the novel object recognition test. Eight days after withdrawal from repeated PCP (10 mg/kg, s.c., for 14 days) treatment, mice were subjected to the novel-object recognition test. Aripiprazole (0.01–1.0 mg/kg, p.o.), haloperidol (0.3–1.0 mg/kg, p.o.), or vehicle (0.1% CMC) was administered 1 h before the training session. **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=8–16). Analysis of variance: $F(6,77)=0.911$, $p=0.49$ for (**b**) training; $F(6,77)=7.304$, $p<0.01$ for (**b**) retention; $F(3,39)=0.303$, $p=0.82$ for (**c**) training; $F(3,39)=8.69$, $p<0.01$ for (**c**) retention; $F(6,77)=5.009$, $p<0.01$ for (**d**) training; $F(6,77)=0.057$, $p=0.99$ for (**d**) retention; $F(3,39)=4.665$, $p<0.01$ for (**e**) training; $F(3,39)=0.600$, $p=0.62$ for (**e**) retention. $^{**}p<0.01$ compared with saline + vehicle group. $^{\#}p<0.05$ and $^{\#\#}p<0.01$ compared with PCP + vehicle group

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.

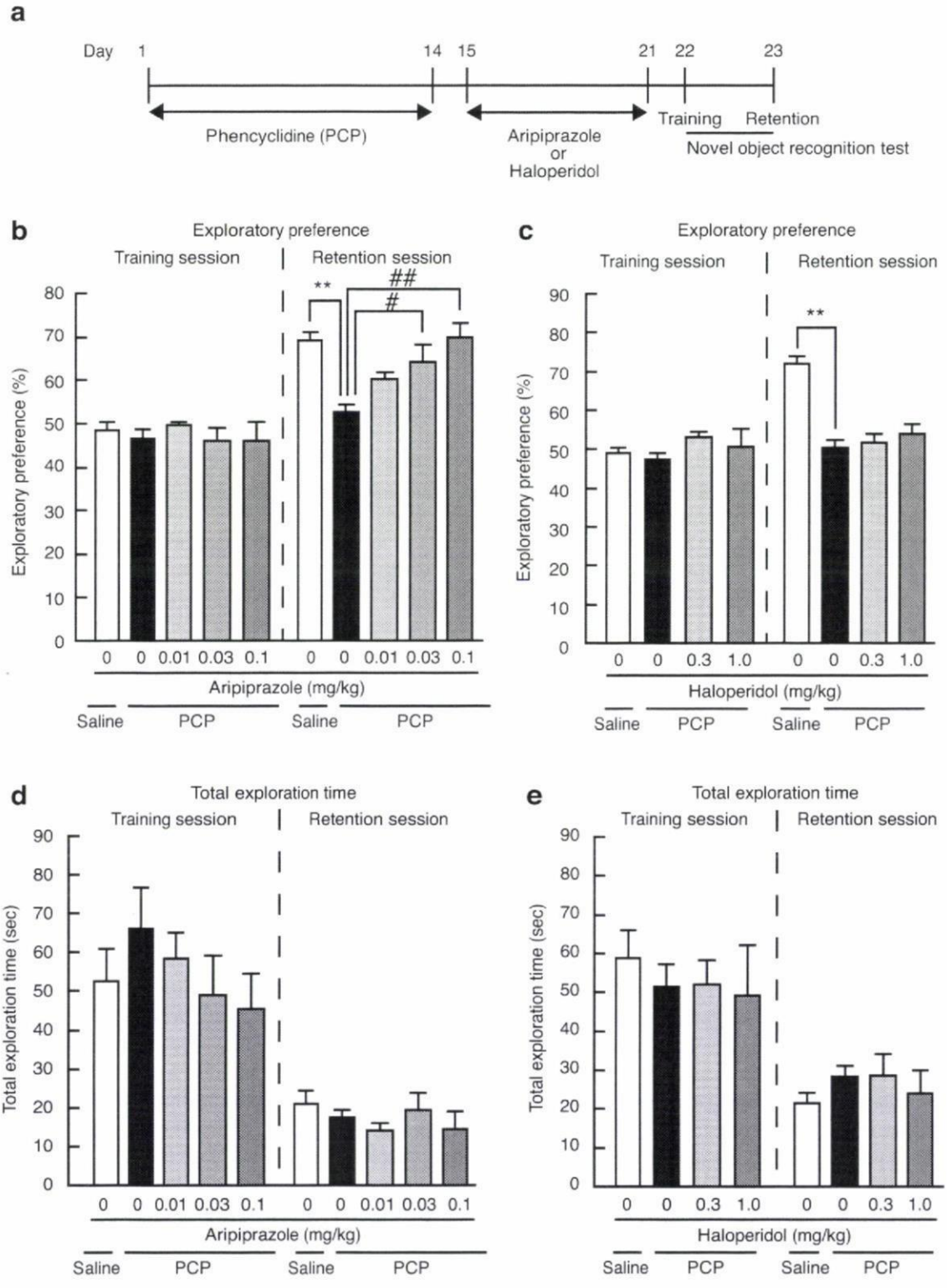
In the training session, treatment with WAY100635 alone did not affect the exploratory preference for objects in PCP-treated mice (Fig. 6b). In the retention session, WAY100635 blocked the ameliorating effect of aripiprazole on the impairment of exploratory preference for a novel object in PCP-treated 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

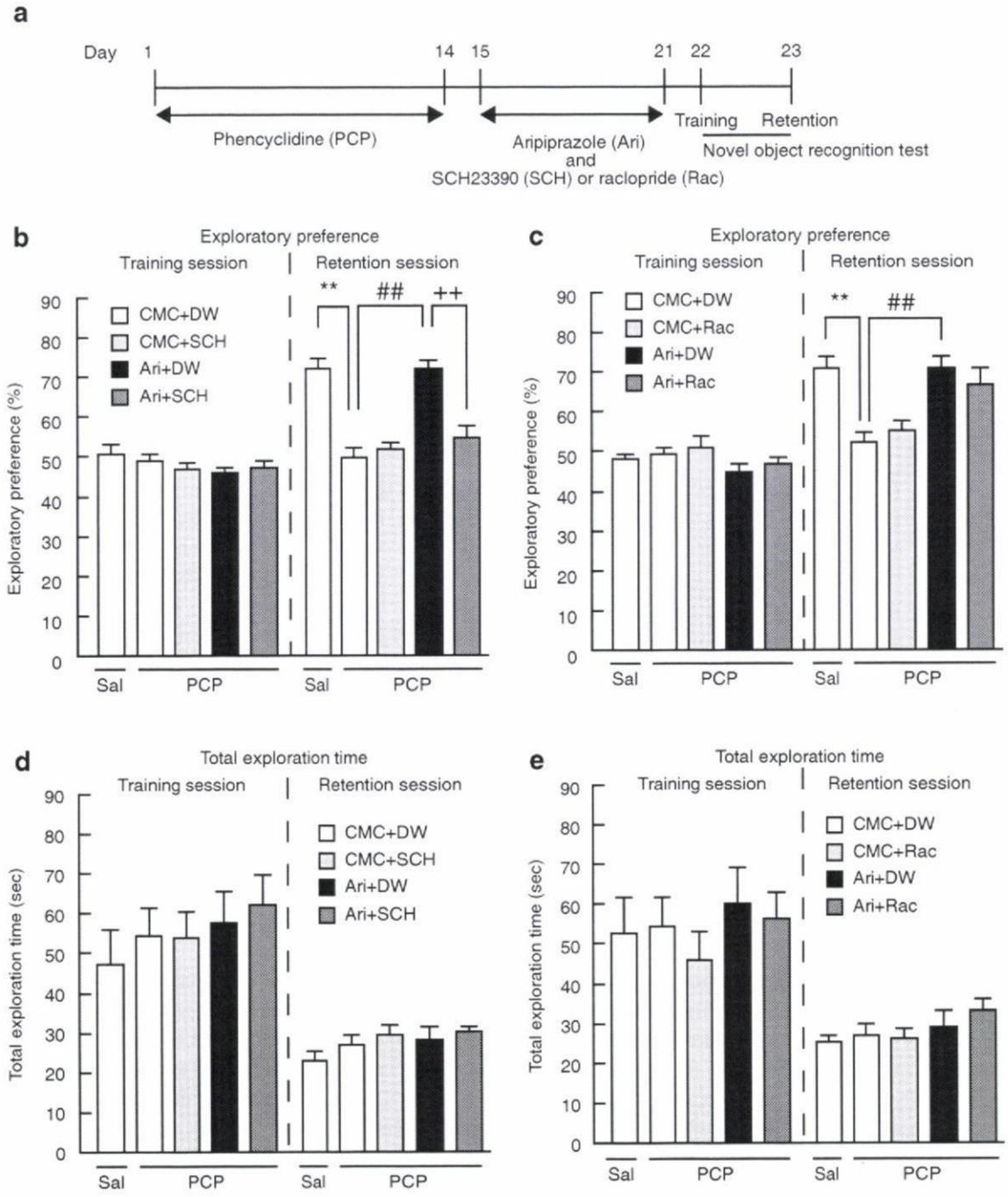


Fig. 5 Effect of dopamine D_1 and D_2 receptor antagonists on ameliorative effect of aripiprazole against PCP-induced cognitive impairment. **a** Experimental schedule for the novel object recognition test using dopamine D_1 and D_2 receptor antagonists. Aripiprazole (Ari, 0.1 mg/kg, p.o.) or vehicle (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

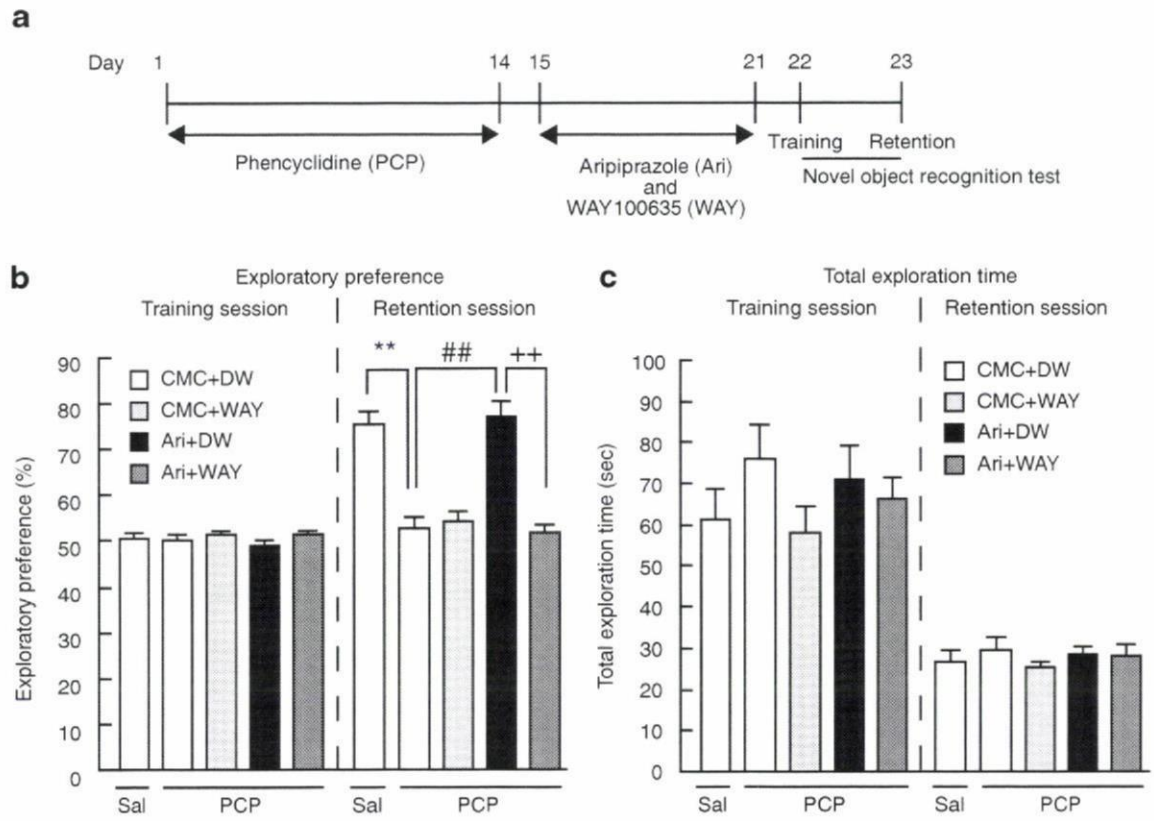


Fig. 6 Effect of serotonin 5-HT_{1A} receptor antagonist on ameliorative effect of aripiprazole against PCP-induced cognitive impairment. **a** Experimental schedule for the novel object recognition test using a serotonin 5-HT_{1A} receptor antagonist. Aripiprazole (Ari, 0.1 mg/kg, p.o.) or vehicle (0.1% 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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