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Phencyclidine-induced cognitive deficits in mice are improved by subsequent subchronic administration of the novel selective $\alpha 7$ nicotinic receptor agonist SSR180711

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Abstract

Background: Accumulating evidence suggests that $\alpha 7$ nicotinic receptor ($\alpha 7$ nAChR) agonists could be potential therapeutic drugs for cognitive deficits in schizophrenia. The present study was undertaken to examine the effects of the novel selective $\alpha 7$ nAChR agonist SSR180711 on cognitive deficits in mice after repeated administration of the NMDA receptor antagonist phencyclidine (PCP).

Methods: Saline or PCP (10 mg/kg/day for 10 days) was administered to mice. Subsequently, vehicle, SSR180711 (0.3 or 3.0 mg/kg/day), SSR180711 (3.0 mg/kg/day) plus the selective $\alpha 7$ nAChR antagonist methyllycaconitine (MLA; 3.0 mg/kg/day) or MLA (3.0 mg/kg/day) was administered i.p. for 2 consecutive weeks. Twenty-four hours after the final administration, a novel object recognition test was performed.

Results: PCP -induced cognitive deficits were significantly improved by subsequent subchronic (2-week) administration of SSR180711 (3.0 mg/kg). The effects of SSR180711 (3.0 mg/kg) were significantly antagonized by co-administration of MLA (3.0 mg/kg). Furthermore, Western blot analysis and immunohistochemistry revealed that levels of $\alpha 7$ nAChRs in the frontal cortex and hippocampus of the PCP (10 mg/kg/day for 10 days)-treated mice were significantly lower than those of saline-treated mice.

Conclusions: These findings suggest that repeated PCP administration significantly decreased

the density of $\alpha 7$ nAChRs in the brain, and that the $\alpha 7$ nAChR agonist SSR180711 could ameliorate cognitive deficits in mice after repeated administration of PCP. Therefore, $\alpha 7$ nAChR agonists including SSR180711 are potential therapeutic drugs for treating cognitive deficits in schizophrenic patients.

INTRODUCTION

Cognitive deficits in patients with schizophrenia are a core feature of the illness, which predicts vocational and social disabilities in patients (1-5). Accumulating evidence suggests that a dysfunction in the glutamatergic neurotransmission via the N-methyl-D-aspartate (NMDA) receptors might be involved in the pathophysiology of schizophrenia (3,6-11). NMDA receptor antagonists such as phencyclidine (PCP) and ketamine are known to induce schizophrenia-like symptoms including cognitive deficits in healthy subjects (6,12). Therefore, PCP has been used as an animal model of cognitive deficits in schizophrenia (13-16). We recently found that PCP-induced cognitive deficits in the novel object recognition test (NORT) could be significantly improved by subsequent subchronic (2-week) administration of clozapine, but not haloperidol (14). These findings suggest that reversal of PCP-induced cognitive deficits as measured by the NORT may be a potential animal model of atypical antipsychotic activity related to the amelioration of cognitive deficits in schizophrenia (14-16).

Several lines of evidence suggest that $\alpha 7$ nicotinic receptors ($\alpha 7$ nAChRs) might play a role in the pathophysiology of schizophrenia, and that $\alpha 7$ nAChR agonists might have therapeutic potential in the treatment of cognitive deficits in patients with schizophrenia (17-26). It has been reported that ARR17779, a full agonist at $\alpha 7$ nAChRs, significantly improved learning and memory as well as social recognition in rats (27,28) although one report did not

find any effects of ARR17779 in the five-choice task (29). Furthermore, it has been reported that the administration of tropisetron or 3-(2,4)-dimethoxybenzylidene anabaseine (DMXB), partial agonists at $\alpha 7$ nAChRs, improved the deficient inhibitory processing of the P20-N40 auditory evoked potential in DBA/2 mice (30,31). Recently, these two drugs have been demonstrated to improve the P50 auditory gating deficits in schizophrenic patients (32,33). Thus, it is likely that $\alpha 7$ nAChR agonists such as tropisetron and DMXB could be therapeutic for cognitive deficits in schizophrenia.

SSR180711, 4-bromophenyl 1,4-diazabicyclo[3.2.2]nonane-4-carboxylate hydrochloride, is a novel selective partial agonist at $\alpha 7$ nAChRs with a high affinity ($K_i=50$ nM for rat $\alpha 7$ nAChRs, $K_i=78$ nM for human $\alpha 7$ nAChRs)(25,34,35). Furthermore, it has been reported that SSR180711 restores the selective attention deficits induced by PCP administration at the neonatal stage and the spatial working memory deficit induced by dizocilpine (35). These findings suggest that SSR180711 has the potential to improve cognitive deficits in schizophrenic patients (25,34,35).

In the present study, using the NORT, we examined the effects of subsequent acute or subchronic (2-week) treatment with SSR180711 on cognitive deficits in mice after repeated administration of PCP. Furthermore, we studied whether repeated PCP treatment alters the density of $\alpha 7$ nAChRs in the mouse brain.

METHODS

Animals

Male ICR mice (6 weeks old) weighing 25–30 g were purchased from SLC Japan (Hamamatsu, Shizuoka, Japan). The mice were housed in clear polycarbonate cages (22.5×33.8×14.0 cm) and in groups of 5 or 6 mice under a controlled 12/12-h light–dark cycle (light from 7:00 AM to 7:00 PM), with room temperature at $23 \pm 1^{\circ}\text{C}$ and humidity at $55 \pm 5\%$. The mice were given free access to water and food pellets. The experimental procedure was approved by the Animal Care and Use Committee of Chiba University Graduate School of Medicine.

Drugs

PCP hydrochloride was synthesized in our laboratory at Chiba University. SSR180711 was synthesized at Nard Institute, Ltd (Amagasaki, Hyogo, Japan). Methyllycaconitine citrate (MLA) was purchased from Sigma-Aldrich Corporation (St. Louis, MI, USA). Other drugs were purchased from commercial sources.

Drug administration

Saline (10 ml/kg) or PCP (10 mg/kg expressed as a hydrochloride salt) was administered subcutaneously (s.c.) for 10 days (once daily on days 1-5, 8-12), with no treatment on days 6, 7, 13 and 14. In the experiment involving acute treatment, 3 days after the final administration of saline or PCP (i.e., on day 15), vehicle (10 ml/kg; saline) or SSR180711 (0.3 or 3.0 mg/kg) was administered intraperitoneally (i.p.). In the experiment involving subchronic (2-week) treatment, 3 days after the final administration of saline or PCP (i.e., on day 15), vehicle (10 ml/kg; saline), SSR180711 (0.3 or 3.0 mg/kg), SSR180711 (3.0 mg/kg) plus MLA (3.0 mg/kg), or MLA (3.0 mg/kg) was administered i.p. for 2 consecutive weeks (once daily on days 15-28). A 3 mg/kg dose of MLA was selected because this dose was effective in the deficient inhibitory processing of the P20-N40 auditory evoked potential in DBA/2 mice (31) or in PCP-induced cognitive deficits (15).

Novel object recognition test (NORT)

In the experiment involving acute treatment, 1 h after the final administration of vehicle or SSR180711, NORT was performed as previously reported (14-16,36). In the experiment involving subchronic (2-week) treatment, a training session for NORT was performed 24 hours after the final administration of vehicle, SSR180711 (0.3 or 3.0 mg/kg), SSR180711 (3.0 mg/kg) plus MLA (3.0 mg/kg), or MLA (3.0 mg/kg). The apparatus for this task consisted of a

black open field box (50.8×50.8×25.4 cm). Before the test, mice were habituated in the box for 3 days. During the training session, two objects (various objects differing in their shape and color but similar in size were used) were placed in the box 35.5 cm apart (symmetrically), and each animal was allowed to explore in the box for 10 min (5 min x 2). The animals were considered to be exploring the object when the head of the animal was facing the object within an inch of the object or when any part of the body, except for the tail, was touching the object. The time that the mice spent exploring each object was recorded. After the training, the mice were immediately returned to their home cages, and the box and objects were cleaned with 75% ethanol to avoid any possible instinctive odorant cues. Retention test sessions were carried out at 1-day intervals following the initial training. During the retention test sessions, each mouse was placed back in the same box, but one of the objects in the box used during the initial training was replaced with a novel one. The mice were then allowed to freely explore for 5 min, and the time spent exploring each object was recorded. Throughout the experiments, the objects were used in a counter-balanced manner in terms of their physical complexity. A preference index, the ratio of the amount of time spent exploring any one of the two objects (training session) or the novel object (retention session) to the total time spent exploring both objects, was used to measure memory performance.

Western blotting

Saline (10 ml/kg/day) or PCP (10 mg/kg/day) was administered s.c. for 10 days (once daily on days 1-5, 8-12). First, three days after the final administration of saline or PCP (10 mg/kg/day for 10 days), i.e. on day 15, the mice were sacrificed by decapitation. Second, three days after the last administration of saline or PCP (i.e., on day 15), vehicle (10 ml/kg/day; saline) or SSR180711 (3.0 mg/kg/day) was administered i.p. into mice for 2 consecutive weeks (once daily on days 15-28). Twenty-four hours after the last administration of vehicle or SSR180711, the mice were sacrificed by decapitation. Then, the frontal cortex and hippocampus were dissected on ice and stored at -80°C.

Briefly, brain tissue was homogenized in 10 vol of 5 mM Tris/HCl (pH 7.4) containing 0.32 M sucrose and centrifuged for 10 min at 1,000 x g. The resulting supernatant was recentrifuged for 10 min at 40,000 x g to obtain the crude membrane fraction. The pellet was washed twice in buffer and resuspended. Aliquots (frontal cortex: 25 µg protein, hippocampus: 40 µg protein) of the membranes were incubated for 5 min at 95°C with an equal volume of 125 mM Tris/HCl, pH 6.8, 20% glycerol, 0.002% bromphenol blue, 10% β-mercaptoethanol, and 4% SDS, and subjected to SDS-PAGE using 10% mini-gels (Mini Protean II; Bio-Rad, Hercules, CA, USA). Proteins were transferred onto PVDF membranes using a Trans Blot Mini Cell (Bio-Rad, Hercules, CA, USA). For immunodetection, the blots were blocked for 1-2 h in TBST (50 mM Tris/HCl, pH 7.8, 0.13 M NaCl, 0.1% Tween 20) containing 5% nonfat dry milk at

room temperature (RT), followed by incubation with rabbit anti- $\alpha 7$ nAChR antibody (1:2,000, Cat. No: AB5637, Chemicon International Inc., Temecula, CA, USA) overnight at 4°C in TBST/5% blocker. The blots were washed five times with TBST. Incubation with the secondary antibody (GE Healthcare Bioscience, UK) was performed for 1 h at RT. After extensive washing, immunoreactivity was detected by ECL plus the Western Blotting Detection system (GE Healthcare Bioscience, UK). Images were captured using a Fuji LAS3000-mini imaging system (Fujifilm, Tokyo, Japan), and immunoreactive bands were quantified. β -Actin immunoreactivity was used to monitor equal sample loading. The levels of $\alpha 7$ nAChRs in the PCP-treated mice were expressed as percentages of those of saline-treated mice (controls).

Immunohistochemistry

Three days after the final administration of saline (10 ml/kg/day for 10 days; days 1-5 and days 8-12) or PCP (10 mg/kg/day for 10 days; days 1-5 and days 8-12), i.e. on day 15, the mice were deeply anesthetized with sodium pentobarbital and perfused transcardially with 10 ml of isotonic saline, followed by 40 ml of ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were removed from the skulls and were postfixed overnight at 4°C in the same fixative. For the immunohistochemical analysis, 50- μ m-thick serial sagittal sections of brain were cut in ice-cold 0.01M phosphate buffer saline (PBS; pH 7.5) using a vibrating blade

microtome (VT1000S, Leica Microsystems AG, Wetzlar, Germany). Free-floating sections were treated with 0.3% H₂O₂ in 0.05M Tris-HCl saline (TBS) for 30 min, and were blocked in TBS containing 0.2 % Triton X-100 (TBST) and 1.5 % normal serum for 1 h at room temperature. The samples were then incubated for 36 hours at 4°C with rabbit anti- $\alpha 7$ nAChR antibody (1:10,000, Cat. No: AB5637, Chemicon International Inc., Temecula, CA, USA). The sections were washed twice in TBST, and were processed according to the avidin-biotin-peroxidase method (Vectastain *Elite* ABC, Vector Laboratories, Inc., Burlingame, CA, USA). The sections were then reacted for 5 min in a solution of 0.15 mg/ml diaminobenzidine containing 0.01 % H₂O₂. Alternate sections, incubated in the absence of primary antibody as an immunohistochemical control, showed no immunostaining. The sections were mounted on gelatinized slides, dehydrated, cleared, and coverslipped under Entellan[®] New (Merck KGaA, Darmstadt, Germany). The sections were imaged, and the intensity of $\alpha 7$ nAChR-immunoreactivity in the frontal cortex and hippocampus was analyzed by using a light microscope equipped with a CCD camera (Olympus IX70) and the SCION IMAGE software package. The $\alpha 7$ nAChR immunoreactivity was quantified in the regions of the frontal cortex and hippocampus in a blinded manner. The levels of $\alpha 7$ nAChRs in the PCP-treated mice were expressed as percentages of those of saline-treated mice (controls).

Determination of PCP and SSR180711 in the mouse brain

In the experiment involving an acute single treatment, mice were sacrificed by decapitation 1 hour after a single administration of vehicle (10 ml, i.p.) or SSR180711 (3 mg/kg, i.p.). In the experiment involving subchronic (2-week) treatment, saline (10 ml/kg/day, s.c.) or PCP (10 mg/kg/day/ s.c.) was administered for 10 days (once daily on days 1-5, 8-12), and no treatment was given on days 6, 7, 13 and 14. Three days after the final administration of saline or PCP (i.e., on day 15), vehicle (10 ml/kg/day; saline) or SSR180711 (3.0 mg/kg/day) was administered i.p. for 2 consecutive weeks (once daily on days 15-28). Twenty-four hours after the final administration of vehicle or SSR180711, the mice were sacrificed by decapitation. Then brains were dissected on ice, and the frontal cortex and hippocampus were weighed.

Briefly, the brain tissues were homogenized in 20 volumes of methanol (HPLC grade) on ice. The homogenates were centrifuged at 4,500 *g* for 10 min, and 20 μ l of supernatant was injected into the high performance liquid chromatography (HPLC) system with UV detector (Shimadzu Co. Ltd, Kyoto, Japan). A column (4.6 x 250 mm; TSKgel ODS-80Ts, Tosoh Corporation, Tokyo, Japan) was used. The UV detector was set at 244 nm. The mobile phases for the determination of PCP and SSR180711 were acetonitrile: 100 mM sodium acetate (30: 70, vol/vol) containing 0.1% triethylamine (final pH 5.6) and acetonitrile: 30 mM ammonium acetate: acetic acid (HPLC grade)(300: 700: 2, vol/vol), respectively. The flow rate was 1.0 ml/min.

Statistical analysis

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

RESULTS

Effects of SSR180711 on PCP-induced cognitive deficits in mice

In the NORT, repeated administration of PCP (10 mg/kg/day for 10 days) caused significant cognitive deficits in the mice, a result that is consistent with previous reports (14-16). During the training session, there were no significant differences among the four groups in the total amount of time spent exploring the two objects nor in the exploratory preference (**Fig. 1A**). In the retention session, the exploratory preference (approximately 40%) of the PCP-treated group was significantly lower than that (approximately 50%) of the saline-treated group, suggesting that the behavior of the PCP-treated mice may not have been a result of memory impairment (14). Therefore, it is likely that our model of PCP-induced cognitive deficits using NORT may show negative symptoms, such as social withdrawal, which are related to cognitive deficits (14). In the retention session, a single administration of SSR180711 (0.3 or 3.0 mg/kg, 1 h) did not alter the reduction of the exploratory preference in mice after repeated administration

of PCP (**Fig. 1B**).

In contrast, PCP-induced cognitive deficits were significantly improved after subsequent subchronic (2-week) administration of the higher dose (3.0 mg/kg/day), but not of the lower dose (0.3 mg/kg/day), of SSR180711. In the training session, the exploratory preferences of the six groups were not significantly different ($F(5,74) = 0.762$, $P=0.580$) (**Fig. 2B**). However, in the retention session, ANOVA analysis revealed that the exploratory preferences of the six groups were significantly different ($F(5,74) = 12.11$, $P<0.001$) (**Fig. 2B**). A *post hoc* Bonferroni test indicated that the exploratory preference of the PCP-treated group was significantly ($p<0.001$) increased after subchronic (2-week) administration of SSR180711 (3.0 mg/kg/day), but not SSR180711 (0.3 mg/kg/day) (**Fig. 2B**). Furthermore, the effect of SSR180711 (3.0 mg/kg) on the PCP-induced cognitive deficits was significantly ($p<0.001$) antagonized by the co-administration of MLA (3 mg/kg/day) (**Fig. 2B**). Moreover, subchronic (2-week) administration of MLA (3.0 mg/kg/day) alone did not alter PCP-induced cognitive deficits in mice (**Fig. 2B**).

Effects of repeated PCP administration on the levels of $\alpha 7$ nAChRs in the mouse brain

Western blot analysis revealed that the levels of $\alpha 7$ nAChRs in the frontal cortex ($t=2.589$, $p=0.021$) and hippocampus ($t=3.024$, $p=0.009$) of the PCP (10 mg/kg/day for 10 days)-treated mice were significantly lower than those of saline-treated mice (**Fig. 3**). Furthermore,

immunohistochemistry revealed that the immunoreactivity of $\alpha 7$ nAChRs in the frontal cortex ($t=3.182$, $p=0.005$) and hippocampus ($t=5.820$, $p<0.001$) of mice treated with PCP (10 mg/kg/day 10 days) was significantly lower than that of the saline-treated group (**Fig. 4**).

Next, we examined whether subsequent subchronic (2-week) administration of SSR180711 (3 mg/kg/day) alters the reduction of $\alpha 7$ nAChRs in the mouse brain after repeated PCP administration. In this study, we found that subchronic administration of SSR180711 (3 mg/kg/day) did not alter the reduction of $\alpha 7$ nAChRs in the frontal cortex and hippocampus after repeated PCP administration (**Fig. 5**). These findings suggest that repeated PCP administration caused a long-term reduction of $\alpha 7$ nAChRs in the mouse brain (more than 2 weeks after the final administration of PCP).

HPLC determination of PCP and SSR180711 levels in the mouse brain

In order to ascertain whether the behavioral effects of SSR180711 are due to the stimulation at $\alpha 7$ nAChRs in the mouse brain or due to another effect (residual PCP) in the mouse brain, we measured the PCP levels in the mouse brain at the time of the behavioral NORT test. In this study, we did not detect the compound PCP in the mouse brain 24 hours after the subsequent subchronic (2-week) administration of SSR180711 (3 mg/kg/day). Therefore, it is likely that the behavioral effect of SSR180711 on PCP-induced cognitive deficits might be due to the stimulation at $\alpha 7$ nAChRs in the mouse brain, but not to residual PCP in the mouse

brain.

Next, we measured the levels of SSR180711 in the mouse brain at the time of the behavioral NORT test. We could detect the compound SSR180711 (SSR180711 treated group: 33.04 ± 10.97 ng/mg tissue (n=6); vehicle treated group: not detected) in the mouse brain at 1 hour after a single acute administration of SSR180711 (3 mg/kg). However, we did not detect the compound SSR180711 in the mouse brain at 24 hours after the subchronic (2-week) administration of SSR180711 (3 mg/kg/day). These findings suggest that the behavioral effect of SSR180711 on PCP-induced cognitive deficits might be due to the subchronic stimulation at $\alpha 7$ nAChRs in the mouse brain, but not to residual SSR180711 in the mouse brain.

DISCUSSION

The major findings of the present study are that repeated PCP administration significantly decreased the density of $\alpha 7$ nAChRs in the mouse brain, and that PCP-induced cognitive deficits could be improved by subsequent subchronic (2-week) administration of the novel selective $\alpha 7$ nAChR agonist SSR180711. We reported recently that, in the NORT, PCP-induced cognitive deficits could be improved by subsequent subchronic (2-week) administration of clozapine, but not of haloperidol, suggesting that the reversal of PCP-induced cognitive deficits using the NORT may be a potential animal model of atypical antipsychotic activity related to the amelioration of cognitive deficits in schizophrenia (14). Very recently, Pichat et al. (35)

reported that SSR180711 significantly reversed dizocilpine-induced deficits in the retention of episodic memory in rats (object recognition test). SSR180711 was co-administered with dizocilpine (0.1 mg/kg, i.p.) in that study (35), whereas SSR180711 was administered after repeated PCP administration in the present study. Taken together, these findings suggest that SSR180711 could ameliorate cognitive deficits after the administration of NMDA receptor antagonists (PCP and dizocilpine), although the treatment schedules differed between the two studies. Nonetheless, it is likely that SSR180711 can be used as a therapeutic drug in the treatment of cognitive deficits in schizophrenia.

In the present study, we found that repeated PCP administration caused the reduction of $\alpha 7$ nAChRs in the mouse brain. This is the first report demonstrating that the administration of PCP significantly decreased the density of $\alpha 7$ nAChRs in the brain. The precise mechanism(s) underlying how repeated PCP administration could modulate $\alpha 7$ nAChRs in the brain are currently unknown. A postmortem human brain study demonstrated decreased expression of hippocampal $\alpha 7$ nAChRs in schizophrenic patients (37), suggesting that schizophrenic patients have fewer $\alpha 7$ nAChRs in the hippocampus, a condition which may lead to the failure of cholinergic activation of the inhibitory interneurons, manifesting clinically as decreased gating of responses to sensory stimulation (18,37). Furthermore, it has been reported that the immunoreactivity of $\alpha 7$ nAChRs in the prefrontal cortex of schizophrenia was significantly

decreased as compared to normal controls (38). Interestingly, $\alpha 7$ nAChR agonists can increase the release of glutamate from the presynaptic terminals, resulting in stimulation of the NMDA receptors on the postsynaptic neurons, suggesting that stimulation at $\alpha 7$ nAChRs may potentiate the NMDA receptors (11,25). Taken together, these findings suggest that $\alpha 7$ nAChRs may interact with the NMDA receptors in the brain (11,25), although further study on the cross-talk between $\alpha 7$ nAChRs and NMDA receptors in the brain is necessary (39).

Deficient inhibitory processing of the P50 auditory evoked potential is a pathophysiological feature of schizophrenia (17,18,40). Several lines of evidence suggest that $\alpha 7$ nAChRs play a critical role in this phenomenon (17-19,22). Similar to schizophrenic patients, DBA/2 mice (decreased levels of $\alpha 7$ nAChRs) spontaneously exhibit a deficit in the inhibitory processing of the P20-N40 auditory evoked potential, which is thought to be a rodent analogue of the human P50 auditory evoked potential (41). We reported that the administration of tropisetron, a partial agonist at $\alpha 7$ nAChRs, improved the deficient inhibitory processing of the P20-N40 auditory evoked potential in DBA/2 mice, and that the co-administration of MLA (3 mg/kg) blocked the normalizing effect of tropisetron, suggesting that $\alpha 7$ nAChRs play a role in the mechanism of action of tropisetron (15). In addition, we also reported that tropisetron improved the deficits of P50 suppression in schizophrenic patients (32). Recently, we also reported that, via $\alpha 7$ nAChRs, tropisetron could improve PCP-induced cognitive deficits in

mice (31). Furthermore, it has been reported that the selective $\alpha 7$ nAChR agonist PNU-282987 restores auditory gating deficits in rats after the administration of amphetamine (42), and that the selective $\alpha 7$ nAChR agonists, (R)-3'-(5-chlorothiophen-2-yl)spiro-1-azabicyclo[2,2,2]octane-3,5'-[1',3']oxazolidin-2'-one (25,43) and W-56203, (R)-3'-(3-methylbenzo[b]thiophen-5-yl)spiro[1-azabicyclo[2.2.2]octane-3,5'-oxazolidin]-2'-one (25,44), significantly improved dizocilpine-induced auditory gating deficits in rats. Therefore, it may be of interest to study the P20-N40 auditory evoked potential in mice with the repeated PCP administration, since repeated PCP administration decreased the density of $\alpha 7$ nAChRs in the mouse brain.

In conclusion, the present findings suggest that repeated administration of PCP decreased the density of $\alpha 7$ nAChRs in the mouse brain, and that SSR180711 could improve the cognitive deficits that occur in mice after the repeated administration of PCP. These findings suggest that $\alpha 7$ nAChR agonists such as SSR180711 could be used as potential therapeutic drugs in the treatment of cognitive deficits in schizophrenia.

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Figure legends**Figure 1. Effects of acute administration of SSR180711 on PCP-induced cognitive deficits**

in mice. Saline (10 ml/kg) or PCP (10 mg/kg) was administered s.c. for 10 days (once daily on days 1-5, 8-12). Three days after the last administration of saline or PCP (i.e., on day 15), vehicle (10 ml/kg; saline) or SSR180711 (3 mg/kg) was administered i.p. to the mice. The novel object recognition test (NORT) was performed 1 hr after the administration. Values are the means $\pm$ S.E.M. (n=7-13). *p<0.05 as compared with the saline-treated group (control).

Figure 2. Effects of subchronic administration of SSR180711 on PCP-induced cognitive

deficits in mice. Saline (10 ml/kg) or PCP (10 mg/kg) was administered s.c. for 10 days (once daily on days 1-5, 8-12). Three days after the last administration of saline or PCP (i.e., on day 15), vehicle (10 ml/kg/day; saline), SSR180711 (0.3 or 3.0 mg/kg/day), SSR180711 (3.0 mg/kg/day) plus MLA (3.0 mg/kg/day), or MLA (3.0 mg/kg/day) was administered i.p. to the mice for 2 consecutive weeks (once daily on days 15-28). On days 29 and 30, the NORT was performed. Values are means $\pm$ S.E.M. (n=7-24). ***p<0.001 as compared with the PCP plus saline-treated group. +++p<0.001 as compared with the PCP plus SSR180711 (3.0 mg/kg)-treated group.

Figure 3. Effects of repeated administration of PCP on $\alpha 7$ nAChR protein in the mouse

brain. Saline (10 ml/kg/day) or PCP (10 mg/kg/day) was administered s.c. for 10 days (once

daily on days 1-5, 8-12). Three days after the last administration of saline or PCP (i.e., on day 15), the mice were sacrificed by decapitation. Western blot analysis using $\alpha 7$ nAChR antibody was performed as described in Methods. The levels of $\alpha 7$ nAChRs in the PCP-treated mice were expressed as percentages of those of the saline-treated mice. Values are the means $\pm$ S.E.M. (n=8). * $p < 0.05$, ** $p < 0.01$ as compared with the saline-treated group (control).

Figure 4. Effects of repeated administration of PCP on the distribution of $\alpha 7$ nAChR

immunoreactivity in the mouse brain. Saline (10 ml/kg/day) or PCP (10 mg/kg/day) was administered s.c. for 10 days (once daily on days 1-5, 8-12). Three days after the last administration of saline or PCP (i.e., on day 15), the mice were perfused.

Immunohistochemistry was performed as described in Methods. Values are the means $\pm$ S.E.M. (n=11). ** $p < 0.01$, *** $p < 0.001$ as compared with the saline-treated group (control).

Figure 5. Effects of subsequent subchronic administration of SSR180711 on the reduction

of $\alpha 7$ nAChR immunoreactivity in the mouse brain after the repeated administration of

PCP. Saline (10 ml/kg/day) or PCP (10 mg/kg/day) was administered s.c. for 10 days (once daily on days 1-5, 8-12). Three days after the last administration of saline or PCP (i.e., on day 15), vehicle (10 ml/kg/day; saline) or SSR180711 (3.0 mg/kg/day) was administered i.p. to the mice for 2 consecutive weeks (once daily on days 15-28). Twenty-four hours after the last administration of vehicle or SSR180711, the mice were sacrificed by decapitation. Western blot

analysis using $\alpha 7$ nAChR antibody was performed as described in Methods. The levels of $\alpha 7$ nAChRs in the PCP-treated mice were expressed as percentages of those of the saline-treated mice. Values are the means $\pm$ S.E.M. (n=8). *p<0.05, **p<0.01 as compared with the saline-treated group (control).

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5