Prenatal exposure to phencyclidine produces abnormal behaviour and NMDA receptor expression in postpubertal mice

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Abstract
Several studies have shown the disruptive effects of non-competitive N-methyl-D-aspartate (NMDA) receptor antagonists on neurobehavioural development. Based on the neurodevelopment hypothesis of schizophrenia, there is growing interest in animal models treated with NMDA antagonists at developing stages to investigate the pathogenesis of psychological disturbances in humans. Previous studies have reported that perinatal treatment with phencyclidine (PCP) impairs the development of neuronal systems and induces schizophrenia-like behaviour. However, the adverse effects of prenatal exposure to PCP on behaviour and the function of NMDA receptors are not well understood. This study investigated the long-term effects of prenatal exposure to PCP in mice. The prenatal PCP-treated mice showed hypersensitivity to a low dose of PCP in locomotor activity and impairment of recognition memory in the novel object recognition test at age 7 wk. Meanwhile, the prenatal exposure reduced the phosphorylation of NR1, although it increased the expression of NR1 itself. Furthermore, these behavioural changes were attenuated by atypical antipsychotic treatment. Taken together, prenatal exposure to PCP produced long-lasting behavioural deficits, accompanied by the abnormal expression and dysfunction of NMDA receptors in postpubertal mice. It is worth investigating the influences of disrupted NMDA receptors during the prenatal period on behaviour in later life.

Key words: Antipsychotic, behaviour, neurodevelopment, NMDA receptor, PCP, prenatal.

Introduction
Neurodevelopmental abnormalities are considered part of the pathogenesis of psychological disturbances. Exposure to environmental insults during pregnancy increases the probability of neuropsychiatric disorders in later life (Brown & Susser, 2002; Green et al. 1994).

According to the neurodevelopmental hypothesis of schizophrenia, disruption of the prenatal brain predisposes the neural systems to long-lasting structural and functional abnormalities, leading to the emergence of psychopathological behaviour in adulthood (Ashdown et al. 2006).

The N-methyl-D-aspartate (NMDA) receptor, a kind of ligand-gated ion channel, is a heteromeric assembly comprising a core NR1 subunit and several modulatory subunits. At the cell surface, including synapses, NMDA receptors are anchored and clustered forming larger complexes (Husi & Grant, 2001). Stimulation of NMDA receptors during development
is critical for the survival, differentiation and migration of immature neurons (Behar et al. 1999; Komuro & Rakic, 1993), and controls structure and plasticity (Scheetz & Constantine-Paton, 1994), as well as establishing normal neural networks in the developing brain (Deutsch et al. 1998). It has been found that pharmacological inhibition of NMDA receptors during development disturbs neural functions in the brain (Bellinger et al. 2002). Post-mortem studies have identified the abnormal expression (Akbarian et al. 1996; Dracheva et al. 2001) and phosphorylation (Emamian et al. 2004) of NMDA receptors in the prefrontal cortex (PFC) of schizophrenia patients.

In clinical tests, abuse of phencyclidine (PCP), a non-competitive NMDA receptor antagonist, causes a schizophrenic psychosis in normal volunteers and exacerbates symptoms in schizophrenia patients (Javitt & Zukin, 1991). In adult rodents, PCP produces abnormal behaviour and biochemical alterations resembling schizophrenia including positive symptoms, negative symptoms, and cognitive deficits (Mouri et al. 2007a, c; Noda et al. 1995). However, several lines of evidence suggest that abnormal architectural arrangements of nerve cells, or cortical layers (Bogerts, 1993), an absence of normal cerebral structural asymmetry (Crow et al. 1989), and gliosis (Jones et al. 1994) are involved in the pathology of schizophrenia. This suggests schizophrenia to be a developmental disorder rather than a progressive degenerative disease (Bogerts, 1993).

Therefore, although many schizophrenia-like symptoms are observed in adult rodents repeatedly treated with PCP, it is unlikely that these abnormalities completely resemble the pathogenesis of schizophrenia, since at least in some cases, they occur in the developing period initiated by prenatal insults (Murray et al. 1992; Pilowski et al. 1993). Therefore, based on the neurodevelopmental hypothesis, several studies have modified this classic ‘PCP animal model’, through treatment with NMDA antagonists early in the development of the brain. For instance, perinatal PCP treatment in rats enhanced hyperlocomotion elicited by PCP and impaired the acquisition of a delayed spatial alternation task in adolescent offspring, associated with the disruption of neurodevelopment (Deutsch et al. 1998; Wang et al. 2001). Prenatal exposure to (+)-MK-801 has been reported to reduce the density of parvalbumin-immunoreactive interneurons and enhance PCP-induced hyperlocomotion in postpubertal rats (Abekawa et al. 2007). However, it is unclear whether prenatal exposure to PCP leads to behavioural and NMDA receptor dysfunction in mice.

In this study, we investigated the influences of prenatal exposure to PCP during the middle and late stages of pregnancy [embryonic days 6–18 (E6–E18)], covering the entire neurodevelopment period in the prenatal brain from neurulation to corticogenesis (Theiler, 1989). PCP-induced hyperlocomotion, recognition memory, and the expression and phosphorylation of NR1 protein were investigated from age 7 wk. In addition, the effects of antipsychotics on these behavioural abnormalities were further evaluated.

**Materials and methods**

**Animals**

Pregnant ICR dams (E5) obtained from SLC Japan (Shizuoka, Japan) were maintained on a 12-h light/dark cycle (lights on 08:00 hours) with free access to food (CE2; Clea Japan Inc., Japan) and water. The dams were randomly divided into saline-treated and PCP-treated groups. All were housed individually until parturition. There was no increase in maternal deaths and resorption or stillbirths on exposure to PCP in this study. At birth [postnatal day 0 (PD 0)], pups were culled to eight per litter with a balance of males and females wherever possible. Pups were weighed weekly until weaning and maternal care behaviour during feeding was monitored. After weaning at PD 21, pups given the same prenatal treatment were mixed by gender and then randomly assigned to each group for behavioural testing at the age of 7–8 wk. All groups of mice had litters of 2–3 and the test was repeated more than three times to reduce the influence of litters. Moreover, a balanced number of males and females were used in each experiment, since there were no significant differences between genders in this study.

The experiments with offspring commenced at the age of 7 wk and were performed in a sound-attenuated, air-conditioned room (23 ± 1 °C, 50 ± 5% humidity). The mice were habituated to the room for 40 min before the behavioural experiments. All the behavioural tests were recorded with a digital camera to re-analyse the results. The experiments were performed in accordance with the Guidelines for Animal Experiments of Meijo University Faculty of Pharmaceutical Sciences and the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmaceutical Society (2008).

**Drugs**

PCP hydrochloride was synthesized according to the method of Maddox et al. (1965) and checked for purity.
The PCP was dissolved in saline prior to use. Clozapine (Sigma, USA) was dissolved in a minimum amount of 0.1 N HCl and then diluted with saline (adjusted to pH 6–7 with 0.1 N NaOH), as previously described (Qiao et al. 2001). An injectable solution of haloperidol (5 mg/ml; Tanabe Seiyaku, Japan) was diluted with saline. All compounds were administered in a volume of 0.1 ml/10 g body weight.

**Drug treatment**

The dams were administered saline or PCP (20 mg/kg s.c.) once daily at 18:00 hours on E6–E18. The injection was made as gentle as possible to minimize potential stress-related influences on dams. In the fetal brain, the density of NMDA receptors is relatively low (Monyer et al. 1994; Watanabe et al. 1992), and the affinity for PCP, as well as the distribution of PCP, remains unclear. According to dose-dependent responses in our preliminary study (2.5–20 mg/kg), the dose of 20 mg/kg was selected in the present study, since it produced more obvious and similar behavioural and biochemical changes in relation to schizophrenia (L. Lu et al., unpublished data).

Based on previous studies (Mouri et al. 2007a), a low dose of PCP (3 mg/kg) or saline was used to challenge mice 30 min after habituation in PCP-induced locomotion; clozapine (1 and 3 mg/kg) or haloperidol (0.1 and 0.3 mg/kg) were injected 30 min before each behavioural test, and PCP (3 mg/kg) was injected into all mice to evaluate the effects of antipsychotics on it.

Different batches of mice were used for different experiments to avoid disruption. The experiments were performed according to the protocol shown in Fig. 1.

**Measurement of locomotor activity**

Locomotor activity was measured at the age of 7 wk. Mice were placed individually in a transparent acrylic cage with a black frosted Plexiglas floor (45 × 26 × 40 cm) for 120 min, and locomotor activity was measured in 5-min intervals using digital counters with infrared sensors (Scanet SV-10; Melquest Ltd, Japan) as previously reported (Lu et al. 2009). Locomotor activity was defined as the total number of beam cuts due to horizontal movement measured by the photo sensors.

**Novel object recognition test (NORT)**

As previously described (Mouri et al. 2007b), the test procedure consisted of three sessions: habituation, training, and retention. Each mouse was individually habituated to the box (L 30 × W 30 × H 35 cm), with 10 min of exploration in the absence of objects for 3 d (habituation session). During the training session, two objects (a red painted triangular prism and a yellow painted quadratic prism) were symmetrically fixed to the floor of the box, 8 cm from the walls, and each animal was allowed to explore the box for 10 min (day 4). An animal was considered to be exploring the object when its head was facing the object or it was touching or sniffing the object at a distance of <2 cm and/or touching it with its nose. The time spent exploring each object was recorded. After training, mice were immediately returned to their home cages.

During the retention session, animals were returned to the same box 24 h (day 5) after the training session, in which one of the familiar objects used during training was replaced with a novel object (a black painted golf ball). The animals were allowed to explore freely for 5 min and the time spent exploring each object was recorded. Throughout the experiments, the objects were used in a counterbalanced manner in terms of their physical complexity and emotional neutrality. A preference index, the ratio of time spent exploring either of the two objects (training session) or the novel object (retention session) over the total amount of time...
spent exploring both objects, was used to assess cognitive function.

Western blot analysis

Western blotting was performed as previously described (Mouri et al. 2007c). Dissected brain tissue obtained 24 h after the NORT test, was homogenized in ice-cold Tris buffer A [10 mM Tris–HCl (pH 7.4), 5 mM EDTA, 320 mM sucrose, 1 mM EGTA, 0.1 mM sodium orthovanadate, 1 mM NaF, 5 μg/ml aprotinin, 5 μg/ml leupeptin, and 5 μg/ml pepstatin] and centrifuged at 700 g for 10 min. The supernatant was centrifuged again at 37 000 g for 40 min, and the membrane-enriched extracts were re-suspended in Tris buffer B [10 mM Tris–HCl (pH 7.4), 0.1 mM sodium orthovanadate, 1 mM NaF, 5 μg/ml aprotinin, 5 μg/ml leupeptin, and 5 μg/ml pepstatin], and the suspension was used.

The protein concentrations were determined using a Pierce BCA Protein Assay kit (Thermo, USA). Samples were boiled at 95 °C for 5 min in the sample buffer [125 mM Tris–HCl (pH 6.8), 10% 2-mercaptoethanol, 4% sodium diphosphate decahydrate, 10% sucrose, and 0.0004% Bromophenol Blue], separated on a polyacrylamide gel, and transferred to polyvinylidene difluoride membranes (Millipore Corporation, USA). The membranes were blocked with a Detector Block kit (Kirkegaard & Perry Laboratories, USA) and probed with a primary antiphospho-NR1 (Ser89) antibody (1:1000; Upstate Biotechnology, USA). Membranes were washed with the washing buffer [50 mM Tris–HCl (pH 7.4), 0.05% Tween-20, and 150 mM NaCl] and subsequently incubated with a secondary horseradish peroxidase-linked antibody (Kirkegaard & Perry Laboratories). The immune complexes were detected with an ECL kit (GE Healthcare, UK) and exposed to X-ray film (Hyperfilm, GE Healthcare). The intensity of bands was analysed by Atto Densitogram Software Library Lane Analyzer (Atto, Japan). After the phosphorylated-NR1 was detected, membranes were stripped with stripping buffer (100 mM 2-mercaptoethanol, 2% SDS, and 62.5 mM Tris–HCl, pH 6.7) at 50 °C for 30 min, and NR1 expression was detected with a primary anti-NR1 antibody (1:1000; Santa Cruz Biotechnology, USA).

Preparation of brain slices and staining

Histological procedures were performed as described with a minor modification (Murai et al. 2007). Mice were anaesthetized with pentobarbital sodium (50 mg/kg i.p.) and perfused transcardially with ice-cold phosphate-buffered saline (PBS), followed by 4% paraformaldehyde and then soaked in 10–30% (w/v) sucrose. Coronal sections (20-μm thick) were cut with a cryostat (CM 1850; Leica, Germany). According to a previous method (Shen et al. 2008), Cresyl Violet staining was performed and the sizes of ventricles and brains were quantified with a computer-based image analysis system (WinRoof, Mitani, Japan). Apoptosis was detected with an in-situ cell-death detection kit, POD (Roche, Germany), and TUNEL-positive cells in layers II/III of the prefrontal cortex were counted using image analysis software. Images were acquired with a microscope (BZ-9000; Keyence, Japan).

Statistical analysis

All data were expressed as the mean ± S.E.M. The statistical significance of differences between two groups was determined by Student’s t test. The significance of differences among more than three groups was determined using a two-way analysis of variance (ANOVA) or ANOVA with repeated measures, followed by Bonferroni’s test. Pearson’s correlation analysis was used to identify the relationship; p < 0.05 was regarded as statistically significant.

Results

Effect of prenatal-PCP treatment on PCP-induced hyperlocomotion

To investigate the effects of prenatal exposure to PCP on drug-induced sensitization, PCP-induced hyperlocomotion was examined at age 7 wk. In the habituation period, no significant differences were observed among groups. After the 30-min habituation, prenatal saline- or PCP-treated mice were administered a low dose of PCP (3 mg/kg) or saline. The time-course of change in prenatal saline-treated mice revealed that the PCP challenge rapidly and significantly increased locomotion compared to the administration of saline. PCP-induced hyperlocomotion was significantly potentiated in the prenatal PCP-treated mice compared to the prenatal saline-treated mice over 5-min intervals after habituation (prenatal treatment: $F_{1,67} = 9.54, p < 0.01$; PCP challenge: $F_{1,67} = 64.85$, $p < 0.01$; prenatal treatment × PCP challenge: $F_{1,67} = 5.73$, $p < 0.05$; time: $F_{17,629} = 45.82$, $p < 0.01$; time × prenatal treatment: $F_{17,629} = 2.33$, $p < 0.01$; time × PCP challenge: $F_{17,629} = 20.20$, $p < 0.01$; time × prenatal treatment × PCP challenge: $F_{17,629} = 1.13$, $p > 0.05$, repeated two-way ANOVA; Fig. 2a), and the entire 90 min (30–120 min) ($F_{(group), (1, 67)} = 9.20$, $p < 0.01$; $F_{(treatment), (1, 67)} = 65.19$, $p < 0.01$; $F_{(group × treatment), (1, 67)} = 5.73$, $p < 0.05$, two-way ANOVA; Fig. 2b).
Changes in the expression and phosphorylation of the NR1 subunit of NMDA receptors of prenatal PCP-treated mice

We postulated that the abnormal behaviour was accompanied by a malfunction of NMDA receptors, since PCP as a non-competitive NMDA antagonist might inhibit NMDA receptors during development. The level of NR1 protein was significantly increased in the prenatal PCP-treated mice compared to that in the prenatal saline-treated mice (PFC: 100.0 vs 100.0, p = 0.01; hippocampus: 100.0 vs 100.0, p = 0.01; striatum: 100.0 vs 100.0, p = 0.01). In addition, the level of NR1 phosphorylated at Ser1887 was significantly increased in the prenatal PCP-treated mice compared to that in the prenatal saline-treated mice (PFC: 10.8% vs 10.8%, p = 0.01; hippocampus: 10.8% vs 10.8%, p = 0.01; striatum: 10.8% vs 10.8%, p = 0.01).
and (11–12 mice (Student’s t test).

(100.0 ± 5.9% vs. 75.4 ± 7.1%; p < 0.05, Fig. 4b; hippocampus: 100.0 ± 10.5% vs. 67.8 ± 9.5%; p < 0.05; Fig. 4f; striatum: 100.0 ± 7.3% vs. 87.1 ± 10.1%; p > 0.05, Fig. 4g). Furthermore, the proportion of phosphorylated-NR1 was also significantly reduced in prenatal PCP-treated mice (PFC: 100.0 ± 8.5% vs. 50.1 ± 6.7%; p < 0.01, Fig. 4c; hippocampus: 100.0 ± 13.4% vs. 48.8 ± 10.5%; p < 0.05, Fig. 4g; striatum: 100.0 ± 14.3% vs. 55.0 ± 5.8%; p < 0.05, Fig. 4f). Moreover, between the cognitive deficit in the NORT and the decreased level of phosphorylated NR1, there was a significant correlation in the PFC (r = 0.587, p = 0.045, Pearson’s correlation; Fig. 4d), and a positive and almost significant correlation in the hippocampus (r = 0.569, p = 0.054, Pearson’s correlation; Fig. 4h), but no correlation in the striatum (r = 0.325, p > 0.05, Pearson’s correlation; Fig. 4f).

However, a lower dose of prenatal PCP exposure (5 mg/kg) did not affect the expression of phosphorylated NR1 in the PFC of postpubertal mice (100 ± 4.23% vs. 91.41 ± 6.69%; p > 0.05, Suppl. Fig. S3). Additionally, the behavioural test itself did not affect the expression or phosphorylation of NR1 (p > 0.05, Suppl. Fig. S4).

**The neurotoxicity of prenatal-PCP treatment in the developing brain**

To evaluate the neurotoxic effects of prenatal PCP treatment during neurodevelopment, the TUNEL-positive cells in the PFC were counted at PD 0, PD 7 and PD 49. As shown by the results, apoptosis was significantly increased at PD 0 (253.4 ± 19.9 vs. 338.8 ± 28.2; p < 0.05, Suppl. Fig. S1a, d), but was not observed at either PD 7 (31.5 ± 3.9 vs. 37.2 ± 3.5; p > 0.05, Suppl. Fig. S1b, c), or PD 49 (35.7 ± 5.1 vs. 39.1 ± 4.0; p > 0.05, Suppl. Fig. S1c, f).

**Effect of antipsychotics on the behavioural abnormalities in prenatal PCP-treated mice**

We evaluated whether the prenatal PCP-induced behavioural changes were sensitive to both the atypical antipsychotic clozapine (Clz) and the typical antipsychotic haloperidol (Hal). The results showed that clozapine selectively attenuated the PCP-induced hypersensitivity over the 5-min intervals after habituation (30–120 min) in the prenatal PCP-treated mice (prenatal treatment: F_{3,62} = 15.41, p < 0.01; Clz: F_{3,62} = 29.07, p < 0.01; prenatal treatment × Clz: F_{3,62} = 5.23, p < 0.01; time: F_{17,1046} = 70.46, p < 0.01; time × prenatal treatment: F_{17,1046} = 1.96, p < 0.05; time × Clz: F_{17,1046} = 3.03, p < 0.01; time × prenatal treatment × Clz: F_{34,1046} = 1.05, p < 0.05, repeated two-way ANOVA; Fig. 5a). However, haloperidol reduced the hyperlocomotion of mice in both the prenatal saline- and PCP-treated groups (prenatal treatment: F_{3,62} = 6.88, p < 0.05; Hal: F_{3,62} = 17.35, p < 0.01; prenatal treatment × Hal: F_{3,62} = 1.30, p > 0.05; time: F_{17,1046} = 29.46, p < 0.01; time × prenatal treatment: F_{17,1046} = 1.66, p < 0.05; time × Hal: F_{17,1046} = 3.15, p < 0.01; time × prenatal treatment × Hal: F_{34,1046} = 0.92, p > 0.05, repeated two-way ANOVA; Fig. 5c). Furthermore, in terms of the entire 120-min period, the higher dose of clozapine (3 mg/kg) and haloperidol (0.1 and 0.3 mg/kg) reduced the locomotion in both the first 30 min and the last 90 min (Clz: 0–30 min: F_{3,62} = 3.23, p > 0.05; F_{3,62} = 5.98, p > 0.05; F_{3,62} = 0.12, p > 0.05, two-way ANOVA; 30–120 min: F_{3,62} = 14.84, p < 0.01; F_{3,62} = 29.07, p < 0.01; F_{3,62} = 5.23, p < 0.01, two-way ANOVA; Fig. 5b; Hal: 0–30 min: F_{3,62} = 1.23, p > 0.05; F_{3,62} = 14.90, p < 0.01; F_{3,62} = 0.56, p > 0.05, two-way ANOVA; 30–120 min: F_{3,62} = 7.43, p < 0.01; F_{3,62} = 17.28, p < 0.01; F_{3,62} = 1.30, p > 0.05, two-way ANOVA).
ANOVA; Fig. 5d). However, the lower dose of clozapine (1 mg/kg) did not affect the locomotion of prenatal saline-treated mice during the 120 min (0–30 min, 30–120 min; \( p > 0.05 \), respectively).

Next, we evaluated the effects of antipsychotics on the impairment of recognition memory. There was no bias in exploratory preference (Clz: \( F_{\text{group}}(1,53) = 0.42, p > 0.05 \); \( F_{\text{treatment}}(2,53) = 0.32, p > 0.05 \); \( F_{\text{group} \times \text{treatment}}(2,53) = 0.23, p > 0.05 \), two-way ANOVA; Fig. 6a; Hal: \( F_{\text{group}}(1,50) = 0.05, p > 0.05 \); \( F_{\text{treatment}}(2,50) = 0.23, p > 0.05 \); \( F_{\text{group} \times \text{treatment}}(2,50) = 1.27, p > 0.05 \), two-way ANOVA; Fig. 6c), or total exploration time after clozapine (1 mg/kg) and haloperidol (0.1 mg/kg) treatment in the training session, although the higher
dose had a slight effect (Clz: $F_{\text{group}(1,54)} = 0.02, p > 0.05$; $F_{\text{treatment}(2,54)} = 4.27, p < 0.05$; $F_{\text{group} \times \text{treatment}(2,54)} = 0.59, p > 0.05$, two-way ANOVA; Fig. 6b); Hal: $F_{\text{treatment}(2,62)} = 3.24, p > 0.05$; $F_{\text{treatment}(2,62)} = 25.84, p < 0.01$; $F_{\text{group} \times \text{treatment}(2,54)} = 0.35, p > 0.05$, two-way ANOVA; Fig. 6f). Interestingly, the impairment of recognition memory in prenatal PCP-treated mice was significantly improved by clozapine (Clz: $F_{\text{group}(1,54)} = 16.11, p < 0.01$; $F_{\text{treatment}(2,54)} = 3.42, p < 0.05$; $F_{\text{group} \times \text{treatment}(2,54)} = 4.04, p < 0.05$, two-way ANOVA; Fig. 6c), but not by haloperidol (Hal: $F_{\text{treatment}(2,54)} = 56.22, p < 0.01$; $F_{\text{treatment}(2,54)} = 0.09, p < 0.05$; $F_{\text{group} \times \text{treatment}(2,54)} = 0.16, p > 0.05$, two-way ANOVA; Fig. 6g). However, there were no differences in total exploration time in the retention sessions (Clz: $F_{\text{group}(1,54)} = 1.72, p > 0.05$; $F_{\text{treatment}(2,54)} = 0.25, p > 0.05$; $F_{\text{group} \times \text{treatment}(2,54)} = 0.16, p > 0.05$, two-way ANOVA; Fig. 6d; Hal: $F_{\text{group}(1,54)} = 1.09, p > 0.05$; $F_{\text{treatment}(2,54)} = 0.20, p > 0.05$; $F_{\text{group} \times \text{treatment}(2,54)} = 0.10, p > 0.05$, two-way ANOVA; Fig. 6h).

**Discussion**

Hypersensitivity to NMDA receptor antagonists has been demonstrated in adult rodents after repeated administration of PCP (Nabeshima et al. 1987; Nagai et al. 2003), and observed in schizophrenia patients. Perinatal exposure to PCP and prenatal exposure to
Fig. 6. Effects of antipsychotics on cognitive dysfunction in prenatal PCP-treated mice. Clozapine (Clz; 1 or 3 mg/kg) and haloperidol (Hal; 0.1 or 0.3 mg/kg) were administered 30 min before the training session. For clozapine treatment: (a) exploratory preference in the training session ($F_{\text{group}(1,30)} = 0.42, p > 0.05$; $F_{\text{treatment}(2,30)} = 0.32, p > 0.05$; $F_{\text{group} \times \text{treatment}(2,30)} = 0.23, p > 0.05$, two-way ANOVA), and (c) retention session ($F_{\text{group}(1,30)} = 16.11, p < 0.01$; $F_{\text{treatment}(2,30)} = 3.42, p < 0.05$; $F_{\text{group} \times \text{treatment}(2,30)} = 4.04, p < 0.05$, two-way ANOVA). Total exploration time in (b) the training session ($F_{\text{group}(1,30)} = 0.02, p > 0.05$; $F_{\text{treatment}(2,30)} = 4.27, p < 0.05$; $F_{\text{group} \times \text{treatment}(2,30)} = 0.59, p > 0.05$, two-way ANOVA) and (d) retention session ($F_{\text{group}(1,30)} = 1.72, p > 0.05$; $F_{\text{treatment}(2,30)} = 0.25, p > 0.05$; $F_{\text{group} \times \text{treatment}(2,30)} = 0.16, p > 0.05$, two-way ANOVA). For haloperidol treatment: (e) exploratory preference in the training session ($F_{\text{group}(1,30)} = 0.05, p > 0.05$; $F_{\text{treatment}(2,30)} = 0.23, p > 0.05$; $F_{\text{group} \times \text{treatment}(2,30)} = 1.27, p > 0.05$, two-way ANOVA) and (g) retention session ($F_{\text{group}(1,30)} = 56.22, p < 0.01$; $F_{\text{treatment}(2,30)} = 0.09, p > 0.05$; $F_{\text{group} \times \text{treatment}(2,30)} = 0.16, p > 0.05$, two-way ANOVA). Total exploration time in (f) the training session ($F_{\text{group}(1,30)} = 3.24, p > 0.05$; $F_{\text{treatment}(2,30)} = 25.84, p < 0.01$; $F_{\text{group} \times \text{treatment}(2,30)} = 0.35, p > 0.05$, two-way ANOVA) and (h) retention session ($F_{\text{group}(1,30)} = 1.09, p > 0.05$; $F_{\text{treatment}(2,30)} = 0.20, p > 0.05$; $F_{\text{group} \times \text{treatment}(2,30)} = -0.10, p > 0.05$, two-way ANOVA). *p < 0.05, **p < 0.01 compared to saline (Sal) treatment. *p < 0.05 compared to the prenatal Sal-treated group. Data are expressed as the mean ± S.E.M for 8–12 mice (Bonferroni’s test).
(+)-MK-801, enhanced PCP-induced hyperlocomotion in rats (Abekawa et al. 2007; Wang et al. 2001). In the present study, mice with prenatal exposure to PCP showed hypersensitivity to PCP at age 7 wk and this hypersensitivity was reversed by antipsychotics. PCP easily crosses the placenta (Kaufman et al. 1983; Nicholas et al. 1982). Fico & Vanderwende (1988) found that PCP was rapidly transported into the fetal brain and disappeared in 8 h after maternal exposure during pregnancy. These findings suggest that prenatal PCP exposure results in a behavioural hypersensitivity similar to the neonatal and adulthood exposure.

A blockade of NMDA receptors by antagonists during development impairs cognitive function. For instance, prenatal exposure to PCP disrupts the passive avoidance response and pole-climbing avoidance response (Nabeshima et al. 1982), and impairs performance in the eight-arm maze and Morris water maze in adult rats (Yanai et al. 1992). In the present study, prenatal PCP exposure caused an impairment of recognition memory. Since NMDA receptors play a critical role in memory formation (Rao & Finkbeiner, 2007) and the hypofunction of NMDA receptors to be involved in the cognitive deficits in PCP-treated adult mice (Enomoto et al. 2005; Mouri et al. 2007c), we postulated that prenatal exposure to PCP results in a disturbance of NMDA receptors, associated with cognitive dysfunction.

To test this hypothesis, we evaluated the expression and function of NMDA receptors. Phosphorylated NRI modulates the activity and function of NMDA receptors (Scott et al. 2003), and its expression is down-regulated in the post-mortem brains of schizophrenia patients (Emamian et al. 2004). In the present study, prenatal PCP-treated mice showed an increase in NRI expression but a reduction in the level and proportion of NRI phosphorylated at Ser897. The up-regulation of NRI expression is consistent with the inhibition of NMDA receptors in the developing brain causes an up-regulation of NMDA receptors (Anastasio & Johnson, 2008; Haberny et al. 2002; Slikker et al. 2007; Wang et al. 2001). It is likely that the up-regulated expression of NRI is due to a compensatory attempt to re-establish the delicate balance of the neurotransmitter network. However, a decreased level of phosphorylated NRI suggests the function of NMDA receptors is impaired. Moreover, there was a clearly shown positive correlation between decreased NRI phosphorylation and memory deficits in the PFC. In addition, D-serine, a NMDA receptor agonist, is reported to reverse the spatial memory deficits in perinatal PCP-treated rats (Andersen & Pouzet, 2004). These results suggest that the impairment of recognition memory is associated with the disturbance of NMDA receptors.

In clinical tests, atypical antipsychotics are used to control both the positive and negative symptoms of schizophrenia, especially cognitive dysfunction. It has been found that atypical antipsychotics attenuate cognitive dysfunction in PCP-treated adult mice (Amitai et al. 2007; Nagai et al. 2009), and perinatal PCP-treated rats (Anastasio & Johnson, 2008; Wang et al. 2001). In the present study, clozapine, but not haloperidol, selectively attenuated the PCP-induced hyperlocomotion and improved the cognitive dysfunction in prenatal PCP-treated mice. Clozapine promotes the function of NMDA receptors by increasing NMDA receptor-mediated excitatory postsynaptic potentials (EPSCs) (Chen & Yang, 2002), regulating protein kinase A (PKA)-cAMP signal transduction (Leveque et al. 2000), and specifically phosphorylating Ser897 of the NR1 subunit (Raman et al. 1996), as well as enhancing NMDA-mediated glutamatergic release (Millan, 2005). Furthermore, clozapine facilitated long-term potentiation in the PFC (Gempeler et al. 2003). Therefore, a reversed hypofunction of NMDA receptors might be responsible for the beneficial effect on schizophrenia-related cognitive deficits caused by prenatal PCP exposure.

Many neurons undergo a stage when they are critically dependent on stimulation by glutamate through the NMDA receptors, and sustained deprivation of this input during development activates apoptosis (Ikonomidou et al. 1999). Apoptosis is dependent on the stage of development, which occurs only in late fetal and early neonatal life (Ikonomidou et al. 1999). In our study, we found that enhanced apoptosis occurred at PD 0, but disappeared at PD 7 and PD 49, and there were no obvious architectural abnormalities of ventricles and brain in adults. These results suggest that neurotoxicity is involved in these behavioural changes, although it is relatively temporary and not sufficiently severe to alter the ventricular architecture. Therefore, it is possible that such neurotoxicity induces developmental changes that give rise to neuronal loss, or results in cytoarchitectural abnormalities implicated in abnormal behaviour in later life. Moreover, other factors implicated in neurodevelopment are also probably involved, since the inhibition of NMDA receptors by antagonists during development disrupts neuronal migration (Komuro & Rakic, 1993), inhibits neuronal proliferation (Behar et al. 1999), and reduces neuronal numbers and volume (Komuro & Rakic, 1993). In addition, abnormalities of some neurodevelopmental markers, such as brain-derived...
neurotropic factor (BDNF) and reelin, which plays a critical role in neurodevelopment and is implicated in schizophrenia (Angelucci et al. 2005; Impagnatiello et al. 1998), are also quite likely to be involved in these changes. However, the exact effects of them need to be investigated further.

In conclusion, our findings suggest that prenatal exposure to PCP produces long-term behavioural changes accompanied by abnormal expression and impaired function of NR1. Since the altered expression of NMDA receptors in the developing brain is considered part of the pathogenesis of schizophrenia, the present study might provide further insight into the influences of neurodevelopmental abnormalities during the prenatal period on behaviour in later life, via the disruption of NMDA receptors.

Note
Supplementary material accompanies this paper on the Journal’s website (http://journals.cambridge.org/pnp).

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Statement of Interest
None.

References
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