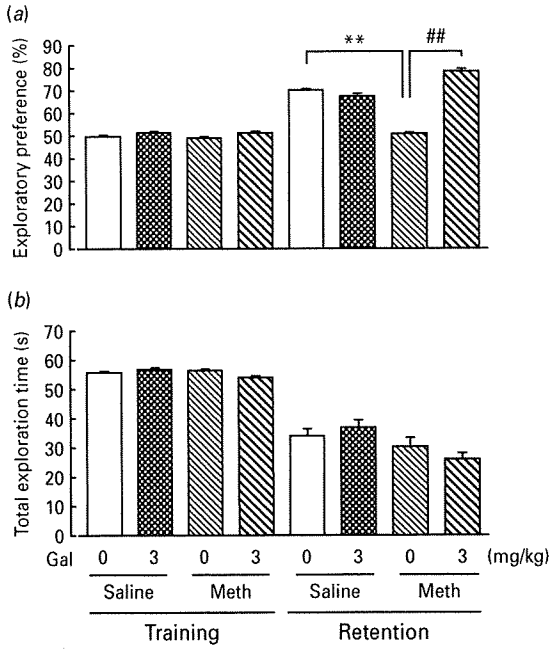

Fig. 2. Effect of galantamine on methamphetamine (Meth)-induced impairment of recognition memory in mice: (*a*) exploratory preference and (*b*) total exploration time. One day after the cessation of repeated Meth (1 mg/kg s.c.) treatment for 7 d, mice underwent the novel object recognition test. Galantamine (3 mg/kg p.o.) or saline was administered 1 h before the training session. Values indicate the mean ± S.E. ($n=10$). One-way ANOVA, (*a*) training: $F(3,36)=1.188$, $p=0.328$; retention: $F(3,36)=63.849$, $p<0.01$; (*b*) training: $F(3,36)=1.241$, $p=0.309$; retention: $F(3,36)=2.396$, $p=0.084$. ** $p<0.01$ compared to saline + saline-treated group (Bonferroni's test). ## $p<0.01$ compared to Meth + saline-treated group (Bonferroni's test).

are due to increase of ACh levels caused by inhibition of AChE, we examined the effect of donepezil, an AChE inhibitor, on the impairment of cognition in Meth-treated mice.

Donepezil at a dose of 1 mg/kg caused about a 2-fold increase in the levels of extracellular ACh in the PFC of Meth-treated mice [$F(1,35)=14.042$, $p<0.01$] (Fig. 3*a*). However, donepezil (1 mg/kg) had no effect on the level of exploratory preference for the objects in the retention sessions in Meth-treated mice (Fig. 3*b*). It also affected neither the level of exploratory preference for the objects in the training session [$F(2,40)=0.159$, $p=0.854$] (Fig. 3*a*) nor the total exploration time in either the training [$F(2,40)=0.296$, $p=0.746$] or retention [$F(2,40)=0.160$, $p=0.215$] sessions in Meth-treated mice (Fig. 3*c*).

Involvement of nicotinic receptors, but not muscarinic receptors in the cognitive-improving effect of galantamine on Meth-treated mice

To determine whether the improving effects of galantamine on Meth-induced cognitive impairment are mediated via nAChRs, but not muscarinic AChRs (mAChRs), we examined the antagonism by using mecamylamine, a nAChR antagonist and scopolamine, a mAChR antagonist, against the cognitive-improving effects of galantamine in Meth-treated mice.

In the training session of the NOR task, there were no differences in exploratory preference for the objects in any of the groups (Fig. 4*a*, *c*). The nAChR antagonist, mecamylamine (3 mg/kg) significantly and completely prevented the improving effects of galantamine on the impairment of recognition memory in Meth-treated mice ($p<0.01$) (Fig. 4*a*). In saline-treated mice, mecamylamine alone at the dose used had no effect on the NOR performances (Fig. 4*a*). The antagonistic effect of mecamylamine on galantamine-induced improvement of exploratory preference in Meth-treated mice was not associated with changes in the total exploration time [training: $F(4,57)=0.516$, $p=0.725$; retention: $F(4,57)=2.403$, $p=0.060$] (Fig. 4*b*).

Scopolamine at a dose of 0.1 mg/kg impaired the performance of saline-treated mice in the NOR task (Fig. 4*c*). However, scopolamine failed to prevent the improving effects of galantamine on the impairment of recognition memory in Meth-treated mice (Fig. 4*c*). Treatment with any compound did not affect the total exploration time in either the training [$F(6,77)=2.193$, $p=0.053$] or retention [$F(6,77)=1.919$, $p=0.088$] sessions (Fig. 4*d*).

Effects of galantamine on the levels of extracellular dopamine in the PFC of Meth-treated mice

We examined whether galantamine at a dose of 3 mg/kg, which improved the cognitive deficit in Meth-treated mice, facilitated dopamine release in the PFC of Meth-treated mice.

There were no differences in the basal levels of extracellular dopamine in the PFC in any of the groups (Fig. 5 insert). As shown in Fig. 5, galantamine (3 mg/kg) caused a marked increase in the levels of extracellular dopamine in the PFC of Meth-treated mice (Fig. 5). The significant increase in the levels of extracellular dopamine was observed from 30 min after galantamine administration ($p<0.01$ by *post hoc* test, Fig. 5). When mecamylamine (3 mg/kg) was injected into Meth-treated mice 20 min after galantamine administration, galantamine-induced elevation of extracellular dopamine levels was significantly diminished

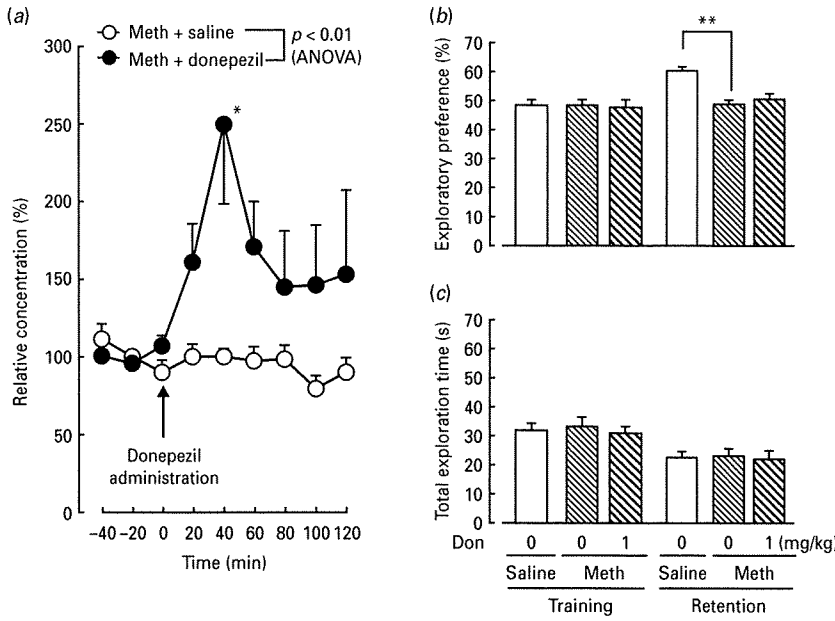

Fig. 3 Effect of donepezil on the extracellular acetylcholine (ACh) levels of the prefrontal cortex (PFC) and the impairment of recognition memory in methamphetamine (Meth)-treated mice. (*a*) Extracellular ACh levels of PFC in microdialysis. *In-vivo* microdialysis was performed 3 d after the final injection of Meth (1 mg/kg s.c.) treatment for 7 d. Donepezil (1 mg/kg p.o.) was administered to the Meth-treated mice (●, Meth + donepezil). In the control group, an equivalent amount of saline was given to the Meth-treated mice (○, Meth + saline). Values indicate the mean ± S.E. ($n = 4$–5). Results with the repeated ANOVA were: time [$F(5, 35) = 1.111$, $p = 0.37$]; treatment [$F(1, 35) = 14.042$, $p < 0.01$]; time × treatment interaction [$F(5, 35) = 0.677$, $p = 0.64$]. * $p < 0.05$ compared to Meth + saline-treated group (Bonferroni's test). The basal levels of ACh in the PFC of the Meth + saline- and Meth + donepezil-treated mice were 0.17 ± 0.05 and 0.12 ± 0.06 pmol/20 μl per 20 min, respectively. (*b*) Exploratory preference in novel object recognition (NOR) test. (*c*) Total exploration time in NOR test. One day after the cessation of repeated Meth (1 mg/kg s.c.) treatment for 7 d, mice underwent the NOR test. Donepezil (1 mg/kg p.o.) or saline was administered 1 h before the training session. Values indicate the mean ± S.E. ($n = 13$–15). One-way ANOVA, (*b*) training: $F(2, 40) = 0.159$, $p = 0.854$; retention: $F(2, 40) = 9.400$, $p < 0.01$; (*c*) training: $F(2, 40) = 0.296$, $p = 0.746$; retention: $F(2, 40) = 0.160$, $p = 0.215$. ** $p < 0.01$ compared to saline + saline-treated group (Bonferroni's test).

(Fig. 5). However, mecamylamine alone did not affect the extracellular dopamine levels in saline-treated mice (data not shown).

Involvement of dopaminergic systems in the cognitive-improving effect of galantamine on Meth-treated mice

Previous studies have shown that the ERK1/2 signalling pathway linked to dopamine D_1 receptors (D_1Rs) (Valjent *et al.* 2000; Zanassi *et al.* 2001) is involved in Meth-associated contextual memory in rats (Mizoguchi *et al.* 2004) and that repeated Meth treatment induces cognitive impairment in the NOR test in mice, which is accompanied by dysfunction of the dopamine D_1R-ERK1/2 pathway in the PFC (Kamei *et al.* 2006). To clarify whether the improving effects of galantamine on Meth-induced cognitive impairment are mediated through the activation of dopamine D_1Rs, we investigated the antagonism by using SCH 23390, a dopamine D_1R antagonist, against the cognitive-improving effects of galantamine in Meth-treated mice.

SCH 23390 (0.02 mg/kg) significantly and completely prevented the improving effects of galantamine on Meth-induced cognitive impairment without affecting the exploratory preference for the objects in the training session (Fig. 6*a*). In saline-treated mice, SCH 23390 alone had no effect on NOR performance (Fig. 6*a*). SCH 23390 also had no effect on the total exploration time in either the training [$F(4, 50) = 1.520$, $p = 0.211$] or retention [$F(4, 55) = 1.943$, $p = 0.116$] sessions of Meth-treated mice (Fig. 6*b*).

Effect of galantamine on the defect of novelty-induced ERK1/2 phosphorylation in the PFC of Meth-treated mice

Kamei *et al.* (2006) have demonstrated novelty-induced ERK1/2 activation in the PFC when mice are

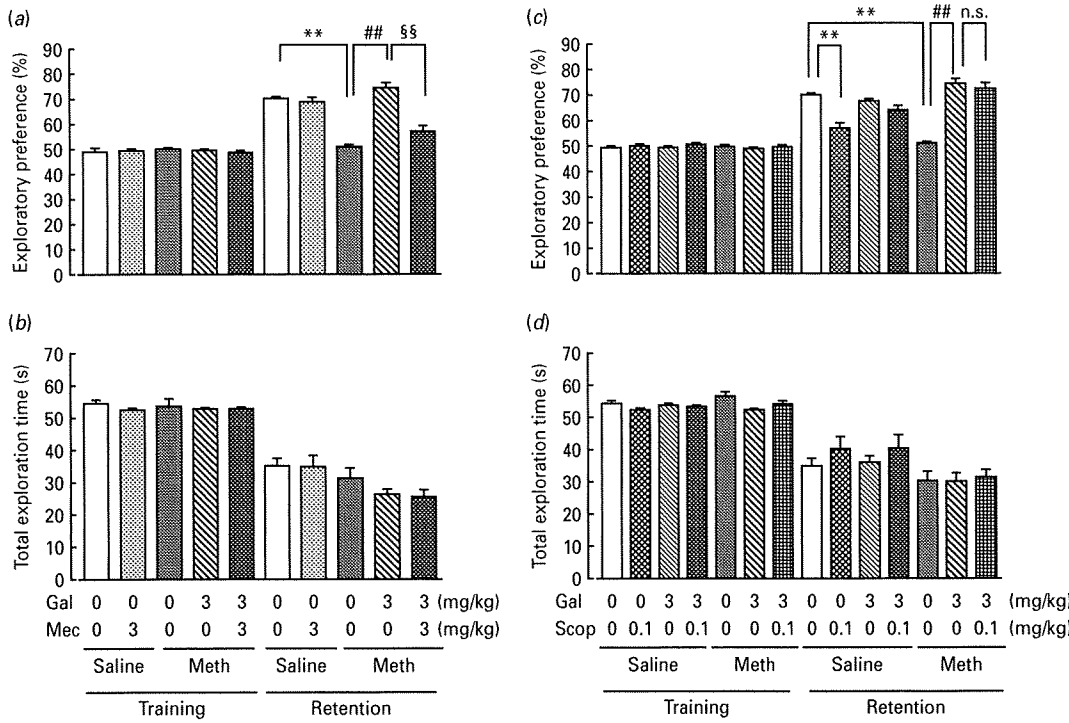

Fig. 4. Involvement of nicotinic receptors, but not muscarinic receptors in the cognitive-improving effect of galantamine on methamphetamine (Meth)-treated mice: (*a*, *c*) exploratory preference and (*b*, *d*) total exploration time. One day after the cessation of repeated Meth (1 mg/kg s.c.) treatment for 7 d, mice underwent the novel object recognition test. Galantamine (Gal; 3 mg/kg p.o.), mecamylamine (Mec; 3 mg/kg s.c.) and/or scopolamine (Scop; 0.1 mg/kg s.c.) were administered to saline- or Meth-treated mice 1 h, 40 min and/or 40 min, respectively, before the training session. Values indicate the mean ± S.E. ($n = 10$–15). One-way ANOVA, (*a*) training: $F(4, 57) = 0.255$, $p = 0.906$; retention: $F(4, 57) = 28.901$, $p < 0.01$; (*b*) training: $F(4, 57) = 0.516$, $p = 0.725$; retention: $F(4, 57) = 2.403$, $p = 0.060$; (*c*) training: $F(6, 77) = 0.429$, $p = 0.858$; retention: $F(6, 77) = 20.277$, $p < 0.01$; (*d*) training: $F(6, 77) = 2.193$, $p = 0.053$; retention: $F(6, 77) = 1.919$, $p = 0.088$. ** $p < 0.01$ compared to saline + saline/saline-treated group (Bonferroni's test). ## $p < 0.01$ compared to Meth + saline/saline-treated group (Bonferroni's test). §§ $p < 0.01$ compared to Meth + galantamine/saline-treated group (Bonferroni's test). n.s., Not significant.

exposed to novel objects, leading to the formation of long-lasting object recognition memory. Further, memory impairment in Meth-treated mice was associated with dysfunction of ERK1/2 signalling in the PFC. In order to examine the mechanism by which galantamine ameliorates the impairment of recognition memory in Meth-treated mice, we examined the effect of galantamine on ERK1/2 phosphorylation in the PFC of Meth-treated mice when they were exposed to novel objects.

A significant increase in phosphorylation of ERK1/2 levels was observed in the PFC of saline-treated mice immediately after a 10-min exposure to novel objects (Fig. 7*a*, *b*) ($p < 0.01$ *vs.* baseline in saline-treated mice, Student's *t* test), and repeated Meth treatment abolished novelty-induced ERK1/2 activation in the PFC in accord with the previous study (Kamei *et al.* 2006) ($p < 0.01$) (Fig. 7*a*). Galantamine (3 mg/kg) significantly recovered the defect of novelty-induced activation of ERK1/2 in the PFC of Meth-treated mice ($p < 0.01$) (Fig. 7*a*). SCH 23390 (0.02 mg/kg) significantly blocked the improving effects of galantamine on the defect of novelty-induced ERK1/2 phosphorylation in the PFC ($p < 0.01$) (Fig. 7*a*). SCH 23390 alone had no effect on the levels of phosphorylation and total ERK1/2 in either the baseline or exposure of saline-treated mice (Fig. 7*b*). The levels of total ERK1/2 did not differ in the exposed groups examined [$F(3, 16) = 1.629$, $p = 0.222$].

Influence of an ERK inhibitor on the cognitive-improving effect of galantamine on Meth-treated mice

We confirmed that PD98059 (2 μg/1 μl/bilateral) has no effect on the phosphorylation of ERK1/2 in the PFC and hippocampus of naive mice (data not shown). Then, we examined the effect of PD98059 (2 μg/1 μl/bilateral) administered before the training session on

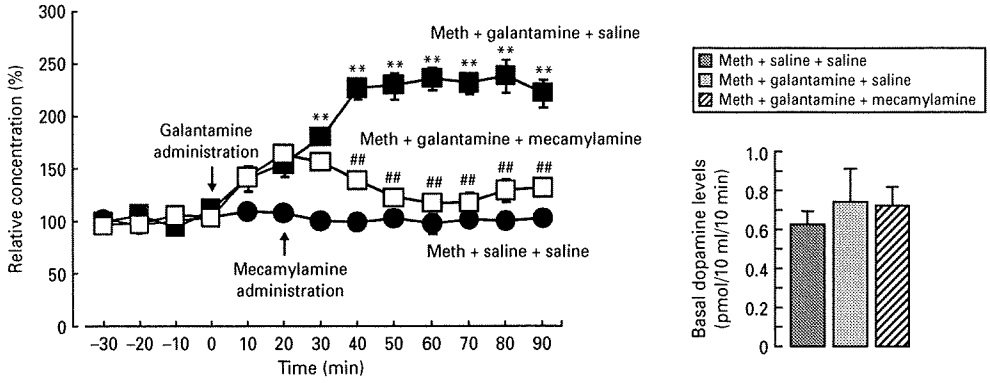

Fig. 5. Effects of galantamine on the levels of the extracellular dopamine in the PFC of methamphetamine (Meth)-treated mice. Meth (1 mg/kg, s.c.) was injected for 7 d, and 3 d after withdrawal, extracellular levels of dopamine were measured in the PFC by *in-vivo* microdialysis. Galantamine (3 mg/kg p.o.) was administered to the Meth-treated mice (■, Meth + galantamine + saline). In the control group, an equivalent amount of saline was given (●, Meth + saline + saline) to the Meth-treated mice. Mecamylamine (3 mg/kg s.c.) was injected 20 min after galantamine (□, Meth + galantamine + mecamylamine) to Meth-treated mice. The basal levels of dopamine in the PFC of the Meth + saline + saline (▩)-, Meth + galantamine + saline (▨)- and Meth + galantamine + mecamylamine (▨)-treated mice were 0.62 ± 0.08, 0.74 ± 0.18 and 0.72 ± 0.10 pmol/10 μl per 10 min, respectively (right-hand panel). Values indicate the mean ± S.E. ($n = 3$). Results with the repeated ANOVA were time [$F(9, 54) = 8.063$, $p < 0.01$], treatment [$F(2, 6) = 73.188$, $p < 0.01$], and time × treatment interaction [$F(18, 54) = 10.802$, $p < 0.01$]. ** $p < 0.01$ compared to Meth + saline + saline-treated group (Bonferroni's test). ## $p < 0.01$ compared to Meth + galantamine + saline-treated group (Bonferroni's test).

the cognitive-improving effect of galantamine in Meth-treated mice to determine the involvement of ERK1/2 activation in the mechanism of action of galantamine.

In the training session, bilateral microinjections of PD98059 into the PFC (1 μg/side) of saline-treated mice did not affect the exploratory preference for the objects (Fig. 8*a*). In the retention session, the level of exploratory preference in PD98059-treated mice was significantly increased as for vehicle-treated mice ($p < 0.01$, Fig. 8*a*), but it was significantly decreased compared to that in vehicle-treated mice ($p < 0.05$, Fig. 8*a*). PD98059 had no effect on the total exploration time in either the training or retention sessions of saline-treated mice (Fig. 8*b*).

In Meth-treated mice, PD98059 completely blocked the ameliorating effect of galantamine on the impairment of exploratory preference for a novel object in the retention session [$F(2, 25) = 27.986$, $p < 0.01$] (Fig. 8*c*). The antagonistic effect of PD98059 on galantamine-induced improvement of exploratory preference in Meth-treated mice was not associated with changes in the total exploration time [training: $F(2, 25) = 0.399$, $p = 0.676$; retention: $F(2, 25) = 0.015$, $p = 0.985$] (Fig. 8*d*).

Discussion

We have reconfirmed that Meth-treated mice show impairments to their novelty discrimination ability in the NOR test that is consistent with previous reports (Ito *et al.* 2007; Kamei *et al.* 2006). It is unlikely that the impairment in performance of Meth-treated mice in learning and memory tasks is due to changes in motivation, although various motivations are involved in the behavioural task. The fact that Meth reduced the exploratory preference for the objects in the retention session could be interpreted as neophobia. However, the possible involvement of motivation and/or neophobia can be excluded because Meth treatment had no effect on total exploration time of novel objects during the training session. Therefore, it is likely that impairment of performance in Meth-treated mice is due to learning and memory deficits.

Galantamine, a drug approved for the treatment of Alzheimer's disease, has a dual mechanism of action; it inhibits AChE and allosterically modulates nAChR as a potent APL (Eisele *et al.* 1993; Santos *et al.* 2002). We have recently reported that galantamine reverses the impairment of object recognition in $A\beta_{25-35}$-infused mice as an animal model of Alzheimer's disease and in repeated PCP-treated mice as an animal model of schizophrenia (Wang *et al.* 2007*a*, *b*). In accord with these findings, in the present study, galantamine significantly ameliorated the cognitive impairments induced by Meth in the NOR test. Galantamine at a dose of 3 mg/kg had no effect on the total exploration time in the training session of the NOR test in Meth-treated

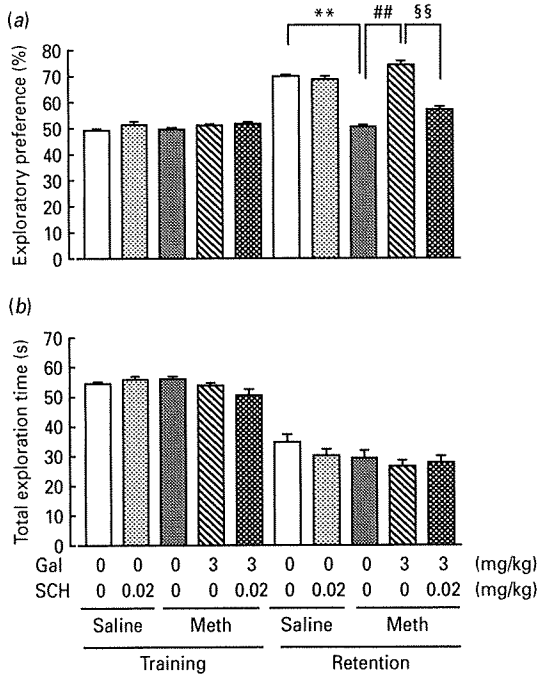

Fig. 6. Involvement of dopaminergic systems in the cognitive-improving effect of galantamine on methamphetamine (Meth)-treated mice: (*a*) exploratory preference and (*b*) total exploration time. One day after the cessation of repeated Meth (1 mg/kg s.c.) treatment for 7 d, mice underwent the novel object recognition test. Galantamine (Gal; 3 mg/kg p.o.) and SCH 23390 (SCH; 0.02 mg/kg s.c.) were administered 1 h and 30 min, respectively, before the training session. Values indicate the mean ± S.E. ($n = 10$–15). One-way ANOVA, (*a*) training: $F(4, 50) = 1.422$, $p = 0.240$; retention: $F(4, 55) = 40.622$, $p < 0.01$; (*b*) training: $F(4, 50) = 1.520$, $p = 0.211$; retention: $F(4, 55) = 1.943$, $p = 0.116$. $^{**}p < 0.01$ compared to saline + saline/saline-treated group (Bonferroni's test). $^{\#\#}p < 0.01$ compared to Meth + saline/saline-treated group (Bonferroni's test). $^{\S\S}p < 0.01$ compared to Meth + galantamine/saline-treated group (Bonferroni's test).

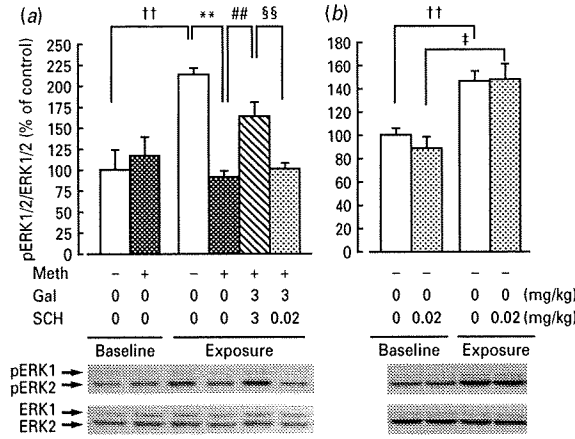

Fig. 7. Effect of galantamine on the defect of novelty-induced ERK1/2 phosphorylation in the PFC of methamphetamine (Meth)-treated mice. One hour before exposure to novel objects, galantamine (Gal; 3 mg/kg p.o.) or saline was administered to mice that had been previously treated with either saline or Meth (1 mg/kg s.c.) for 7 d. SCH 23390 (SCH; 0.02 mg/kg s.c.) was administered 30 min before exposure to novel objects. Values indicate the mean ± S.E. ($n = 4$–5). $^{\dagger\dagger}p < 0.01$ compared to saline + saline/saline-treated group that was not exposed to novel objects (baseline) (Student's *t* test). $^{\ddagger}p < 0.05$ compared to saline + saline/SCH23390-treated group that was not exposed to novel objects (baseline) (Student's *t* test). One-way ANOVA: $F(3, 16) = 28.286$, $p < 0.01$. $^{**}p < 0.01$ compared to saline + saline/saline-treated group (exposure) (Bonferroni's test). $^{\#\#}p < 0.01$ compared to Meth + saline/saline-treated group (exposure) (Bonferroni's test). $^{\S\S}p < 0.01$ compared to Meth + galantamine/saline-treated group (exposure) (Bonferroni's test).

mice. Therefore, it is unlikely that the observed improvement in performance in the task brought about by galantamine is due to changes in motivation in Meth-treated mice, and it is apparently true that galantamine ameliorates learning and memory deficits caused by repeated Meth treatment in mice. The improving effects of galantamine on the performance of Meth-treated mice were prevented by treatment with mecamylamine, a nAChR antagonist, at a dose that did not significantly affect the performance of saline-treated mice. These findings support the notion that galantamine improves Meth-induced cognitive impairment via activation of nAChRs. Alternatively, the roles of mAChRs in the effects of galantamine were also investigated in the present study. The effects of galantamine on the performance of Meth-treated mice in the NOR task were not blocked by scopolamine at the dose that impaired the performance of saline-treated mice. Although mAChR agonists improve cognitive dysfunctions in patients with Alzheimer's disease and schizophrenia (Friedman, 2004), the present result indicated that mAChRs have little influence on the effects of galantamine for this particular cognitive task. On the other hand, the activation of nAChRs may be due to an increase in the levels of ACh caused by AChE inhibition of galantamine. We investigated the effect of donepezil, which is 3–15 times more potent in AChE inhibition than that of galantamine *in vivo* (Geerts *et al.* 2005), on Meth-induced cognitive impairment. Although donepezil at 1 mg/kg caused about a 2-fold increase from basal extracellular ACh levels in the PFC of Meth-treated mice, it had no effect on behavioural performance in Meth-treated mice. From the

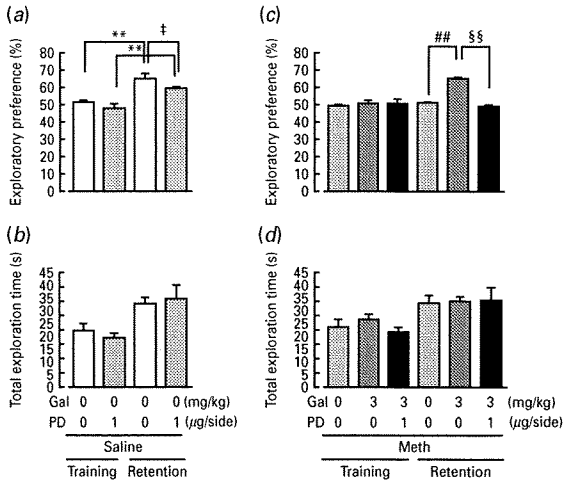

Fig. 8. Influence of an ERK inhibitor on the cognitive-improving effect of galantamine on methamphetamine (Meth)-treated mice: (*a*, *c*) exploratory preference and (*b*, *d*) total exploration time. One day after the cessation of repeated Meth (1 mg/kg s.c.) treatment for 7 d, mice underwent the novel object recognition test. Galantamine (Gal; 3 mg/kg p.o.) and PD98059 (PD; 1 μg/0.5 μl per side) were administered 1 h and 30 min, respectively, before the training session. Values indicate the mean ± S.E. (*a*, *b*; $n=8$) (*c*, *d*; $n=9$–10). One-way ANOVA, (*c*) training: $F(2, 25)=0.309$, $p=0.737$; retention: $F(2, 25)=27.986$, $p<0.01$; (*d*) training: $F(2, 25)=0.399$, $p=0.676$; retention: $F(2, 25)=0.015$, $p=0.985$. ** $p<0.01$ compared to corresponding saline-treated training group (Student's *t* test). ‡ $p<0.05$ compared to saline + saline/vehicle-treated retention group (Student's *t* test). ## $p<0.01$ compared to Meth + saline/vehicle-treated group (Bonferroni's test). §§ $p<0.01$ compared to Meth + galantamine/vehicle-treated group (Bonferroni's test).

present results and a report that there is only 1–12% brain AChE inhibition 1 h after s.c. injection of 3 mg/kg galantamine (Geerts *et al.* 2005), our conclusion is that galantamine induces the ameliorating effect on impairment of memory mainly by allosterically modulating the function of nAChRs, but not by AChE inhibition. However, further experiments are needed to exclude the involvement of AChE inhibition by galantamine in the ameliorating effect of it on cognitive impairment in Meth-treated mice, since the allosteric potentiating effect of nAChRs can be detected at lower doses (Geerts *et al.* 2005).

Accumulating evidence suggests that the dopaminergic system in the PFC is involved in cognitive function. For instance, disruption of dopamine transmission in the PFC by infusions of dopamine D_1R antagonists or by excitotoxic lesions impairs the performance of object retrieval-detour tasks, as well as delayed response tasks in non-human primates (Dias *et al.* 1996*a*,*b*; Sawaguchi & Goldman-Rakic, 1991). A previous study with functional magnetic resonance imaging has shown that dysfunction in the PFC of Meth abusers is related to cognitive impairment (Paulus *et al.* 2002). Accordingly, cognitive impairment in Meth abusers may be associated with deficits in dopamine transmission in the PFC. Our previous findings in *in-vivo* microdialysis experiments demonstrated that galantamine increases the extracellular dopamine release in the hippocampus and PFC and that the increasing effects of galantamine on dopamine release in the hippocampus are potentiated by nicotine and antagonized by mecamylamine (Wang *et al.* 2007*a*). The present *in-vivo* microdialysis experiment show that galantamine significantly increased extracellular dopamine release in the PFC of Meth-treated mice. The effects of galantamine on increasing dopamine release were antagonized by mecamylamine. These results strongly suggest that galantamine ameliorates Meth-induced learning and memory deficits by activating nAChRs, and thereby stimulates release of dopamine in the PFC. Further, we found that the improving effects of galantamine were prevented by SCH 23390, a dopamine D_1R antagonist. Galantamine enhances dopaminergic neurotransmission *in vivo* via allosteric potentiation of nAChRs. These findings provide the *in-vivo* evidence that galantamine augments dopaminergic neurotransmission in the PFC through the allosteric activation of nAChRs. The present results are supported by the results published by Schilström *et al.* (2007) that effects of galantamine on dopamine cell firing are mediated by allosteric potentiation of nAChRs. Taken together, our results suggest that the PFC-dependent behaviour task was impaired due to dysfunction of dopaminergic systems induced by Meth, since the PFC is involved in object recognition behaviour (Kamei *et al.* 2006). In fact, Kamei *et al.* (2006) have already demonstrated that repeated administration of Meth in mice induces object recognition impairment, which is associated with the dopamine D_1Rs, but not dopamine D_2Rs in the PFC. However, the object recognition memory is ascribed to the perirhinal cortex and its interactions with the hippocampus (Winters *et al.* 2008). We will investigate the functional role of the perirhinal cortex in Meth-induced cognitive deficits, in the ameliorating effects of galantamine and D_1R/ERK signalling in the NOR test.

Previous studies have demonstrated that the ERK1/2 signalling pathway linked to dopamine D_1Rs (Valjent *et al.* 2000; Zanassi *et al.* 2001) is involved in the rewarding effects induced by Meth (Mizoguchi *et al.* 2004) and the behavioural sensitization and

rewarding effects induced by cocaine (Valjent *et al.* 2000). Regarding the mechanism underlying the repeated Meth-induced memory impairment, Kamei *et al.* (2006) have already demonstrated dysfunction of the ERK1/2 pathway in the PFC. Hyperphosphorylation of ERK1/2 was found in the PFC when control mice were exposed to novel objects, whereas this activation was abolished in repeated Meth-treated mice. Inhibition of ERK1/2 by the microinjection of PD98059 (4 μg/mouse/bilateral), a selective MEK inhibitor, into the PFC resulted in cognitive impairment (Kamei *et al.* 2006). Ito *et al.* (2007) have also found that another MEK1/2 inhibitor, SL327 (30 and 50 mg/kg i.p.), significantly impairs long-term recognition memory 24 h after a training session in naive mice. In this study, galantamine ameliorated the Meth-induced defect of ERK1/2 hyperphosphorylation in the PFC of mice exposed to novel objects. In addition, the ameliorating effect of galantamine on Meth-induced object recognition impairment was completely blocked by pretreatment with the ERK inhibitor PD98059 at the dose used, slightly affecting the performance of saline-treated mice. Accordingly, these results suggest that the ameliorating effect of galantamine on Meth-induced cognitive impairment is related to the activation of ERK1/2 in the PFC.

As discussed above, our findings suggest that dopamine D_1R-ERK1/2 systems are required for the effects of galantamine. Since dopamine the D_1R antagonist and ERK inhibitor impaired recognition memory based on phosphorylation of ERK in the PFC of normal mice (Kamei *et al.* 2006), dopamine D_1R-ERK1/2 systems are critical in recognition memory. If the action site of galantamine is downstream of dopamine D_1R-ERK1/2 systems, dopamine D_1R antagonists or the ERK inhibitor would fail to reverse the effect of galantamine. Accordingly, our data suggest that galantamine acts upstream of dopamine D_1R-ERK1/2 systems.

In conclusion, the ameliorating effect of galantamine on Meth-induced memory impairment is associated with indirect activation of dopamine D_1R-ERK1/2 following augmentation with dopaminergic neurotransmission in the PFC through the allosteric activation of nAChRs. Galantamine could prove to be a useful therapeutic drug for treating cognitive deficits in schizophrenia/Meth psychosis, as well as Alzheimer's disease.

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Statement of Interest

None.

References

Cretzmeyer M, Sarrazin MV, Huber DL, Block RI, *et al.* (2003). Treatment of methamphetamine abuse: research findings and clinical directions. *Journal of Substance Abuse Treatment* **24**, 267–277.

Dias R, Robbins TW, Roberts AC (1996*a*). Dissociation in prefrontal cortex of affective and attentional shifts. *Nature* **380**, 69–72.

Dias R, Robbins TW, Roberts AC (1996*b*). Primate analogue of the Wisconsin card sorting test: effects of excitotoxic lesions of the prefrontal cortex in the marmoset. *Behavioral Neuroscience* **110**, 872–886.

Eisele JL, Bertrand S, Galzi JL, Devillers-Thiery A, *et al.* (1993). Chimaeric nicotinic-serotoninergic receptor combines distinct ligand binding and channel specificities. *Nature* **366**, 479–483.

Franklin KBJ, Paxinos G (1996). *The Mouse Brain in Stereotaxic Coordinates*. San Diego: Academic Press.

Friedman JI (2004). Cholinergic targets for cognitive enhancement in schizophrenia: focus on cholinesterase inhibitors and muscarinic agonists. *Psychopharmacology (Berlin)* **174**, 45–53.

Geerts H, Guillaumat PO, Grantham C, Bode W, *et al.* (2005). Brain levels and acetylcholinesterase inhibition with galantamine and donepezil in rats, mice and rabbits. *Brain Research* **1033**, 186–193.

Ito Y, Takuma K, Mizoguchi H, Nagai T, *et al.* (2007). A novel azaindolizinone derivative ZSET1446 [spiro[imidazo[1,2-a]pyridine-3,2-indan]-2(3H)-one] improves methamphetamine-induced impairment of recognition memory in mice by activating extracellular signal-regulated kinase 1/2. *Journal of Pharmacology and Experimental Therapeutics* **320**, 819–827.

Kalechstein AD, Newton TF, Green M (2003). Methamphetamine dependence is associated with neurocognitive impairment in the initial phases of abstinence. *Journal of Neuropsychiatry and Clinical Neurosciences* **15**, 215–220.

Kamei H, Nagai T, Nakano H, Togan Y, *et al.* (2006). Repeated methamphetamine treatment impairs recognition memory through a failure of novelty-induced ERK1/2 activation in the prefrontal cortex of mice. *Biological Psychiatry* **59**, 75–84.

Mizoguchi H, Yamada K, Mizuno M, Mizuno T, *et al.* (2004). Regulations of methamphetamine reward by extracellular signal-regulated kinase 1/2/ets-like gene-1 signaling pathway via the activation of dopamine receptors. *Molecular Pharmacology* **65**, 1293–1301.

Mouri A, Noda Y, Noda A, Nakamura T, *et al.* (2007). Involvement of a dysfunctional dopamine-D1/*N*-methyl-*d*-aspartate-NR1 and Ca^{2+}/calmodulin-dependent protein kinase II pathway in the impairment of latent learning in a model of schizophrenia induced by phencyclidine. *Molecular Pharmacology* **71**, 1598–1609.

Mouri A, Zou LB, Iwata N, Saido TC, *et al.* (2006). Inhibition of neprilysin by thiorphan (i.c.v.) causes an accumulation of amyloid beta and impairment of learning and memory. *Behavioral Brain Research* **168**, 83–91.

Nordahl TE, Salo R, Leamon M (2003). Neuropsychological effects of chronic methamphetamine use on neurotransmitters and cognition: a review. *Journal of Neuropsychiatry and Clinical Neurosciences* **15**, 317–325.

Paulus MP, Hozack NE, Zauscher BE, Frank L, *et al.* (2002). Behavioral and functional neuroimaging evidence for prefrontal dysfunction in methamphetamine-dependent subjects. *Neuropsychopharmacology* **26**, 53–63.

Rawson RA, Gonzales R, Brethen P (2002). Treatment of methamphetamine use disorders: an update. *Journal of Substance Abuse Treatment* **23**, 145–150.

Santos MD, Alkondon M, Aracava Y, Eisenberg HM, *et al.* (2002). The nicotinic allosteric potentiating ligand galantamine facilitates synaptic transmission in the mammalian central nervous system. *Molecular Pharmacology* **61**, 1222–1234.

Sato M, Chen CC, Akiyama K, Otsuki S (1983). Acute exacerbation of paranoid psychotic state after long-term abstinence in patients with previous methamphetamine psychosis. *Biological Psychiatry* **18**, 429–440.

Sawaguchi T, Goldman-Rakic PS (1991). D1 dopamine receptors in prefrontal cortex: involvement in working memory. *Science* **251**, 947–950.

Schilström B, Ivanov VB, Wiker C, Svensson TH (2007). Galantamine enhances dopaminergic neurotransmission in vivo via allosteric potentiation of nicotinic acetylcholine receptors. *Neuropsychopharmacology* **32**, 43–53.

Shintani F, Kanba S, Nakaki T, Nibuya M, *et al.* (1993). Interleukin-1b augments release of norepinephrine, dopamine, and serotonin in the rat anterior hypothalamus. *Journal of Neuroscience* **13**, 3574–3581.

Simon SL, Domier C, Carnell J, Brethen P, *et al.* (2000). Cognitive impairment in individuals currently using methamphetamine. *American Journal on Addictions* **9**, 222–231.

Srisurapanont M, Ali R, Marsden J, Sunga A, *et al.* (2003). Psychotic symptoms in methamphetamine psychotic in-patients. *International Journal of Neuropsychopharmacology* **6**, 347–352.

Valjent E, Corvol JC, Pages C, Besson MJ, *et al.* (2000). Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties. *Journal of Neuroscience* **20**, 8701–8709.

Wang D, Noda Y, Zhou Y, Mouri A, *et al.* (2007*a*). The allosteric potentiation of nicotinic acetylcholine receptors by galantamine ameliorates the cognitive dysfunction in beta amyloid$_{25\text{-}35}$ i.c.v.-injected mice: involvement of dopaminergic systems. *Neuropsychopharmacology* **32**, 1261–1271.

Wang D, Noda Y, Zhou Y, Nitta A, *et al.* (2007*b*). Synergistic effect of combined treatment with risperidone and galantamine on phencyclidine-induced impairment of latent visuospatial learning and memory: role of nAChR activation-dependent increase of dopamine D1 receptor-mediated neurotransmission. *Neuropharmacology* **53**, 379–389.

Winters BD, Saksida LM, Bussey TJ (2008). Object recognition memory: neurobiological mechanisms of encoding, consolidation and retrieval. *Neuroscience and Biobehavioral Reviews* **32**, 1055–1070.

Yui K, Ikemoto S, Goto K, Nishijima K, *et al.* (2002). Spontaneous recurrence of methamphetamine-induced paranoid-hallucinatory states in female subjects: susceptibility to psychotic states and implications for relapse of schizophrenia. *Pharmacopsychiatry* **35**, 62–71.

Zanassi P, Paolillo M, Feliciello A, Avvedimento EV, *et al.* (2001). cAMP-dependent protein kinase induces cAMP-response element-binding protein phosphorylation via an intracellular calcium release/ERK-dependent pathway in striatal neurons. *Journal of Biological Chemistry* **276**, 11487–11495.

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ARTICLE

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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.

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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

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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.* 2007*a*, *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 Pharmacological Society (2008).

Drugs

PCP hydrochloride was synthesized according to the method of Maddox *et al.* (1965) and checked for purity.

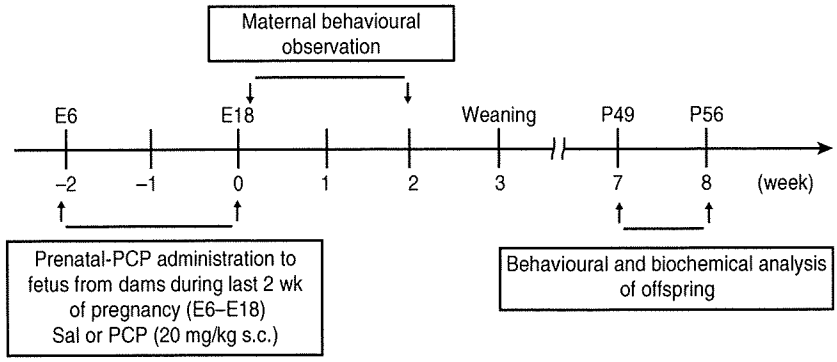

Fig. 1. Experimental protocol of this study.

The PCP was dissolved in saline prior to use. Clozapine (Sigma, USA) was dissolved in a minimum amount of 0.1 N HCI 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.* 2007*a*), 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.* 2007*b*), 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.* 2007*c*). 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 membrance-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 (Ser^{897}) 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 prelimbic area 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: $F_{1,37}=9.54$, $p<0.01$; PCP challenge: $F_{1,37}=64.85$, $p<0.01$; prenatal treatment × PCP challenge: $F_{1,37}=5.73$, $p<0.05$; 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. 2*a*), and the entire 90 min (30–120 min) ($F_{\text{group}(1,37)}=9.20$, $p<0.01$; $F_{\text{treatment}(1,37)}=65.19$, $p<0.01$; $F_{\text{group}\times\text{treatment}(1,37)}=5.73$, $p<0.05$, two-way ANOVA; Fig. 2*b*).

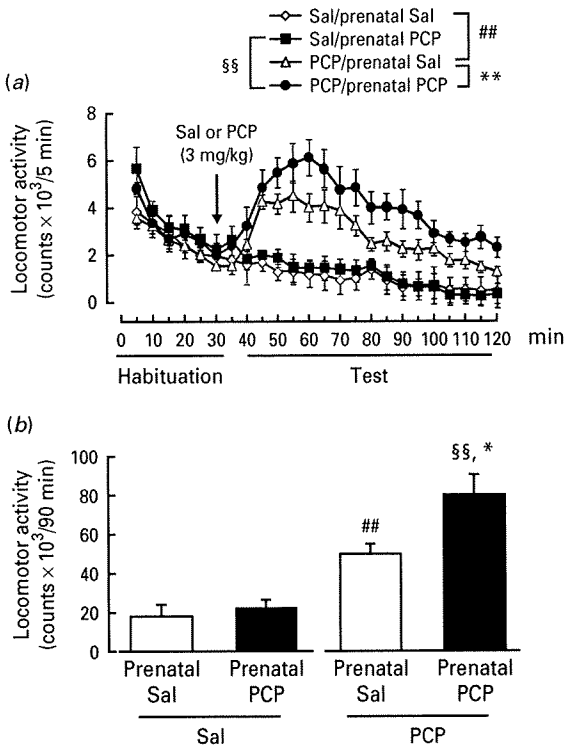

Fig. 2. Effect of prenatal phencyclidine (PCP) treatment on PCP-induced hyperlocomotion. PCP (3 mg/kg) or saline (Sal) was administered after 30 min of habituation. The locomotor activity of mice was assessed over 5-min intervals during the last 90 min after habituation (prenatal treatment: $F_{1,37}=9.54$, $p<0.01$; PCP challenge: $F_{1,37}=64.85$, $p<0.01$; prenatal treatment × PCP challenge: $F_{1,37}=5.73$, $p<0.05$; 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) (*a*) and the entire 90 min (30–120 min) ($F_{\text{group}(1,37)}=9.20$, $p<0.01$; $F_{\text{treatment}(1,37)}=65.19$, $p<0.01$; $F_{\text{group}\times\text{treatment}(1,37)}=5.73$, $p<0.05$, two-way ANOVA) (*b*). $^{\#\#}$ $p<0.01$ compared to Sal/prenatal Sal group. * $p<0.05$, ** $p<0.01$ compared to PCP/prenatal Sal group. §§ $p<0.01$ compared to Sal/prenatal PCP group. Data are expressed as the mean ± S.E.M. for 10–11 mice (Bonferroni's test).

Effect of prenatal-PCP treatment on cognitive function in the NORT

To investigate the effects of prenatal PCP treatment on cognitive function, recognition memory was evaluated in the NORT. In the training session, the prenatal saline- or PCP-treated mice spent equal amounts of time exploring either of the two objects, and there was no biased exploratory preference in each group (prenatal saline-treated mice, 50.4±1.7%; prenatal PCP-treated mice, 53.6±2.6%; $p>0.05$, Fig. 3*a*). In addition, the total time spent in exploration of objects in the training session did not differ between these two groups (prenatal saline-treated mice, 28.3±2.7 s; prenatal PCP-treated mice, 32.0±3.7 s; $p>0.05$, Fig. 3*b*). However, when retention performance was tested, the prenatal PCP-treated mice showed a reduced level of exploratory preference for the novel objects compared to the prenatal saline-treated group (prenatal saline-treated mice, 70.6±2.1%; prenatal PCP-treated mice, 54.5±2.9%; $p<0.01$, Fig. 3*c*). There was no significant difference in total exploration time in the retention session (prenatal saline-treated mice, 17.4±2.3 s; prenatal PCP-treated mice, 16.6±2.3 s; $p>0.05$, Fig. 3*d*).

The sizes of lateral ventricles and brain in prenatal PCP-treated mice

We examined whether prenatal exposure to PCP induced any architectural abnormalities of lateral ventricles and brain at age 7 wk. However, there were no obvious differences between the prenatal saline- and PCP-treated mice in the ratio of brain to body weight (prenatal saline-treated mice, 6.12±0.15%; prenatal PCP-treated mice, 6.02±0.17%; $p>0.05$, Supplementary Fig. S2*e*, available online), the size of lateral ventricles (prenatal treatment: $F_{1,6}=1.93$, $p>0.05$; bregma: $F_{3,18}=493.88$, $p<0.01$; prenatal treatment × bregma: $F_{3,18}=0.85$, $p>0.05$, repeated one-way ANOVA; Suppl. Fig. S2*f*) and of whole brain (prenatal treatment: $F_{1,6}=0.25$, $p>0.05$; bregma: $F_{3,18}=4.44$, $p<0.05$; prenatal treatment × bregma: $F_{3,18}=0.14$, $p>0.05$, repeated one-way ANOVA; Suppl. Fig. S2*g*), as well as the ratio of lateral ventricles to brain size (prenatal treatment: $F_{1,6}=3.93$, $p>0.05$; bregma: $F_{3,18}=564.37$, $p<0.01$; prenatal treatment × bregma: $F_{3,18}=1.98$, $p>0.05$, repeated one-way ANOVA; Suppl. Fig. S2*h*). These suggested the architecture of lateral ventricles was not affected by the prenatal treatment.

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±7.6% *vs.* 152.0±12.9%; $p<0.01$, Fig. 4*a*; hippocampus: 100.0±10.8% *vs.* 140.9±12.2%; $p<0.05$, Fig. 5*e*; striatum: 100.0±10.9% *vs.* 138.3±10.8%; $p<0.05$, Fig. 4*i*). In contrast, the level of NR1 phosphorylated at Ser^{897} was

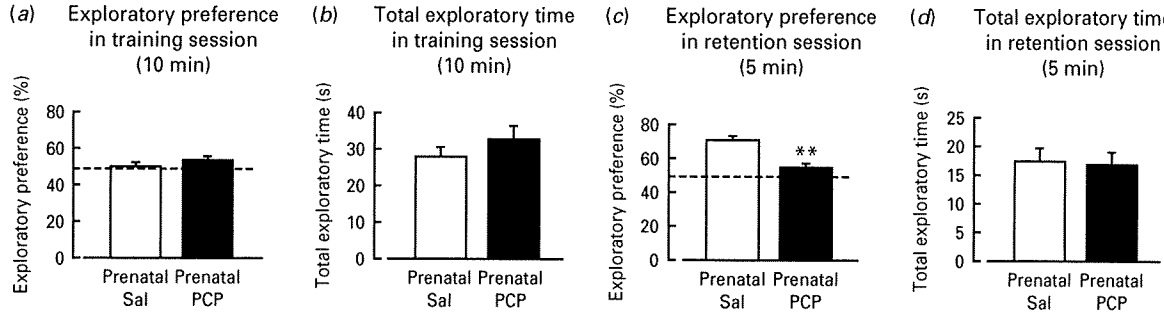

Fig. 3. Effect of prenatal phencyclidine (PCP) treatment on cognitive function in the novel object recognition test. Exploratory preference in (*a*) the training session and (*c*) the retention session. Total time spent exploring the objects in (*b*) the training session and (*d*) the retention session. ** $p<0.01$ compared to the prenatal saline (Sal) group. Data are expressed as the mean ± S.E.M. for 11–12 mice (Student's *t* test).

decreased in prenatal PCP-treated mice (PFC: 100.0 ± 5.9% *vs.* 75.4 ± 7.1%; $p<0.05$, Fig. 4*b*; hippocampus: 100.0 ± 10.5% *vs.* 67.8 ± 9.5%; $p<0.05$; Fig. 4*f*; striatum: 100.0 ± 7.3% *vs.* 87.1 ± 10.1%; $p>0.05$, Fig. 4*j*). 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. 4*c*; hippocampus: 100.0 ± 13.4% *vs.* 48.8 ± 10.5%; $p<0.05$, Fig. 4*g*; striatum: 100.0 ± 14.3% *vs.* 55.0 ± 5.8%; $p<0.05$, Fig. 4*k*). 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. 4*d*), and a positive and almost significant correlation in the hippocampus ($r=0.569$, $p=0.054$, Pearson's correlation; Fig. 4*h*), but no correlation in the striatum ($r=0.325$, $p>0.05$, Pearson's correlation; Fig. 4*l*).

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. S1*a*, *d*), but was not observed at either PD 7 (31.5 ± 3.9 *vs.* 37.2 ± 3.5; $p>0.05$, Suppl. Fig. S1*b*, *e*), or PD 49 (35.7 ± 5.1 *vs.* 39.1 ± 4.0; $p>0.05$, Suppl. Fig. S1*c*, *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: $F_{1,62}=15.41$, $p<0.01$; Clz: $F_{2,62}=29.07$, $p<0.01$; prenatal treatment × Clz: $F_{2,62}=5.23$, $p<0.01$; time: $F_{\text{time}(17,1054)}=70.46$, $p<0.01$; time × prenatal treatment: $F_{17,1054}=1.96$, $p<0.05$; time × Clz: $F_{34,1054}=3.03$, $p<0.01$; time × prenatal treatment × Clz: $F_{34,1054}=1.05$, $p>0.05$, repeated two-way ANOVA; Fig. 5*a*). However, haloperidol reduced the hyperlocomotion of mice in both the prenatal saline- and PCP-treated groups (prenatal treatment: $F_{1,63}=6.88$, $p<0.05$; Hal: $F_{2,63}=17.35$, $p<0.01$; prenatal treatment × Hal: $F_{2,63}=1.30$, $p>0.05$; time: $F_{\text{time }17,1071}=29.46$, $p<0.01$; time × prenatal treatment: $F_{17,1071}=1.66$, $p<0.05$; time × Hal: $F_{34,1071}=3.15$, $p<0.01$; time × prenatal treatment × Hal: $F_{34,1071}=0.92$, $p>0.05$, repeated two-way ANOVA; Fig. 5*c*). 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: $F_{\text{group}(1,62)}=3.23$, $p>0.05$; $F_{\text{treatment}(2,62)}=5.98$, $p<0.01$; $F_{\text{group}\times\text{treatment}(2,62)}=0.12$, $p>0.05$, two-way ANOVA; 30–120 min: $F_{\text{group}(1,62)}=14.84$, $p<0.01$; $F_{\text{treatment}(2,62)}=29.07$, $p<0.01$; $F_{\text{group}\times\text{treatment}(2,62)}=5.23$, $p<0.01$, two-way ANOVA; Fig. 5*b*; Hal: 0–30 min: $F_{\text{group}(1,63)}=1.23$, $p>0.05$; $F_{\text{treatment}(2,63)}=14.90$, $p<0.01$; $F_{\text{group}\times\text{treatment}(2,63)}=0.56$, $p>0.05$, two-way ANOVA; 30–120 min: $F_{\text{group}(1,63)}=7.43$, $p<0.01$; $F_{\text{treatment}(2,63)}=17.28$, $p<0.01$; $F_{\text{group}\times\text{treatment}(2,63)}=1.30$, $p>0.05$, two-way

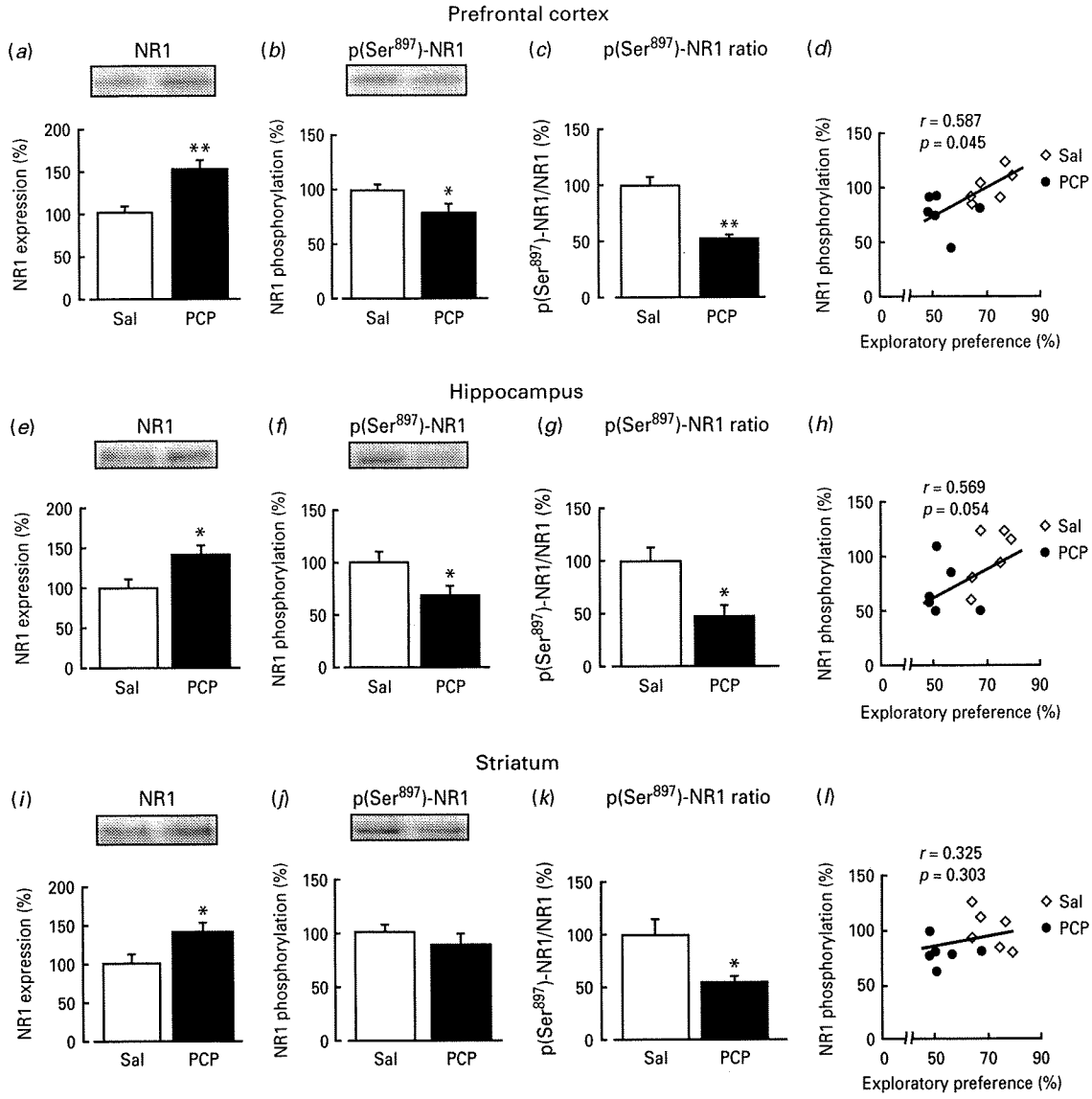

Fig. 4. Changes in the expression and phosphorylation of the NR1 subunit of the NMDA receptor of prenatal phencyclidine (PCP)-treated mice. Expression of NR1 and phosphorylated NR1 [p(Ser^{897})-NR1] was detected by Western blotting. Loaded protein was normalized to β-actin. The phosphorylation ratio was calculated as NR1 phosphorylation *vs.* NR1 expression. Results are represented as the level of NR1 expression in (*a*) the PFC, (*e*) hippocampus and (*i*) striatum; the level of NR1 phosphorylation (Ser^{897}) in (*b*) the PFC, (*f*) hippocampus and (*j*) striatum; and the ratio of NR1 phosphorylation *vs.* NR1 expression in (*c*) the PFC, (*g*) hippocampus and (*k*) striatum. The correlation of phosphorylated NR1 (Ser^{897}) with exploratory preference in the retention session of the novel object recognition test in (*d*) the PFC, (*h*) hippocampus, and (*l*) striatum. * $p<0.05$, ** $p<0.01$ compared to the prenatal saline (Sal) group. Data are expressed as the mean ± S.E.M. for six mice in each group (Student's *t* test).

ANOVA; Fig. 5*d*). 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. 6*a*; 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. 6*e*), or total exploration time after clozapine (1 mg/kg) and haloperidol (0.1 mg/kg) treatment in the training session, although the higher

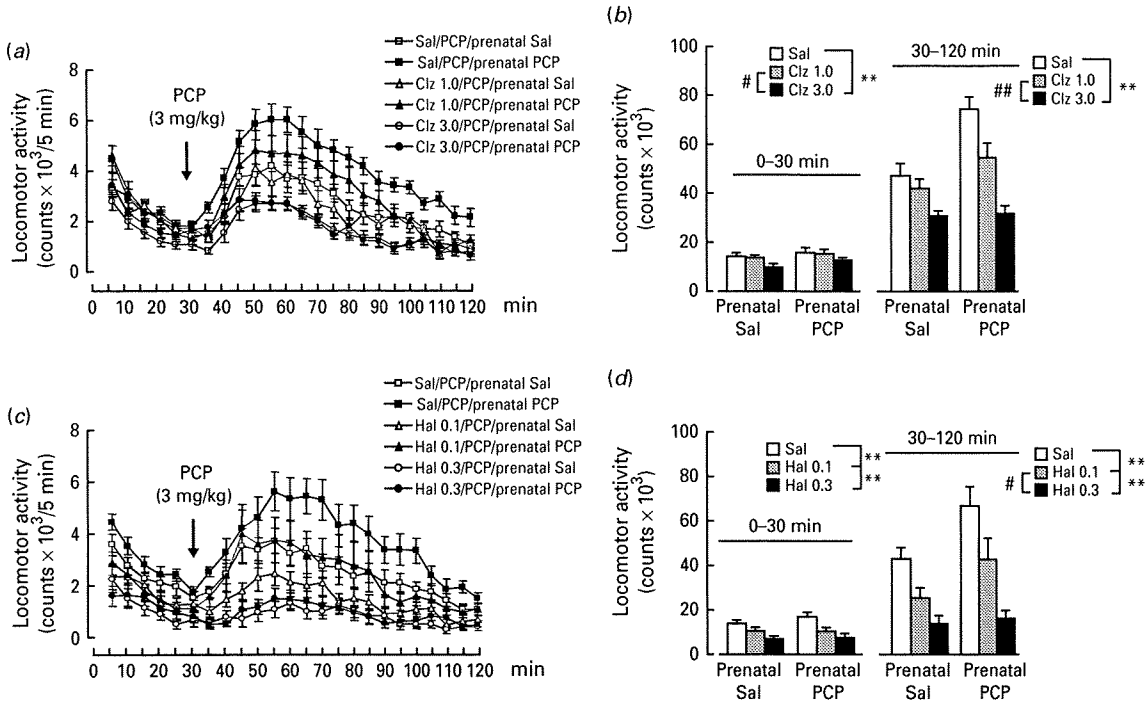

Fig. 5. Effects of antipsychotics on phencyclidine (PCP)-induced hyperlocomotion in prenatal PCP-treated mice. Clozapine (Clz; 1 or 3 mg/kg) and haloperidol (Hal; 0.1 or 0.3 mg/kg) were administered to mice 30 min before the test. After 30 min habituation, mice were challenged with PCP (3 mg/kg). The effects of clozapine or haloperidol on the PCP-induced hyperlocomotion were assessed over 5-min intervals during the last 90 min after habituation (prenatal treatment: $F_{1,62}=15.41$, $p<0.01$; Clz: $F_{2,62}=29.07$, $p<0.01$; prenatal treatment × Clz: $F_{2,62}=5.23$, $p<0.01$; time: $F_{\text{time}(17,1054)}=70.46$, $p<0.01$; time × prenatal treatment: $F_{17,1054}=1.96$, $p<0.05$; time × Clz: $F_{34,1054}=3.03$, $p<0.01$; time × prenatal treatment × Clz: $F_{34,1054}=1.05$, $p>0.05$, repeated two-way ANOVA) (*a*) and haloperidol treatment (prenatal treatment: $F_{1,63}=6.88$, $p<0.05$; Hal: $F_{2,63}=17.35$, $p<0.01$; prenatal treatment × Hal: $F_{2,63}=1.30$, $p>0.05$; time: $F_{\text{time}(17,1071)}=29.46$, $p<0.01$; time × prenatal treatment: $F_{17,1071}=1.66$, $p<0.05$; time × Hal: $F_{34,1071}=3.15$, $p<0.01$; time × prenatal treatment × Hal: $F_{34,1071}=0.92$, $p>0.05$, repeated two-way ANOVA) (*c*); and the entire 120 min (0–30 min, 30–120 min) by clozapine treatment (Clz: 0–30 min: $F_{\text{group}(1,62)}=3.23$, $p>0.05$; $F_{\text{treatment}(2,62)}=5.98$, $p<0.01$; $F_{\text{group}\times\text{treatment}(2,62)}=0.12$, $p>0.05$, two-way ANOVA; 30–120 min: $F_{\text{group}(1,62)}=14.84$, $p<0.01$; $F_{\text{treatment}(2,62)}=29.07$, $p<0.01$; $F_{\text{group}\times\text{treatment}(2,62)}=5.23$, $p<0.01$, two-way ANOVA) (*b*); and haloperidol treatment (Hal: 0–30 min: $F_{\text{group}(1,63)}=1.23$, $p>0.05$; $F_{\text{treatment}(2,63)}=14.90$, $p<0.01$; $F_{\text{group}\times\text{treatment}(2,63)}=0.56$, $p>0.05$, two-way ANOVA; 30–120 min: $F_{\text{group}(1,63)}=7.42$, $p>0.05$; $F_{\text{treatment}(2,63)}=17.28$, $p<0.01$; $F_{\text{group}\times\text{treatment}(2,63)}=1.30$, $p>0.05$, two-way ANOVA) (*d*). ** $p<0.01$ compared to saline (Sal) treatment. # $p<0.05$, ## $p<0.01$ compared to the lower dose of clozapine (1 mg/kg) or haloperidol (0.1 mg/kg) treatments. Data are expressed as the mean ± S.E.M. for 8–14 mice (Bonferroni's test).

dose had a slight effect (Clz: $F_{\text{group}(1,53)}=0.02$, $p>0.05$; $F_{\text{treatment}(2,53)}=4.27$, $p<0.05$; $F_{\text{group}\times\text{treatment}(2,53)}=0.59$, $p>0.05$, two-way ANOVA; Fig. 6*b*; Hal: $F_{\text{group}(1,50)}=3.24$, $p>0.05$; $F_{\text{treatment}(2,50)}=25.84$, $p<0.01$; $F_{\text{group}\times\text{treatment}(2,50)}=0.35$, $p>0.05$, two-way ANOVA; Fig. 6*f*). Interestingly, the impairment of recognition memory in prenatal PCP-treated mice was significantly improved by clozapine (Clz: $F_{\text{group}(1,53)}=16.11$, $p<0.01$; $F_{\text{treatment}(2,53)}=3.42$, $p<0.05$; $F_{\text{group}\times\text{treatment}(2,53)}=4.04$, $p<0.05$, two-way ANOVA; Fig. 6*c*), but not by haloperidol (Hal: $F_{\text{group}(1,50)}=56.22$, $p<0.01$; $F_{\text{treatment}(2,50)}=0.09$, $p>0.05$; $F_{\text{group}\times\text{treatment}(2,50)}=0.16$, $p>0.05$, two-way ANOVA; Fig. 6*g*). However, there were no differences in total exploration time in the retention sessions (Clz: $F_{\text{group}(1,53)}=1.72$, $p>0.05$; $F_{\text{treatment}(2,53)}=0.25$, $p>0.05$; $F_{\text{group}\times\text{treatment}(2,53)}=0.16$, $p>0.05$, two-way ANOVA; Fig. 6*d*; Hal: $F_{\text{group}(1,50)}=1.09$, $p>0.05$; $F_{\text{treatment}(2,50)}=0.20$, $p>0.05$; $F_{\text{group}\times\text{treatment}(2,50)}=0.10$, $p>0.05$, two-way ANOVA; Fig. 6*h*).

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

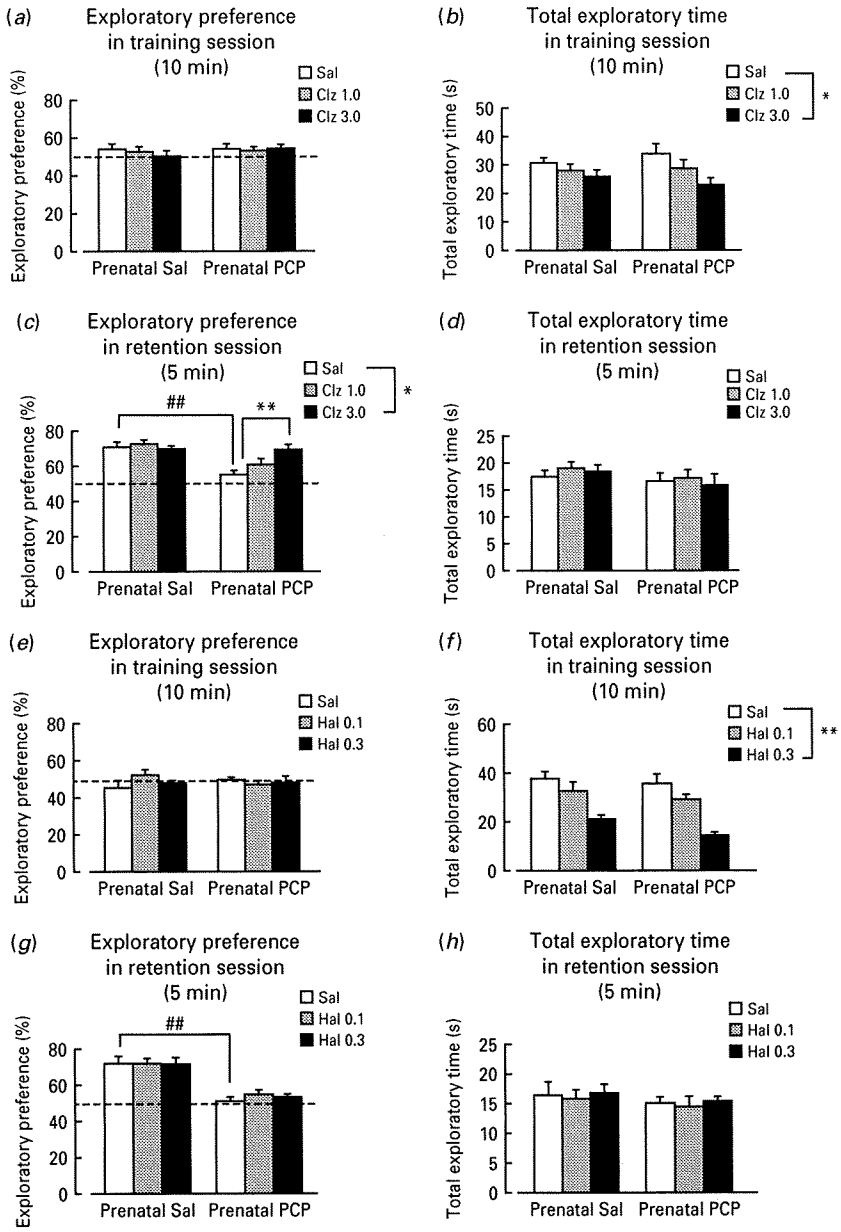

Fig. 6. Effects of antipsychotics on cognitive dysfunction in prenatal PCP-treated mice. Clozapine (Clz; 1 or 3 mg/kg) and haloperidol (Hal; 0.1 or 0.3 mg/kg) were administered 30 min before the training session. For clozapine treatment: (*a*) exploratory preference in the training session ($F_{group(1,53)}=0.42$, $p>0.05$; $F_{treatment(2,53)}=0.32$, $p>0.05$; $F_{group\times treatment(2,53)}=0.23$, $p>0.05$, two-way ANOVA), and (*c*) retention session ($F_{group(1,53)}=16.11$, $p<0.01$; $F_{treatment(2,53)}=3.42$, $p<0.05$; $F_{group\times treatment(2,53)}=4.04$, $p<0.05$, two-way ANOVA). Total exploration time in (*b*) the training session ($F_{group(1,53)}=0.02$, $p>0.05$; $F_{treatment(2,53)}=4.27$, $p<0.05$; $F_{group\times treatment(2,53)}=0.59$, $p>0.05$, two-way ANOVA) and (*d*) retention session ($F_{group(1,53)}=1.72$, $p>0.05$; $F_{treatment(2,53)}=0.25$, $p>0.05$; $F_{group\times treatment(2,53)}=0.16$, $p>0.05$, two-way ANOVA). For haloperidol treatment: (*e*) exploratory preference in the training session ($F_{group(1,50)}=0.05$, $p>0.05$; $F_{treatment(2,50)}=0.23$, $p>0.05$; $F_{group\times treatment(2,50)}=1.27$, $p>0.05$, two-way ANOVA) and (*g*) retention session ($F_{group(1,50)}=56.22$, $p<0.01$; $F_{treatment(2,50)}=0.09$, $p>0.05$; $F_{group\times treatment(2,50)}=0.16$, $p>0.05$, two-way ANOVA). Total exploration time in (*f*) the training session ($F_{group(1,50)}=3.24$, $p>0.05$; $F_{treatment(2,50)}=25.84$, $p<0.01$; $F_{group\times treatment(2,50)}=0.35$, $p>0.05$, two-way ANOVA) and (*h*) retention session ($F_{group(1,50)}=1.09$, $p>0.05$; $F_{treatment(2,50)}=0.20$, $p>0.05$; $F_{group\times treatment(2,50)}=0.10$, $p>0.05$, two-way ANOVA). * $p<0.05$, ** $p<0.01$ compared to saline (Sal) treatment. ## $p<0.01$ 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.* 1988), 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.* 2007*c*), 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 NR1 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 NR1 expression but a reduction in the level and proportion of NR1 phosphorylated at Ser^{897}. The up-regulation of NR1 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 NR1 is due to a compensatory attempt to re-establish the delicate balance of the neurotransmitter network. However, a decreased level of phosphorylated NR1 suggests the function of NMDA receptors is impaired. Moreover, there was a clearly shown positive correlation between decreased NR1 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 Ser^{897} 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 (Gemperle *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

Abekawa T, Ito K, Nakagawa S, Koyama T (2007). Prenatal exposure to an NMDA receptor antagonist, MK-801 reduces density of parvalbumin-immunoreactive GABAergic neurons in the medial prefrontal cortex and enhances phencyclidine-induced hyperlocomotion but not behavioral sensitization to methamphetamine in postpubertal rats. *Psychopharmacology (Berlin)* **192**, 303–316.

Akbarian S, Sucher NJ, Bradley D, Tafazzoli A, *et al.* (1996). Selective alterations in gene expression for NMDA receptor subunits in prefrontal cortex of schizophrenics. *Journal of Neuroscience* **16**, 19–30.

Amitai N, Semenova S, Markou A (2007). Cognitive-disruptive effects of the psychotomimetic phencyclidine and attenuation by atypical antipsychotic medications in rats. *Psychopharmacology (Berlin)* **193**, 521–537.

Anastasio NC, Johnson KM (2008). Differential regulation of the NMDA receptor by acute and sub-chronic phencyclidine administration in the developing rat. *Journal of Neurochemistry* **104**, 1210–1218.

Andersen JD, Pouzet B (2004). Spatial memory deficits induced by perinatal treatment of rats with PCP and reversal effect of D-serine. *Neuropsychopharmacology* **29**, 1080–1090.

Angelucci F, Brenè S, Mathé AA (2005). BDNF in schizophrenia, depression and corresponding animal models. *Molecular Psychiatry* **10**, 345–352.

Ashdown H, Dumont Y, Ng M, Poole S, *et al.* (2006). The role of cytokines in mediating effects of prenatal infection on the fetus: implications for schizophrenia. *Molecular Psychiatry* **11**, 47–55.

Behar TN, Scott CA, Greene CL, Wen X, *et al.* (1999). Glutamate acting at NMDA receptors stimulates embryonic cortical neuronal migration. *Journal of Neuroscience* **19**, 4449–4461.

Bellinger FP, Wilce PA, Bedi KS, Wilson P (2002). Long-lasting synaptic modification in the rat hippocampus resulting from NMDA receptor blockade during development. *Synapse* **43**, 95–101.

Bogerts B (1993). Recent advances in the neuropathology of schizophrenia. *Schizophrenia Bulletin* **19**, 431–445.

Brown AS, Susser ES (2002). In utero infection and adult schizophrenia. *Mental Retardation and Developmental Disabilities Research Reviews* **8**, 51–57.

Chen L, Yang CR (2002). Interaction of dopamine D1 and NMDA receptors mediates acute clozapine potentiation of glutamate EPSPs in rat prefrontal cortex. *Journal of Neurophysiology* **87**, 2324–2336.

Crow TJ, Ball J, Bloom SR, Brown R, *et al.* (1989). Schizophrenia as an anomaly of development of cerebral asymmetry. A postmortem study and a proposal concerning the genetic basis of the disease. *Archives of General Psychiatry* **46**, 1145–1150.

Deutsch SI, Mastropaolo J, Rosse RB (1998). Neurodevelopmental consequences of early exposure to phencyclidine and related drugs. *Clinical Neuropharmacology* **21**, 320–332.

Dracheva S, Marras SA, Elhakem SL, Kramer FR, *et al.* (2001). N-methyl-D-aspartic acid receptor expression in the dorsolateral prefrontal cortex of elderly patients with schizophrenia. *American Journal of Psychiatry* **158**, 1400–1410.

Emamian ES, Karayiorgou M, Gogos JA (2004). Decreased phosphorylation of NMDA receptor type 1 at serine 897 in brains of patients with schizophrenia. *Journal of Neuroscience* **24**, 1561–1564.

Enomoto T, Noda Y, Mouri A, Shin EJ, *et al.* (2005). Long-lasting impairment of associative learning is correlated with a dysfunction of N-methyl-D-aspartate-extracellular signaling-regulated kinase signaling in mice after withdrawal from repeated administration of phencyclidine. *Molecular Pharmacology* **68**, 1765–7174.

Fico TA, Vanderwende C (1988). Phencyclidine during pregnancy: fetal brain levels and neurobehavioral effects. *Neurotoxicology and Teratology* **10**, 349–354.

Gemperle AY, Enz A, Pozza MF, Luthi A, Olpe HR (2003). Effects of clozapine, haloperidol and iloperidone on neurotransmission and synaptic plasticity in prefrontal cortex and their accumulation in brain tissue: an in vitro study. *Neuroscience* **117**, 681–695.

Green MF, Bracha HS, Satz P, Christenson CD (1994). Preliminary evidence for an association between minor physical anomalies and second trimester neurodevelopment in schizophrenia. *Psychiatry Research* **53**, 119–127.

Haberny KA, Paule MG, Scallet AC, Sistare FD, *et al.* (2002). Ontogeny of the N-methyl-D-aspartate (NMDA) receptor system and susceptibility to neurotoxicity. *Toxicological Sciences* **68**, 9–17.

Husi H, Grant SG (2001). Proteomics of the nervous system. *Trends Neuroscience* **24**, 259–266.

Ikonomidou C, Bosch F, Miksa M, Bittigau P, *et al.* (1999). Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. *Science* **283**, 70–74.

Impagnatiello F, Guidotti AR, Pesold C, Dwivedi Y, *et al.* (1998). A decrease of reelin expression as a putative vulnerability factor in schizophrenia. *Proceedings of the National Academy of Sciences USA* **95**, 15718–15723.

Javitt DC, Zukin SR (1991). Recent advances in the phencyclidine model of schizophrenia. *American Journal of Psychiatry* **148**, 1301–1308.

Jones P, Rodgers B, Murray R, Marmot M (1994). Child development risk factors for adult schizophrenia in the British 1946 birth cohort. *Lancet* **344**, 1398–1402.

Kaufman KR, Petrucha RA, Pitts Jr. FN, Weekes ME (1983). PCP in amniotic fluid and breast milk: case report. *Journal of Clinical Psychiatry* **44**, 269–270.

Komuro H, Rakic P (1993). Modulation of neuronal migration by NMDA receptors. *Science* **260**, 95–97.

Leveque JC, Macías W, Rajadhyaksha A, Carlson RR, *et al.* (2000). Intracellular modulation of NMDA receptor function by antipsychotic drugs. *Journal of Neuroscience* **20**, 4011–4020.

Lu P, Mamiya T, Lu LL, Mouri A, *et al.* (2009). Silibinin prevents amyloid β peptide-induced memory impairment and oxidative stress in mice. *British Journal of Pharmacology* **157**, 1270–1277.

Maddox VH, Godefroi EF, Parcell RF (1965). The synthesis of phencyclidine and other 1-arylcyclohexylamines. *Journal of Medicinal Chemistry* **8**, 230–235.

Millan MJ (2005). N-methyl-D-aspartate receptors as a target for improved antipsychotic agents: novel insight and clinical perspectives. *Psychopharmacology* **179**, 30–53.

Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH (1994). Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron* **12**, 529–540.

Mouri A, Noda Y, Enomoto T, Nabeshima T (2007*a*). Phencyclidine animal models of schizophrenia: approaches from abnormality of glutamatergic neurotransmission and neurodevelopment. *Neurochemistry International* **51**, 173–184.

Mouri A, Noda Y, Hara H, Mizoguchi H, *et al.* (2007*b*). Oral vaccination with a viral vector containing Aβ cDNA attenuates age-related Aβ accumulation and memory deficits without causing inflammation in a mouse Alzheimer model. *Journal of the Federation of American Societies for Experimental Biology* **21**, 2135–2148.

Mouri A, Noda Y, Noda A, Nakamura T, *et al.* (2007*c*). Involvement of a dysfunctional dopamine-D1/N-methyl-d-aspartate-NR1 and Ca^{2+}/calmodulin-dependent protein kinase II pathway in the impairment of latent learning in a model of schizophrenia induced by phencyclidine. *Molecular Pharmacology* **71**, 1598–1609.

Murai R, Noda Y, Matsui K, Kamei H, *et al.* (2007). Hypofunctional glutamatergic neurotransmission in the prefrontal cortex is involved in the emotional deficit induced by repeated treatment with phencyclidine in mice: implications for abnormalities of glutamate release and NMDA-CaMKII signaling. *Behavioral Brain Research* **180**, 152–160.

Murray RM, O'Callaghan E, Castle DJ, Lewis SW (1992). A neurodevelopmental approach to the classification of schizophrenia. *Schizophrenia Bulletin* **18**, 319–332.

Nabeshima T, Fukaya H, Yamaguchi K, Ishikawa K, *et al.* (1987). Development of tolerance and supersensitivity to phencyclidine in rats after repeated administration of phencyclidine. *European Journal of Pharmacology* **135**, 23–33.

Nabeshima T, Hiramatsu M, Yamaguchi K, Kasugai M, *et al.* (1988). Effects of prenatal administration of phencyclidine on the learning and memory processes of rat offspring. *Journal of Pharmacobiodynamics* **11**, 816–823.

Nagai T, Murai R, Matsui K, Kamei H, *et al.* (2009). Aripiprazole ameliorates phencyclidine-induced impairment of recognition memory through dopamine D_1 and serotonin 5-HT_{1A} receptors. *Psychopharmacology* **202**, 315–328.

Nagai T, Noda Y, Une T, Furukawa K, *et al.* (2003). Effect of AD-5423 on animal models of schizophrenia: phencyclidine-induced behavioral changes in mice. *Neuroreport* **14**, 269–272.

Nicholas JM, Lipshitz J, Schreiber EC (1982). Phencyclidine: its transfer across the placenta as well as into breast milk. *American Journal of Obstetrics and Gynecology* **143**, 143–146.

Noda Y, Yamada K, Furukawa H, Nabeshima T (1995). Enhancement of immobility in a forced swimming test by subacute or repeated treatment with phencyclidine: a new model of schizophrenia. *British Journal of Pharmacology* **116**, 2531–2537.