	Ikeda K, Hayashida M, Nishizawa D, Sora I. Method of evaluating drug-sensitivity by analyzing the GIRK channel genes. US20090092972. 8-08. 2010年7月28日
	Ikeda K, Hayashida M, Nishizawa D, Sora I. Method of evaluating drug-sensitivity by analyzing the GIRK channel genes. EP 1895016 B1 2010年7月14日

International Journal of Neuropsychopharmacology, Page 1 of 13. © CINP 2011
doi:10.1017/S146114571100085X

ARTICLE

CINP

Mouse strain differences in phencyclidine-induced behavioural changes

Akihiro Mouri[1,2,3], Takenao Koseki[1,3], Shiho Narusawa[1,3], Minae Niwa[1,3,4,5], Takayoshi Mamiya[1,3], Shin-ichi Kano[5], Akira Sawa[5,6,7,8] and Toshitaka Nabeshima[1,3]

[1] *Department of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Meijo University, Nagoya, Japan*
[2] *Division of Scientific Affairs, Japanese Society of Pharmacopoeia, Tokyo, Japan*
[3] *The Academic Frontier Project for Private Universities, Comparative Cognitive Science Institutes, Meijo University, Nagoya, Japan*
[4] *Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[5] *Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD, USA*
[6] *Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, USA*
[7] *Graduate Program in Cellular and Molecular Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA*
[8] *Graduate Program in Human Genetics, Johns Hopkins University School of Medicine, Baltimore, MD, USA*

Abstract

Administration of phencyclidine (PCP) is acknowledged to generate a model of psychosis in animals. With the identification of genetic susceptibility factors for schizophrenia and bipolar disorder, great efforts have been made to generate genetic animal models for major mental illnesses. As these disorders are multifactorial, comparisons among drug-induced (non-genetic) and genetic models are becoming an important issue in biological psychiatry. A major barrier is that the standard mouse strain used in the generation of genetic models is C57BL/6, whereas almost all studies with PCP-induced models have utilized other strains. To fill this technical gap, we systematically compared the behavioural changes upon PCP administration in different mouse strains, including C57BL/6N, C57BL/6J, ddY, and ICR. We observed strain differences in PCP-induced hyperlocomotion and enhanced immobility in the forced swim test (ddY >> C57BL/6N and 6J > ICR). In contrast, there was no strain difference in the impairment of recognition memory in the novel object recognition memory test after withdrawal of chronic PCP administration. This study provides practical guidance for comparing genetic with PCP-induced models of psychosis in C57BL/6. Furthermore, such strain differences may provide a clue to the biological mechanisms underlying PCP-induced endophenotypes possibly relevant to major mental illnesses.

Received 28 December 2010; Reviewed 7 February 2011; Revised 20 April 2011; Accepted 5 May 2011

Key words: Behavioural sensitization, forced swim test, mouse strain difference, phencyclidine, schizophrenia.

Introduction

It is challenging to model major psychiatric disorders, such as schizophrenia, in mice (Flint & Shifman, 2008). At present, two approaches are utilized. First, on the basis of similarities between drug-induced psychosis in adults and schizophrenia (Javitt & Zukin, 1991; Luby *et al.* 1959), drug-treated, especially phencyclidine (PCP)-treated animals are utilized (Jentsch & Roth, 1999; Mouri *et al.* 2007*a*). PCP is an antagonist of the NMDA-type glutamate receptor, and PCP-induced models have been used for screening many compounds in neuropsychopharmacology (Hashimoto *et al.* 2005; Kunitachi *et al.* 2009; Mouri *et al.* 2007*a*; Noda *et al.* 1995). A major drawback of these models is that, even if they mimic the pathophysiology of schizophrenia, they do not encompass the neurodevelopmental abnormalities that underlie the pathogenesis of the disease (Fatemi & Folsom, 2009). Second, on the basis of disease-associated susceptibility genes for schizophrenia, many groups have generated genetically engineered mice as possible

Address for correspondence: T. Nabeshima, Ph.D., Department of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Meijo University, 150 Yagotoyama, Tempaku-ku, Nagoya 468-8503, Japan.
Tel.: 81-52-839-2735 *Fax*: 81-52-839-2738
Email: tnabeshi@meijo-u.ac.jp

models for the disease (Desbonnet *et al.* 2009; Hikida *et al.* 2007; Mohn *et al.* 1999). An advantage of this approach is that mice with genetic changes in disease susceptibility factors involved in neurodevelopment can offer the potential to address the aetiology-associated biology during development (Fatemi & Folsom, 2009). Furthermore, with rapid advances in controlling spatial- and temporal-specific genetic alternations, genetic models offer the potential to dissect neuronal circuitry-dependent phenotypic changes in detail (Pletnikov, 2009). Nonetheless, caution should be exercised in the interpretation of data because there is currently no specific causal gene for schizophrenia (Glessner & Hakonarson, 2009). Therefore a strategy to compare both genetic and non-genetic (in most cases, drug-induced) animal models is clearly warranted.

A crucial criterion in characterizing the behaviour of these genetically engineered models is that the mice are backcrossed with C57BL/6 and standardized to be in the C57BL/6 genetic background (Hikida *et al.* 2007; Mohn *et al.* 1999). Despite some reports on the effect of repeated PCP treatment on cognition in C57BL/6J strain (Beraki *et al.* 2008, 2009), most studies with PCP-induced endophenotypes have used strains other than C57BL/6, such as ddY (Mouri *et al.* 2007*b*; Murai *et al.* 2007; Noda *et al.* 1995). The present study was designed to fill this gap and enable systematic experiments in both genetic and non-genetic (PCP-induced) models in parallel with those in the C57BL/6 strain. Therefore, we compared behavioural changes and sensitivity to chronic PCP administration in different strains, including C57BL/6N, C57BL/6J, ddY, and ICR.

Methods

Mice

Male mice of the ddY, ICR, C57BL/6N, and C57BL/6J strains (6-wk-old) were obtained from Japan SLC (Shizuoka, Japan). The animals were housed in plastic cages and kept in a regulated environment (24±1 °C, 50±5% humidity), with a 12-h light/dark cycle (lights on at 08:00 hours). Food and tap water were available *ad libitum*. All experiments were performed in accordance with the Guidelines for Animal Experiments of Meijo University Faculty of Pharmaceutical Sciences. The procedures involving animals and their care were conducted in conformity with the international guidelines, Principles of Laboratory Animal Care (National Institutes of Health publication 85–23, revised 1985).

Drugs

Phencyclidine hydrochloride [1-(1-phenylcyclohexyl) piperidine hydrochloride (PCP)] was synthesized by the authors according to the method of Maddox *et al.* (1965) and checked for purity. PCP was dissolved in a saline (0.9% NaCl) solution and administered in a volume of 0.1 ml/10 g body weight. The mice (6-wk-old) were treated with PCP (10 and 15 mg/kg s.c.) once a day for 14 d.

Locomotor activity test

To measure spontaneous locomotor activity in a novel environment, a mouse was placed in a transparent acrylic cage with a black frosted Plexiglas floor (45 × 26 × 40 cm), and locomotion was measured for 120 min using digital counters with infrared sensors (Scanet SV-10; Melquest Co. Ltd, Japan). One day after measurement of spontaneous locomotor activity, mice were given saline or PCP at a dose of 10 mg/kg s.c. for 14 d. Locomotor activity was immediately measured for 2 h after PCP treatment on days 1 and 14. PCP challenge-induced hyperactivity was measured separately from locomotor activity during the 14-d treatment. To exclude any effect of PCP remaining in the brain on the challenge-induced hyperactivity, the test was performed 5 d after withdrawal from chronic PCP treatment. This is because the half-life of PCP in the brain was 30.5 min in rats treated repeatedly (Nabeshima *et al.* 1987) and PCP-treated rats did not show withdrawal syndrome behaviour 4 d after the final treatment (Nabeshima *et al.* 1986). One day after habituation to the apparatus, locomotor activity was measured for 120 min immediately after PCP (1 and 3 mg/kg) challenge.

Forced swim test

The forced swim test was conducted according to previous reports (Murai *et al.* 2007; Noda *et al.* 1995) with a minor modification. The test was performed 1 d and 3 d after withdrawal from 14-d PCP treatment as reported previously (Murai *et al.* 2007; Noda *et al.* 1995). A mouse was placed in a transparent glass cylinder (20 cm high, 15 cm diameter), which contained water at 22–23 °C to a depth of 15 cm, and was forced to swim for 360 s. The duration of swimming was measured by a SCANET MV-10 AQ apparatus (Melquest Co. Ltd). Immobility was calculated as follows (in seconds): total time − swimming time = immobility time.

Novel-object recognition test

The test procedure consisted of three sessions: habituation, training, and retention. One day after the final PCP treatment, each mouse was individually habituated to the Plexiglas box (30 × 30 × 35 high cm), and allowed 10 min exploration in the absence of objects for 3 d (habituation). After habituation (4 d after the final PCP treatment), mice were subjected to a training trial. During the training trial, two objects were placed in the back corner of the box. The objects used were a golf ball, wooden cylinders, and square pyramids, which were different in shape and colour but similar in size. A mouse was then placed midway at the front of the box and the total time spent exploring the two objects was recorded for 10 min. An animal was considered to be exploring the object when its head was facing the object or it was touching or sniffing the object. During the retention trial, the animals were returned to the same box 24 h after the training trial, in which one of the familiar objects used during training was replaced with a novel object. The animals were then allowed to explore freely for 10 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, a ratio of the amount of time spent exploring any one of the two objects (training trial) or the novel object (retention trial) over the total time spent exploring both objects, was used to measure cognitive function.

Western blot analysis

Western blot analysis was performed as described previously (Mouri *et al.* 2007*b*). One and three days after withdrawal from chronic PCP treatment, mice were euthanized by decapitation immediately after the forced swim test, and their brains removed. The frontal cortices were rapidly dissected out, frozen, and stored at −80 °C until used. Cortices were homogenized by sonication in ice-cold lysis buffer [20 mM Tris–HCl (pH 7.4), 150 mM NaCl, 50 mM NaF, 2 mM EDTA, 0.1% sodium dodecyl sulfate (SDS), 1% sodium deoxycholate, 1% NP-40, 1 mM sodium orthovanadate, 20 μg/ml pepstatin, 20 μg/ml aprotinin, and 20 μg/ml leupeptin], followed by centrifugation at 13000 *g* for 20 min to collect soluble fractions. Samples (20 μg protein) were separated by SDS–PAGE and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, USA). The membranes were blocked with a Detector Block kit (Kirkegaard and Perry Laboratories, USA), probed with anti-phospho-CaM kinase IIα/β antibody (T286/287) (1:1000, Millipore), and subsequently incubated with horseradish peroxidase-linked anti-rabbit IgG (1:2000, Kirkegaard and Perry Laboratories). The immune complexes were detected by ChemiDoc XRS (Bio-Rad, USA) based on chemiluminescence (ECL Plus Western blotting detection system, GE Healthcare, UK). The band intensities were analysed by densitometry with the ATTO Densitograph Software Library Lane Analyser (ATTO, Japan). Membranes were stripped and total CaM kinase II expression was detected using anti-CaM kinase IIα antibody (1:2000, Sigma-Aldrich, USA).

Immunohistochemistry

Histological procedures were performed as previously described with a minor modification (Murai *et al.* 2007). One day after withdrawal from 14-d PCP treatment, mice were anaesthetized immediately after the forced swim test with chloral hydrate (150 mg/kg i.p.) and perfused transcardially with ice-cold phosphate-buffered saline (PBS), followed by 4% paraformaldehyde in PBS. The brains were removed, post-fixed in the same fixative for 12 h, and soaked in 20% (w/v) sucrose in PBS. Coronal sections, 20-μm-thick, were cut with a Cryostar HM560 cryostat (Microm International, Germany). For immunohistochemistry, the following primary antibodies were used: rabbit anti-phospho-CaM kinase IIα/β (T286/287) (1:500, Millipore); mouse anti-neuron-specific nuclear antigen (NeuN) (1:500, Millipore); mouse anti-GFAP (1:500, Millipore); and goat anti-NMDAζ1 (C-20) (1:250, Santa Cruz Biotechnology, USA). Fluorescently conjugated secondary antibodies (Alexa 488, 546; Invitrogen, USA) were used for chromogen detection. Images were acquired with a confocal microscope (LSM510; Carl Zeiss, Germany).

Statistical analysis

All results were expressed as the mean ± S.E.M. for each group. The difference between groups was analysed with a one-way, two-way, or repeated-measures ANOVA, followed by the Bonferroni/Dunn multiple range test. Student's *t* test was used to compare two sets of data.

Results

Supersensitivity in PCP-induced hyperlocomotion in C57BL/6N and C57BL/6J strains 5 d after withdrawal from chronic PCP administration

Repeated systemic PCP administration in rodents results not only in an enduring supersensitivity to a

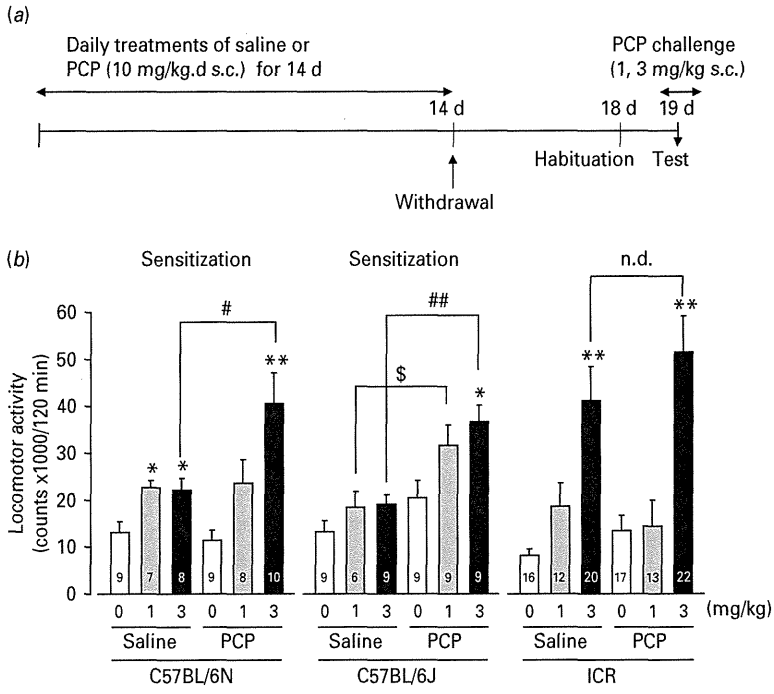

Fig. 1. Supersensitivity in hyperlocomotion induced by low doses of phencyclidine (PCP) was observed in C57BL/6N and C57BL/6J but not ICR strains 5 d after withdrawal from chronic PCP treatment. (*a*) Experimental design. One day after habituation, a locomotor activity test was performed 5 d after cessation of chronic PCP treatment (10 mg/kg s.c. once a day for 14 d). (*b*) Locomotor activities were measured for 120 min immediately after PCP (1 and 3 mg/kg) challenge. $^{\$}p<0.05$ compared to PCP (1 mg/kg)-challenged, saline-treated mice; $^{\#}p<0.05$, $^{\#\#}p<0.01$ compared to PCP (3 mg/kg)-challenged, saline-treated mice; $^{**}p<0.01$, $^{*}p<0.05$ compared to corresponding saline-challenged mice. Values indicate mean ± S.E.M. The number of animals is indicated within the columns. n.d., No difference.

subsequent psychostimulant challenge, but also changes in the brain and behaviour that are particularly pronounced following a prolonged period of withdrawal (Jentsch *et al.* 1998; Nagai *et al.* 2003). Thus, animals in withdrawal represent a drug-free but altered brain state and may be more suitable for the study of psychosis than animals acutely injected with PCP (Jentsch & Roth, 1999). We previously reported that the ddY strain showed sensitization in hyperlocomotion induced by PCP challenge in the withdrawal period after repeated PCP administration (Nagai *et al.* 2003). We thus first examined the strain difference in locomotor activity induced by a low dose of PCP after withdrawal from chronic PCP administration (10 mg/kg.d for 14 d). Four days after the final PCP treatment mice were habituated to the apparatus for 120 min. The next day, locomotor activity was measured for 120 min immediately after a low-dose PCP (1 and 3 mg/kg) challenge (Fig. 1*a*). In the C57BL/6N strain, the PCP challenge increased locomotor activity in the group chronically treated with saline (Fig. 1*b*, two-way ANOVA, chronic PCP treatment: $F_{1,45}=4.47$, $p<0.05$; PCP challenge: $F_{2,45}=12.61$, $p<0.01$; treatment × challenge interaction: $F_{2,45}=3.71$, $p<0.05$), which was significantly augmented after withdrawal from chronic PCP treatment. This supersensitivity of locomotor activity induced by a PCP challenge was also observed in the C57BL/6J strain after withdrawal from chronic PCP treatment, although the challenge did not increase locomotor activity after chronic treatment with saline in this strain (Fig. 1*b*, two-way ANOVA, chronic PCP treatment: $F_{1,45}=22.29$, $p<0.01$; PCP challenge: $F_{2,45}=6.31$, $p<0.01$; treatment × challenge interaction: $F_{2,45}=1.32$, $p=0.27$). In the ICR strain, the PCP challenge significantly increased locomotor activity in mice chronically treated with saline (Fig. 1*b*, two-way ANOVA, chronic PCP treatment: $F_{1,94}=1.09$, $p=0.30$; PCP challenge: $F_{2,94}=21.87$, $p<0.01$; treatment × challenge interaction: $F_{2,94}=0.65$, $p=0.52$). Behavioural sensitization, however, was not observed in the group chronically treated with PCP. Although the ddY strain showed significantly less spontaneous locomotor activity than did the other strains [Supplementary Fig. S1(*a*, *b*)], all the strains showed a marked increase in locomotor activity on acute PCP treatment (10 mg/kg at day 1)

and sensitization in locomotor activity after chronic PCP administration (at day 14) (Supplementary Fig. S1*c*). These results suggested that chronic PCP treatment altered the supersensitivity of locomotor activity induced by PCP in the withdrawal period in the C57BL/6N and C57BL/6J strains as well as in the ddY strain, but not in the ICR strain.

Enhanced immobility in ddY, C57BL/6N, and C57BL/6J strains in the forced swim test 1 d after withdrawal from chronic PCP administration

Several groups including ours reported previously that PCP-induced enhancement of immobility in the forced swim test was observed in the ddY strain after drug withdrawal, and this behavioural change was attenuated by second-generation antipsychotics but not by antidepressants (Abdel-Naby Sayed *et al.* 2001; Murai *et al.* 2007; Noda *et al.* 1995, 1997). Thus, we chronically administered PCP (10 mg/kg.d for 14 d) to C57BL/6N, C57BL/6J, and ICR strains and examined their immobility in the forced swim test after withdrawal compared to that in the ddY strain. Immobility time was measured for 6 min, 1 d after withdrawal from chronic PCP treatment (Fig. 2*a*). The C57BL/6N and C57BL/6J strains showed PCP (10 mg/kg.d for 14 d) enhanced immobility as did the ddY strain, although the extent of enhancement was milder (Fig. 2*b*, two-way ANOVA, 10 mg/kg PCP: $F_{1,112}=35.72$, $p<0.01$; strain: $F_{3,112}=5.13$, $p<0.01$; PCP × strain interaction: $F_{3,112}=3.24$, $p<0.05$). In sharp contrast, even higher doses of PCP (15 mg/kg.d for 14 d) failed to enhance immobility in the ICR strain (Fig. 2*b*, two-way ANOVA, 15 mg/kg PCP: $F_{1,81}=13.79$, $p<0.01$; strain: $F_{2,81}=1.10$, $p=0.34$; PCP × strain interaction: $F_{2,81}=0.55$, $p=0.58$). Detailed analysis of immobility time, measured at 3 min and 6 min, showed that PCP significantly enhanced immobility during the first 3 min of the measurement (0–3 min) in the C57BL/6N and C57BL/6J strains (Supplementary Fig. S2).

The forced swim test typically consists of two swimming tests: a pretest and a test, because the immobile response can be potentiated by a previous exposure to stress (Porsolt *et al.* 1977). To explore the strain difference in the effect of the pretest on PCP-enhanced immobility, each mouse was placed again in the water 1 d after the initial forced swim test and forced to swim for 6 min (Fig. 2*c*). Immobility time in the 2-d forced swim test was prolonged compared to that in the 1-d test in saline-treated C57BL/6N and C57BL/6J strains (Supplementary Fig. S3). In the 2-d test, PCP-enhanced immobility was observed in the C57BL/6J strain at two different doses of PCP (10 and 15 mg/kg.d) as in the ddY strain (Fig. 2*c*, 10 mg/kg, two-way ANOVA, 10 mg/kg PCP: $F_{1,112}=14.13$, $p<0.01$; strain: $F_{3,112}=4.63$, $p<0.01$; PCP × strain interaction: $F_{3,112}=2.75$, $p<0.05$; 15 mg/kg, two-way ANOVA, 15 mg/kg PCP: $F_{1,81}=3.64$, $p=0.06$; strain: $F_{2,81}=8.30$, $p<0.01$; PCP × strain interaction: $F_{2,81}=2.94$, $p=0.06$). We observed no difference in immobility time between saline- and PCP-treated C57BL/6N strain; however, PCP-enhanced immobility in the C57BL/6N strain was prolonged in the 2-d test compared to that in ICR strain, which did not show any PCP-enhanced immobility in either test (Fig. 2*b*, *c*). It has been suggested that two consecutive trials prolong immobility in saline-treated mice and mask PCP-enhanced immobility in the C57BL/6N strain. These results clearly showed the strain difference (ddY > C57BL/6 > ICR) in the effect of PCP on immobility in the forced swim test.

Impaired phosphorylation of CaMKII in the frontal cortex associated with enhanced immobility in the forced swim test

Chronic PCP treatment impaired NMDA-Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) signalling in the prefrontal cortex (Molteni *et al.* 2008). We have shown that a malfunction of the NMDA-NR1 and CaMKII pathways in the prefrontal cortex was involved in PCP-enhanced immobility in the forced swim test in the ddY strain (Murai *et al.* 2007). We examined CaMKII phosphorylation in the frontal cortex of C57BL/6N, ICR, and ddY strains after withdrawal from chronic PCP treatment. One day after the final PCP treatment, phosphorylated CaMKII was detected immediately after the forced swim test (Fig. 3*a*). Levels of phosphorylated CaMKII in the frontal cortex increased significantly after swimming in mice chronically treated with saline in the C57BL/6 and ICR strains as well as the ddY strain (Fig. 3*b*, two-way ANOVA, PCP: $F_{1,26}=0.39$, $p=0.54$; swimming: $F_{1,26}=1.75$, $p=0.20$; PCP × swimming interaction: $F_{1,26}=7.26$, $p<0.05$). Nonetheless, there was no increase in swimming-associated CaMKII phosphorylation levels in the ddY and C57BL/6N strains that were chronically treated with PCP and showed enhanced immobility in the forced swim test (Fig. 3*b*). Interestingly, a swimming-associated increase in phosphorylation of CaMKII was observed in the ICR strain that did not show enhanced immobility after chronic PCP treatment (Fig. 3*b*, two-way ANOVA, C57BL/6N, PCP: $F_{1,28}=2.64$, $p=0.12$; swimming: $F_{1,28}=4.07$, $p=0.05$; PCP × swimming interaction: $F_{1,28}=5.25$, $p<0.05$; ICR,

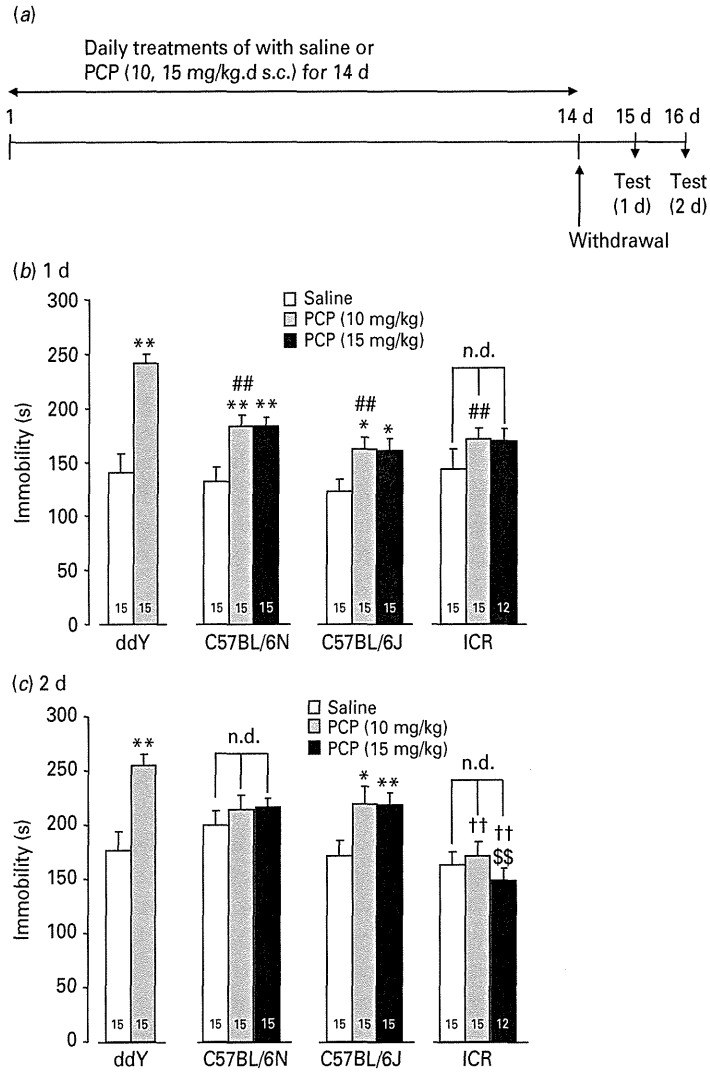

Fig. 2. Enhanced immobility was observed in ddY, C57BL/6N, and C57BL/6J but not ICR strains in the forced swim test 1 d after withdrawal from chronic phencyclidine (PCP) treatment. (*a*) Experimental design. Mice were sequentially subjected to forced swim tests 1 and 2 d after the final PCP treatment. (*b*, *c*) Immobility time was measured at 3 and 6 min in ddY, C57BL/6N, C57BL/6J, and ICR strains at (*b*) 1 d and (*c*) 2 d after withdrawal from chronic PCP treatment (10 and 15 mg/kg s.c. once a day for 14 d). * $p<0.05$, ** $p<0.01$ *vs.* corresponding saline-treated mice; $^{\#\#}$ $p<0.01$ compared to corresponding ddY strain; †† $p<0.01$ compared to corresponding C57BL/6N strain; $^{\$\$}$ $p<0.01$ compared to corresponding C57BL/6J strain. Values indicate mean ± S.E.M. The number of animals is indicated within the columns. n.d., No difference.

PCP: $F_{1,28}=3.26$, $p=0.08$; swimming: $F_{1,28}=11.86$, $p<0.01$; PCP × swimming interaction: $F_{1,28}=0.10$, $p=0.75$). We found a significant inverse correlation between the duration of immobility and swimming-associated increase in CaMKII phosphorylation (Fig. 3*c*, Pearson's correlation coefficient test: $r_{47}=-2.79$, $p<0.01$). We also observed by immunohistochemistry that phospho-CaMKII-positive cells in the prefrontal cortex increased after swimming in C57BL6/N strain treated with saline but not in the strain treated with PCP (Supplementary Fig. S4*b*). We noted that phospho-CaMKII immunoreactivity was co-localized with the immunoreactivity of NeuN and NR1 but not with GFAP (Supplementary Fig. S4*c*, *d*). Thus, swimming-associated CaMKII phosphorylation occurred in neurons expressing NR1. These results suggest a strain difference in PCP-impaired swimming-associated CaMKII phosphorylation that underlies the strain difference in PCP-enhanced immobility in the forced swim test.

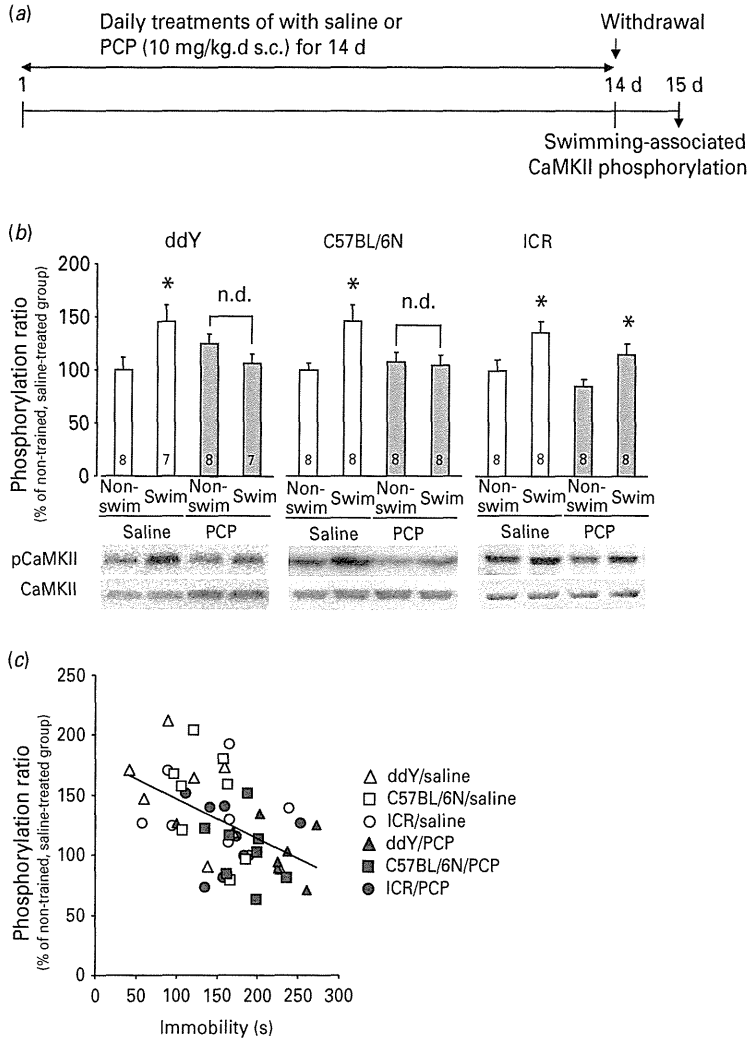

Fig. 3. Impairment of swimming-associated CaMKII phosphorylation in the frontal cortex of mice showing enhanced immobility in the forced swim test after withdrawal from chronic phencyclidine (PCP) treatment. (*a*) Experimental design. A forced swim test was performed 1 d after cessation of chronic PCP treatment (10 mg/kg s.c. once a day for 14 d). Immediately after the forced swim test, mice were euthanized by decapitation, and CaMKII phosphorylated at Thr^{286} and αCaMKII in the frontal cortex detected by Western blotting. (*b*) Impairment of swimming-associated CaMKII phosphorylation in the frontal cortex of ddY, C57BL/6N, and ICR strains after withdrawal of chronic PCP treatment. * $p<0.05$ compared to corresponding non-swim mice. Values indicate mean $\pm$ S.E.M. The number of animals is indicated within the columns. (*c*) Relationship between duration of immobility in the forced swim test and the swimming-associated CaMKII phosphorylation ratio. The solid line represents the regression line, which was estimated by plotting changes in immobility and swimming-associated CaMKII phosphorylation. n.d., No difference.

Strain difference in the persistence of enhanced immobility in the forced swim test after withdrawal from chronic PCP treatment

We next explored the sustained effects of PCP in different strains. We previously reported that PCP-enhanced immobility in the forced swim test persisted for at least 21 d after withdrawal of the drug in the ddY strain (Noda *et al.* 1995). Consistent with this report, the ddY strain showed PCP (10 mg/kg.d) enhanced immobility 3 d after withdrawal (Fig. 4*a*, *b*, two-way ANOVA, 10 mg/kg PCP: $F_{1,79}=9.27$, $p<0.01$; strain: $F_{2,79}=5.57$, $p<0.01$; PCP $\times$ strain interaction: $F_{2,79}=2.21$, $p=0.12$). C57BL/6N and C57BL/6J mice did not show enhanced immobility 3 d after withdrawal; however, even at higher doses of chronic

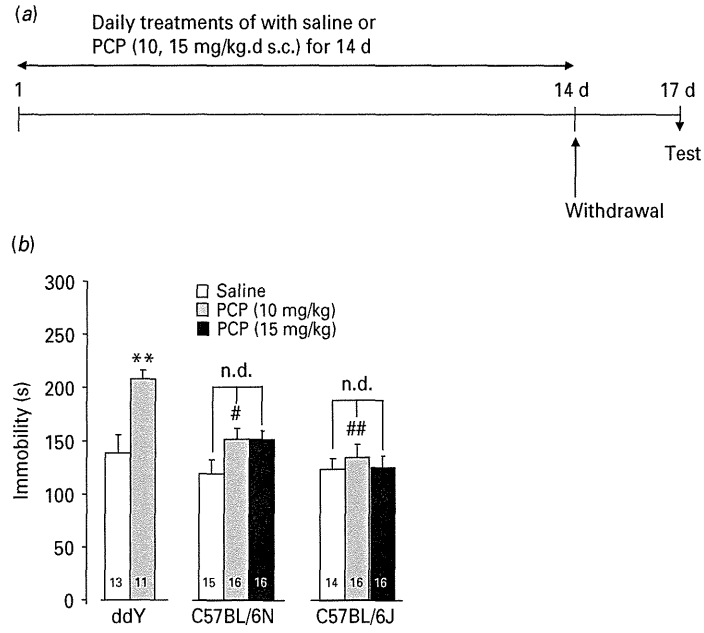

Fig. 4. Enhancement of immobility in the forced swim test was observed in ddY but not C57BL/6N and C57BL/6J strains 3 d after withdrawal from chronic phencyclidine (PCP) treatment. (*a*) Experimental design. Mice were individually subjected to a forced swim test 3 d after the final PCP treatment, and immobility time was measured for 6 min. (*b*) Enhanced immobility in the forced swim test was measured in ddY, C57BL/6N, and C57BL/6J strains 3 d after withdrawal from chronic PCP treatment (10 and 15 mg/kg s.c. once a day for 14 d). ** $p<0.01$ *vs.* corresponding saline-treated mice; ## $p<0.01$, # $p<0.05$ compared to corresponding ddY strain. Values indicate mean ± S.E.M. The number of animals is indicated within the columns. n.d., No difference.

PCP treatment (15 mg/kg.d) (Fig. 4*b*, *c*, chronic PCP treatment: $F_{1,58}=1.91$, $p=0.17$; strain: $F_{1,58}=0.79$, $p=0.38$; treatment × strain interaction: $F_{1,58}=1.89$, $p=0.17$). The level of swimming-associated CaMKII phosphorylation in the frontal cortex recovered in C57BL/6N strain 3 d after withdrawal while it was still impaired in the ddY strain (Supplementary Fig. S5*a*, *b*). Thus, PCP-enhanced immobility in C57BL/6N and C57BL/6J strains was more transient than that in the ddY strain.

No strain difference in the impairment of object recognition memory in the novel object recognition test after withdrawal from chronic PCP administration

PCP-induced impairment of object recognition memory in the novel object recognition test is observed in the ICR strain after drug withdrawal, and this behavioural change is attenuated by second- but not first-generation antipsychotics (Hagiwara *et al.* 2008; Nagai *et al.* 2009). Although repeated PCP treatment induces impairments of spatial learning and working memory in the water maze test in C57BL/6J strain (Beraki *et al.* 2008, 2009), the impairment of object recognition memory in this strain has not been confirmed. Thus, we chronically administered PCP (10 mg/kg.d for 14 d) to ddY, C57BL/6N, C57BL/6J and ICR strains and examined their object recognition memory in the novel object recognition test 4 d after withdrawal. In the training trial, PCP- and saline-treated mice spent equal amounts of time exploring either of the two objects in all strains (Fig. 5*b*, two-way ANOVA, 10 mg/kg PCP: $F_{1,57}=0.75$, $p=0.38$; strain: $F_{3,57}=0.98$, $p=0.40$; PCP × strain interaction: $F_{3,57}=0.76$, $p=0.52$). When the retention trial was performed 24 h after the training trial, the level of exploratory preference for the novel objects in PCP-treated mice was significantly decreased compared to that in saline-treated mice in all strains and there was no strain difference (Fig. 5*b*, two-way ANOVA, 10 mg/kg PCP: $F_{1,57}=0.75$, $p=0.38$; strain: $F_{3,57}=0.98$, $p=0.40$; PCP × strain interaction: $F_{3,57}=0.76$, $p=0.52$). There was a strain difference in total exploration time in the training (Fig. 5*c*, two-way ANOVA, 10 mg/kg PCP: $F_{1,57}=1.65$, $p=0.20$; strain: $F_{3,57}=7.21$, $p<0.01$; PCP × strain interaction: $F_{3,57}=1.28$, $p=0.28$) and retention (Fig. 5*c*, two-way ANOVA, 10 mg/kg PCP: $F_{1,57}=2.22$, $p=0.14$; strain: $F_{3,57}=12.37$,

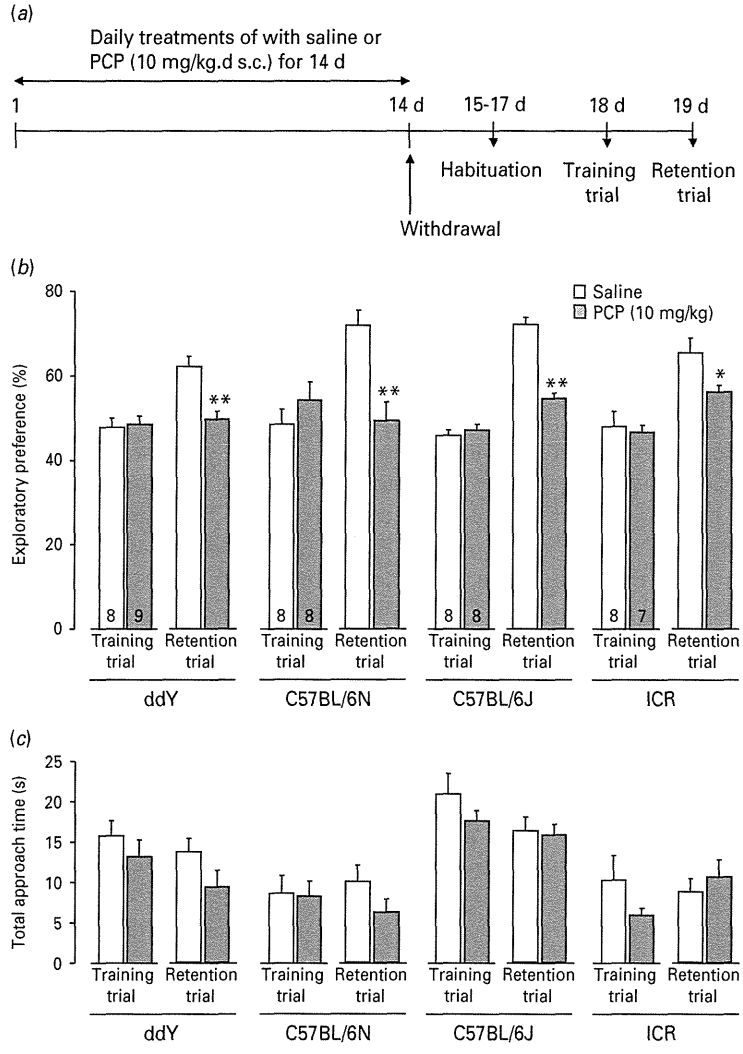

Fig. 5. No strain difference in the impairment of object recognition memory in the novel object recognition test after withdrawal from chronic phencyclidine (PCP) administration. (*a*) Experimental design. Mice were habituated to the apparatus for 3 d and then subjected to a novel object recognition test 4–5 d after the final PCP treatment, and exploratory preference and total approach time for objects were measured for 10 min. (*b*, *c*) Exploratory preference and total approach time, respectively, were measured in ddY, C57BL/6N, C57BL/6J, and ICR strains 4–5 d after withdrawal from chronic PCP treatment (10 mg/kg s.c. once a day for 14 d). ** $p<0.01$, * $p<0.05$ *vs.* corresponding saline-treated mice. Values indicate mean ± S.E.M. The number of animals is indicated within the columns.

$p<0.01$; PCP × strain interaction: $F_{3,57}=0.03$, $p=0.99$) trials, but total time spent in the exploration of objects in the training and retention sessions did not differ between PCP- and saline-treated mice in any strains (Fig. 5*c*). It is possible that there are strain differences in motivation and curiosity for objects but chronic PCP treatment has no effect on them in any of the strains. Taken together, these results suggest that chronic 14-d PCP treatment induces an impairment of object recognition memory 4 d after withdrawal and there was no strain difference in this impairment.

Discussion

In this study, we compared behavioural changes and sensitivity to the chronic administration of PCP in different mouse strains, including C57BL/6N, C57BL/6J, ddY, and ICR. Supersensitivity in hyperlocomotion upon PCP treatment was consistently observed in ddY mice (Nagai *et al.* 2003), but was significantly lower in the C57BL/6N and C57BL/6J strains, and was not detectable in the ICR strain. Hyperfunction of mesolimbic dopaminergic neurons might be implicated in

the enhanced hyperlocomotion induced by chronic PCP treatment (Balla *et al.* 2001; Nabeshima *et al.* 1989*a*, *b*). It is possible that chronic PCP treatment modulates the mesolimbic dopaminergic system in a strain-specific manner.

The administration of dopaminergic psychotomimetic agents to animals is the most widely used model of the positive symptoms of schizophrenia (Snyder, 1988). However, the absence of animal models for the negative symptoms is a major problem in the study of the neurobiology of schizophrenia (Jentsch *et al.* 1997; Mouri *et al.* 2007*a*). Chronic PCP-treated animals show enhanced immobility in the forced swim test, a paradigm considered to be a useful animal model of the negative symptoms of schizophrenia (Noda *et al.* 1995). Enhanced immobility in the forced swim test after withdrawal of chronic PCP administration has been fully established in the ddY strain (Abdel-Naby Sayed *et al.* 2001; Noda *et al.* 1995, 1997). In contrast, no enhanced immobility was observed 3 d after withdrawal in the other strains we tested. These differences in strains were also observed in a molecular marker known to correlate with enhanced immobility, phosphorylation of Ca^{2+}/calmodulin-dependent protein kinase II in NR1-immunopositive neurons in the prefrontal cortex. Chronic PCP treatment disrupts glutamatergic neurotransmission in the prefrontal cortex and such changes are likely to associate with the enhanced immobility in the forced swim test (Murai *et al.* 2007). These results suggest that differences in altered glutamatergic function in the prefrontal cortex might underlie the strain difference in PCP-enhanced immobility in the forced swim test. Notably, it has been demonstrated that acute treatment with a NMDA receptor antagonist (ketamine) produced antidepressant-like effects as shown by shortened immobility in the forced swim test (Li *et al.* 2010; Maeng *et al.* 2008), which involve enhanced AMPA receptor (Maeng *et al.* 2008) and mTOR (mammalian target of rapamycin)-dependent synaptogenesis (Li *et al.* 2010). These findings seemed to be inconsistent with our findings. A selective NR2B antagonist also exerted these antidepressant-like effects (Li *et al.* 2010; Maeng *et al.* 2008). Chronic treatment with ketamine resulted in the development of tolerance to its antidepressant-like effects (Popik *et al.* 2008). Enhanced immobility in the forced swim test induced by PCP was observed only on chronic treatment (Noda *et al.* 1995). This inconsistently might depend on the differences in affinity for the NMDA receptor subunit and duration of treatment.

Cognitive deficits induced by chronic PCP administration have been demonstrated in C57BL/6J (Beraki *et al.* 2008, 2009), ICR (Hagiwara *et al.* 2008; Nagai *et al.* 2009), and ddY (Mouri *et al.* 2007*b*) strains using various behavioural tests. Although strain differences were observed in PCP-induced supersensitivity in hyperlocomotion and enhanced immobility in the forced swim test, there was no strain difference in the impairment of objective recognition memory. It was suggested that strain differences in PCP-induced endophenotypes depend on the behavioural tasks and the impairment of objective recognition memory is more sensitive than PCP-induced supersensitivity in hyperlocomotion and enhanced immobility in the forced swim test.

We have suggested that one possible mechanism of strain difference in PCP-induced endophenotypes is a difference in the phosphorylation of CaMKII under stress, but no other mechanism was elucidated in the present study. D-amino acid oxidase (DAO) regulates glutamatergic function by degrading D-serine, a co-agonist of the NMDA receptor (Schell, 2004). Further, DAO activity is higher in male ddY strain than male C57/BL6 strain (Konno & Yasumura, 1983). DAO transgenic mice show an enhancement of PCP-induced hyperactivity (Otte *et al.* 2009), but ddY strain lacking DAO activity do not show PCP-induced hyperactivity (Almond *et al.* 2006). These results suggest that a strain difference in DAO activity contributes to PCP-induced endophenotypes. In addition, the NMDA receptor hypofunction hypothesis in schizophrenia has evolved from neurotoxic and psychomimetic effects of PCP (Olney *et al.* 1999). Acute and repeated low-dose (5 mg/kg) treatment with PCP induces a transient and reversible vacuolation but not cell death in layers III and IV of the posterior cingulate cortex (Olney *et al.* 1989). Acute treatment with a high dose (50 mg/kg) of PCP induces prolonged severe NMDA receptor hypofunction which causes irreversible degeneration and death of neurons in many cerebrocotical and corticolimbic brain regions (Corso *et al.* 1997). We have found cell shrinkage and glial activation without cell death in the prefrontal cortex 1 d after repeated PCP (10 mg/kg) treatment in the ddY strain (Murai *et al.* 2007). It is possible that reversibility for PCP-induced neuronal toxicity but not cell death is involved in the strain difference in PCP-induced endophenotypes. There were strain differences between C57BL/6N and C57BL/6J strains in supersensitivity of their hyperlocomotion upon PCP (1 mg/kg) treatment and PCP-enhanced immobility in the 2-d forced swim test. There are some single nucleotide polymorphisms in C57BL/6N and C57BL/6J strains and a deletion of nicotinamide nucleotide transhydrogenase in C57BL/6J mice (Mekada *et al.* 2009).

The gene polymorphisms and deletion might contribute to the difference in C57BL/6 substrains in PCP-induced endophenotypes.

The major rationale for studying differences in response to PCP in mouse strains is that C57BL/6, the standard strain with which genetically engineered mice are characterized (Hikida *et al.* 2007; Mohn *et al.* 1999), has seldom been used in experiments with PCP. In contrast, the ddY strain has been most frequently used in studies with PCP (Abdel-Naby Sayed *et al.* 2001; Mouri *et al.* 2007*b*; Murai *et al.* 2007; Nagai *et al.* 2003; Noda *et al.* 1995, 1997). Our systematic study suggests that the frequent use of ddY in the past may be due to its greater response to PCP compared to other strains. Although the response to PCP is less robust, we report here that two C57BL/6 lines (C57BL/6N and C57BL/6J) also show behavioural changes. Thus, this study will provide practical guidance in comparing genetic models to PCP-induced models of psychosis in C57BL/6 strain.

What are the mechanisms underlying the behavioural changes upon treatment with PCP. Genetic polymorphisms existing among these strains may account for the differences. Further study using other strains such as DBA2 and BALB/c will provide more valuable information about strain differences in PCP-induced endophenotypes, since the DBA2 strain shows a deficit of prepulse inhibition and enhanced immobility in the forced swim test (Olivier *et al.* 2001; Popova *et al.* 2009) and the BALB/c strain shows behavioural hypersensitivity to MK-801 (another non-competitive NMDA receptor antagonist) and could be useful for studying 'psychosis-peoneness' (Deutsch *et al.* 1997; Perera *et al.* 2008). Therefore, by addressing such strain differences in further genetic studies, we may be able to obtain important clues as to the biological mechanisms underlying PCP-induced endophenotypes that are possibly relevant to major mental illnesses.

Note

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

Acknowledgements

We thank Dr Pamela Talalay and Ms. Yukiko Lema for help with the critical reading and organization of the manuscript. This study was supported in part by the Academic Frontier Project for Private Universities 2007–2011 from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by an International Research Project supported by The Meijo Asian Research Center (MARC), by a Grant-in-aid for Exploratory Research from Japan Society for the Promotion of Science (JSPS), by a Grant-in-aid for Young Scientists (B) from JSPS, by a Grant-in-aid for Scientific Research (B) from JSPS, by a Grant-in-aid on Priority Areas from JSPS, by a Health and Labour Sciences Research Grant for Research on Regulatory Science of Pharmaceuticals and Medical Devices from the Ministry of Health, Labour and Welfare, Japan, by Solution Oriented Research for Science and Technology (SORST) of the Japan Science and Technology Agency (JST), by a Grant-in-aid for Creative Scientific Research from JSPS, by the Regional Joint Research Programme supported by grants to Private Universities to Cover Current Expenses from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), and by a Smoking Research Foundation Grant for Biomedical Research. It was also supported by NIH grants of Silvo O. Conte Center MH-084018 (A.S.), MH-069853 (A.S.), MH-088753 (A.S.), as well as grants from Stanley (A.S.), CHDI (A.S.), HighQ (A.S.), S-R (A.S.), and NARSAD (A.S.). S.K. was supported by an Uehara Memorial Foundation postdoctoral fellowship and is currently a JSPS Postdoctoral Fellow for Research Abroad.

Statement of Interest

None.

References

Abdel-Naby Sayed M, Noda Y, Mahmoud Hamdy M, Mamiya T, *et al.* (2001). Enhancement of immobility induced by repeated phencyclidine injection: association with c-Fos protein in the mouse brain. *Behavioural Brain Research* **124**, 71–76.

Almond SL, Fradley RL, Armstrong EJ, Heavens RB, *et al.* (2006). Behavioral and biochemical characterization of a mutant mouse strain lacking D-amino acid oxidase activity and its implications for schizophrenia. *Molecular and Cellular Neurosciences* **32**, 324–334.

Balla A, Koneru R, Smiley J, Sershen H, *et al.* (2001). Continuous phencyclidine treatment induces schizophrenia-like hyperreactivity of striatal dopamine release. *Neuropsychopharmacology* **25**, 157–164.

Beraki S, Diaz-Heijtz R, Tai F, Ogren SO (2009). Effects of repeated treatment of phencyclidine on cognition and gene expression in C57BL/6 mice. *International Journal of Neuropsychopharmacology* **12**, 243–255.

Beraki S, Kuzmin A, Tai F, Ogren SO (2008). Repeated low dose of phencyclidine administration impairs spatial learning in mice: blockade by clozapine but not by haloperidol. *European Neuropsychopharmacology* **18**, 486–497.

Corso TD, Sesma MA, Tenkova TI, Der TC, *et al.* (1997). Multifocal brain damage induced by phencyclidine is augmented by pilocarpine. *Brain Research* **752**, 1–14.

Desbonnet L, Waddington JL, Tuathaigh CM (2009). Mice mutant for genes associated with schizophrenia: common phenotype or distinct endophenotypes? *Behavioural Brain Research* **204**, 258–273.

Deutsch SI, Rosse RB, Paul SM, Riggs RL, *et al.* (1997). Inbred mouse strains differ in sensitivity to 'popping' behavior elicited by MK-801. *Pharmacology, Biochemistry and Behavior* **57**, 315–317.

Fatemi SH, Folsom TD (2009). The neurodevelopmental hypothesis of schizophrenia, revisited. *Schizophrenia Bulletin* **35**, 528–548.

Flint J, Shifman S (2008). Animal models of psychiatric disease. *Current Opinion in Genetics and Development* **18**, 235–240.

Glessner JT, Hakonarson H (2009). Common variants in polygenic schizophrenia. *Genome Biol* **10**, 236.

Hagiwara H, Fujita Y, Ishima T, Kunitachi S, *et al.* (2008). Phencyclidine-induced cognitive deficits in mice are improved by subsequent subchronic administration of the antipsychotic drug perospirone: role of serotonin 5-HT1A receptors. *European Neuropsychopharmacology* **18**, 448–454.

Hashimoto K, Fujita Y, Shimizu E, Iyo M (2005). Phencyclidine-induced cognitive deficits in mice are improved by subsequent subchronic administration of clozapine, but not haloperidol. *European Journal of Pharmacology* **519**, 114–117.

Hikida T, Jaaro-Peled H, Seshadri S, Oishi K, *et al.* (2007). Dominant-negative DISC1 transgenic mice display schizophrenia-associated phenotypes detected by measures translatable to humans. *Proceedings of the National Academy of Sciences USA* **104**, 14501–14506.

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

Jentsch JD, Roth RH (1999). The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology* **20**, 201–225.

Jentsch JD, Taylor JR, Roth RH (1998). Subchronic phencyclidine administration increases mesolimbic dopaminergic system responsivity and augments stress- and psychostimulant-induced hyperlocomotion. *Neuropsychopharmacology* **19**, 105–113.

Jentsch JD, Tran A, Le D, Youngren KD, *et al.* (1997). Subchronic phencyclidine administration reduces mesoprefrontal dopamine utilization and impairs prefrontal cortical-dependent cognition in the rat. *Neuropsychopharmacology* **17**, 92–99.

Konno R, Yasumura Y (1983). Mouse mutant deficient in D-amino acid oxidase activity. *Genetics* **103**, 277–285.

Kunitachi S, Fujita Y, Ishima T, Kohno M, *et al.* (2009). Phencyclidine-induced cognitive deficits in mice are ameliorated by subsequent subchronic administration of donepezil: role of sigma-1 receptors. *Brain Research* **1279**, 189–196.

Li N, Lee B, Liu RJ, Banasr M, *et al.* (2010). mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science* **329**, 959–964.

Luby ED, Cohen BD, Rosenbaum G, Gottlieb JS, *et al.* (1959). Study of a new schizophrenomimetic drug; sernyl. *AMA Archives of Neurology and Psychiatry* **81**, 363–369.

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

Maeng S, Zarate CA, Du J, Schloesser RJ, *et al.* (2008). Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biological Psychiatry* **63**, 349–352.

Mekada K, Abe K, Murakami A, Nakamura S, *et al.* (2009). Genetic differences among C57BL/6 substrains. *Experimental Animals* **58**, 141–149.

Mohn AR, Gainetdinov RR, Caron MG, Koller BH (1999). Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. *Cell* **98**, 427–436.

Molteni R, Pasini M, Moraschi S, Gennarelli M, *et al.* (2008). Reduced activation of intracellular signaling pathways in rat prefrontal cortex after chronic phencyclidine administration. *Pharmacological Research* **57**, 296–302.

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, Noda A, Nakamura T, *et al.* (2007*b*). 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. *Behavioural Brain Research* **180**, 152–160.

Nabeshima T, Fukaya H, Kamei H, Ishikawa K, *et al.* (1989*a*). Cross sensitization between phencyclidine and methamphetamine: I repeated administration of methamphetamine at a low dose. *Research Communications in Substances of Abuse* **10**, 62–76.

Nabeshima T, Fukaya H, Kamei H, Ishikawa K, *et al.* (1989*b*). Cross sensitization between phencyclidine and methamphetamine: II repeated administration of methamphetamine at a high dose. *Research Communications in Substances of Abuse* **10**, 95–107.

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, Ishikawa K, Yamaguchi K, Furukawa H, *et al.* (1986). Methysergide-induced precipitated withdrawal syndrome in phencyclidine-dependent rats. *Neuroscience Letters* **69**, 275–278.

Nagai T, Murai R, Matsui K, Kamei H, *et al.* (2009). Aripiprazole ameliorates phencyclidine-induced impairment of recognition memory through dopamine D1 and serotonin 5-HT1A 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.

Noda Y, Mamiya T, Furukawa H, Nabeshima T (1997). Effects of antidepressants on phencyclidine-induced enhancement of immobility in a forced swimming test in mice. *European Journal of Pharmacology* **324**, 135–140.

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.

Olivier B, Leahy C, Mullen T, Paylor R, *et al.* (2001). The DBA/2J strain and prepulse inhibition of startle: a model system to test antipsychotics? *Psychopharmacology* **156**, 284–290.

Olney JW, Labruyere J, Price MT (1989). Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. *Science* **244**, 1360–1362.

Olney JW, Newcomer JW, Farber NB (1999). NMDA receptor hypofunction model of schizophrenia. *Journal of Psychiatric Research* **33**, 523–533.

Otte DM, Bilkei-Gorzo A, Filiou MD, Turck CW, *et al.* (2009). Behavioral changes in G72/G30 transgenic mice. *European Neuropsychopharmacology* **19**, 339–348.

Perera PY, Lichy JH, Mastropaolo J, Rosse RB, *et al.* (2008). Expression of NR1, NR2A and NR2B NMDA receptor subunits is not altered in the genetically-inbred Balb/c mouse strain with heightened behavioral sensitivity to MK-801, a noncompetitive NMDA receptor antagonist. *European Neuropsychopharmacology* **18**, 814–819.

Pletnikov MV (2009). Inducible and conditional transgenic mouse models of schizophrenia. *Progress in Brain Research* **179**, 35–47.

Popik P, Kos T, Sowa-Kucma M, Nowak G (2008). Lack of persistent effects of ketamine in rodent models of depression. *Psychopharmacology (Berlin)* **198**, 421–430.

Popova NK, Naumenko VS, Tibeikina MA, Kulikov AV (2009). Serotonin transporter, 5-HT1A receptor, and behavior in DBA/2J mice in comparison with four inbred mouse strains. *Journal of Neuroscience Research* **87**, 3649–3657.

Porsolt RD, Le Pichon M, Jalfre M (1977). Depression: a new animal model sensitive to antidepressant treatments. *Nature* **266**, 730–732.

Schell MJ (2004). The N-methyl D-aspartate receptor glycine site and D-serine metabolism: an evolutionary perspective. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **359**, 943–964.

Snyder SH (1988). Psychotogenic drugs as models for schizophrenia. *Neuropsychopharmacology* **1**, 197–199.

International Journal of Neuropsychopharmacology (2012), **15**, 1489–1501. © CINP 2011
doi:10.1017/S1461145711001672

ARTICLE

CINP

Exposure to enriched environments during adolescence prevents abnormal behaviours associated with histone deacetylation in phencyclidine-treated mice

Takenao Koseki[1,2], Akihiro Mouri[1,3], Takayoshi Mamiya[1,2], Yuki Aoyama[1], Kazuya Toriumi[1,2], Shizuka Suzuki[1], Azusa Nakajima[1], Takuma Yamada[1], Taku Nagai[3] and Toshitaka Nabeshima[1,2]

[1] *Department of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Meijo University, Nagoya, Japan*
[2] *The Academic Frontier Project for Private Universities, Comparative Cognitive Science Institutes, Meijo University, Nagoya, Japan*
[3] *Department of Neuropsychopharmacology and Hospital Pharmacy, Graduate School of Medicine, Nagoya University, Nagoya, Japan*

Abstract

Enriched environments (EEs) during development have been shown to influence adult behaviour. Environmental conditions during childhood may contribute to the onset and/or pathology of schizophrenia; however, it remains unclear whether EE might prevent the development of schizophrenia. Herein, we investigated the effects of EE during adolescence on phencyclidine (PCP)-induced abnormal behaviour, a proposed schizophrenic endophenotype. Male ICR mice (3 wk old) were exposed to an EE for 4 wk and then treated with PCP for 2 wk. The EE potentiated the acute PCP treatment-induced hyperlocomotion in the locomotor test and prevented chronic PCP treatment-induced impairments of social behaviour and recognition memory in the social interaction and novel object recognition tests. It also prevented the PCP-induced decrease of acetylated Lys^9 in histone H3-positive cells and increase of the histone deacetylase (HDAC)5 level in the prefrontal cortex. To investigate whether the histone modification during adolescence might be critical for the effect of EE, 3-wk-old mice were first treated with sodium butyrate (SB; an HDAC inhibitor) for 4 wk and then treated with PCP for 2 wk. Chronic SB treatment during adolescence mimicked the effects of EE, including potentiation of hyperlocomotion induced by acute PCP treatment and prevention of social and cognitive impairments, decrease of acetylated Lys^9 in histone H3-positive cells and increase of the HDAC5 level in the prefrontal cortex associated with chronic PCP treatment. Our results suggest that EEs prevent PCP-induced abnormal behaviour associated with histone deacetylation. EEs during childhood might prove to be a novel strategy for prophylaxis against schizophrenia.

Received 1 April 2011; Reviewed 13 May 2011; Revised 8 September 2011; Accepted 9 October 2011; First published online 18 November 2011

Key words: Enriched environment, histone acetylation, phencyclidine, schizophrenia.

Introduction

Schizophrenia has an approximately 50% concordance rate in monozygotic twins and 1% prevalence in the general population (McGuffin *et al.* 1995). This report, together with some other findings, suggests that not only genetic factors but also environmental factors, especially early life experiences during the critical period of brain development, might contribute to the onset and/or progression of schizophrenia (van Os *et al.* 2008). Conversely, environmental interventions may protect against the onset of schizophrenia, offering the opportunity for development of unprecedented therapeutic approaches for schizophrenia. Little is known about how environmental factors and the associated experience-dependent plasticity, especially

Address for correspondence: T. Nabeshima, Ph.D., Department of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Meijo University, 150 Yagotoyama, Tempaku-ku, Nagoya 468-8503, Japan.
Tel.: 81-52-839-2735 *Fax*: 81-52-834-2738
Email: tnabeshi@meijo-u.ac.jp

enriched and/or favourable growing environments, might modulate the pathogenesis of schizophrenia and/or of the pattern of disease progression (Laviola *et al.* 2008).

'Enriched environment' (EE) refers to housing conditions in which a combination of complex inanimate and social stimulations (Rosenzweig *et al.* 1978) is provided, generally in large cages, with running wheels and toys that are periodically changed to stimulate curiosity and exploration (Rosenzweig & Bennett, 1996). An EE facilitates brain development and functions, including of sensory, cognitive and motor function, relative to a standard environment (Chapillon *et al.* 1999; Faherty *et al.* 2003; Kozorovitskiy *et al.* 2005; Leggio *et al.* 2005). It has been reported that EEs improve memory function and synaptic plasticity through histone acetylation, which has been implicated in the transcriptional regulation of gene expressions via chromatin remodelling (Fischer *et al.* 2007). These findings suggest that regulation of chromatin structure through histone acetylation may be involved in the beneficial effects of EE exposure.

Exposure to an EE has been shown to have beneficial effects not only in normal animals but also in models of central neurological disorders (Adlard *et al.* 2005; Bezard *et al.* 2003; van Dellen *et al.* 2000). In a clinical study, early exposure to a nutritional, educational and physical exercise enrichment programme prevented the development of a schizotypal personality (Raine *et al.* 2003). However, it remains unclear whether exposure to an EE might also prevent abnormal behaviour in a schizophrenic model.

Administration of phencyclidine (PCP), a non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, has been shown to reproduce a schizophrenia-like psychosis, including positive symptoms, negative symptoms and cognitive dysfunction (Allen & Young, 1978; Javitt & Zukin, 1991; Luby *et al.* 1959; Rainey & Crowder, 1975). In rodents, repeated-dose PCP administration was demonstrated to induce schizophrenic endophenotypes, such as impaired sociality and cognitive functions (Javitt & Zukin, 1991; Nagai *et al.* 2009; Noda *et al.* 1995; Qiao *et al.* 2001). Therefore, it has been suggested that PCP-treated animals might be a useful pharmacological model of schizophrenia (Mouri *et al.* 2007*a*).

In the present study, we designed experiments to investigate whether exposure to an EE during adolescence might prevent PCP-induced behavioural abnormalities through histone modifications: (i) we examined whether the abnormal behaviour of PCP-treated mice could be prevented by housing the animals in an EE, using the locomotor test, social interaction test and novel object recognition test; (ii) we attempted to investigate the changes in histone acetylation and histone deacetylase (HDAC) levels in the prefrontal cortex (PFC) induced by exposure to an EE in mice subjected to chronic treatment with PCP; (iii) we verified the importance of histone modifications during adolescence by investigating whether administration of a HDAC inhibitor might prevent the behavioural and biochemical changes induced by PCP.

Methods

Animals

Male ICR mice (age 3 wk) were obtained from Japan SLC (Japan). The animals were kept in a regulated environment (23±1 °C, 50±5% humidity), under a 12-h light/dark cycle (lights on at 07:00 hours). Food and tap water were available *ad libitum*. All experiments were performed in accordance with the Guidelines for Animal Experiments of Meijo University. The procedures involving animals and their care were in conformity with international guidelines, namely, Principles of Laboratory Animal Care (National Institutes of Health publication 85–23, revised 1985).

Drug treatment

Phencyclidine hydrochloride [1-(1-phenylcyclohexyl) piperidine hydrochloride; PCP] was synthesized by Professor H. Furukawa according to the method reported by Maddox *et al.* (1965). PCP and sodium butyrate (SB; Wako, Japan) were dissolved in a saline (Sal) and administered at a volume of 0.1 ml/10 g body weight. PCP (10 mg/kg.d s.c.) was injected once per day for 14 consecutive days from age 7 wk. SB (1 g/kg.d i.p.) was injected once per day for 4 wk from age 3 wk.

Housing conditions

The mice were divided into two groups at age 3 wk, namely, the EE group and the standard environment (SE) group. The animals of the EE group ($n=8$) were exposed for 12 h/d to a large acrylic box (50 × 70 × 20 cm), containing two different running wheels (11 cm and 15 cm in diameter, respectively) and five objects, including toys, tunnels and hiding places (Fig. 1*b*) and moved back to normal conditions for the remaining 12 h, for 4 wk. More than three objects were changed daily to maintain the environmental

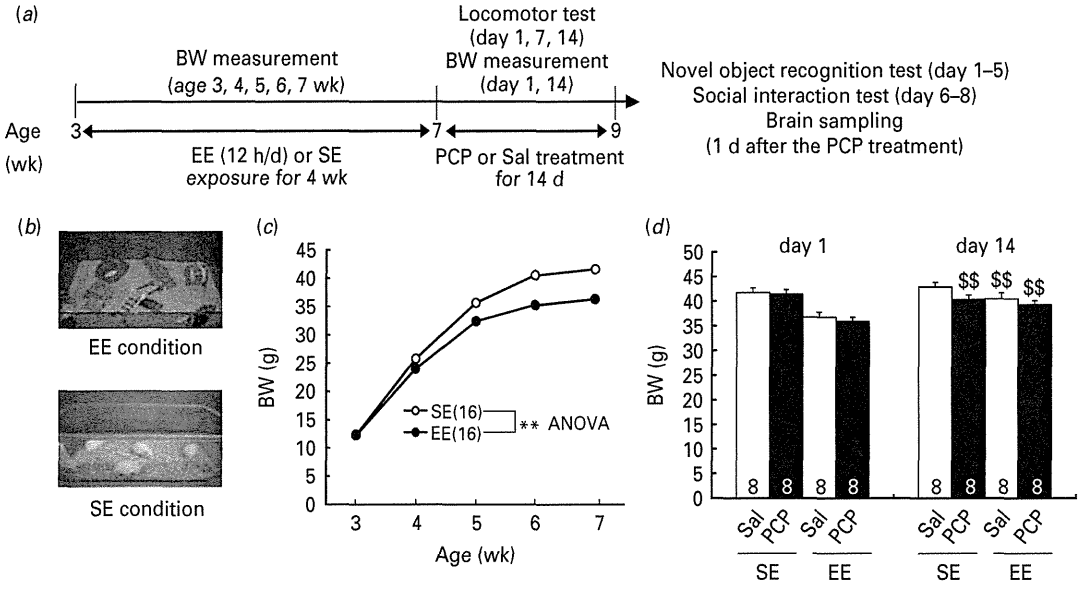

Fig. 1. Experimental design for the enriched environment (EE) exposure experiment and body weight (BW) changes. (*a*) Experimental design. Mice were exposed to EE (12 h/d) or standard environment (SE) conditions for 4 wk from age 3 wk and administered phencyclidine (PCP) (10 mg/kg.d s.c.) for 14 consecutive days until age 9 wk. Locomotor activity was measured on days 1, 7 and 14 of the PCP treatment (locomotor test). The novel object recognition and social interaction tests were conducted after the PCP treatment period. (*b*) EE and SE conditions. (*c*) Gain in BW during exposure to an EE or SE. The BWs of the mice were measured at age 3, 4, 5, 6 and 7 wk. Values indicate the means ± S.E. The number of mice is indicated in parentheses. Results of two-way repeated-measures analysis of variance (ANOVA) were environment: $F_{1,120}=71.28$, $p<0.01$; week: $F_{4,120}=966$, $p<0.01$; environment × week: $F_{4,120}=9.865$, $p<0.01$. (*d*) Gain in BW during the PCP treatment period. After exposure to the EE or SE, the mice were divided into PCP or saline (Sal) treatment groups and weighed on days 1 and 14 of the drug treatment period. Values indicate the means ± S.E. The number of mice is indicated within the columns. Results of two-way repeated-measures ANOVA were group: $F_{3,28}=5.444$, $p<0.01$; day: $F_{1,28}=36.68$, $p<0.01$; group × day: $F_{3,28}=13.56$, $p<0.01$. $^{\$\$}p<0.01$ compared to BW on day 1 in the same group.

novelty. The animals of the SE group ($n=8$) were housed in wire-topped clear plastic cages (27 × 44 × 18 cm). For the SB treatment experiment, the mice ($n=8$) were housed in cages similar to those for the SE group.

Locomotor test

Mice were habituated for 30 min in an apparatus consisting of transparent acrylic walls and a black frosting Plexiglas floor (45 × 26 × 40 cm). Immediately after the habituation, the mice were picked out and treated with PCP or Sal and returned to the apparatus. Locomotion was measured for 90 min using digital counters with infrared sensors (Scanet SV-10; Melquest Co. Ltd, Japan). For the locomotor test, the mice were prepared separately from that for the other behavioural and biochemical experiments. In these mice, the body weights were measured during the differential environment exposure or SB treatment and PCP treatment periods ($n=7$–8 in each group).

Social interaction test

The social interaction test was conducted as described previously (Qiao *et al.* 2001) with minor modifications. The used apparatus consisted of a square open arena (30 × 25 × 25 cm) with no top, made of grey non-reflecting acrylic. Before the test, each mouse (including unfamiliar partner mice) was placed alone in the apparatus for 10 min on two consecutive days (habituation). On the test day, each mouse was randomly assigned to a male ICR mouse of similar body weight, as an unfamiliar partner. The two mice were placed in the test box for 10 min and the time spent by the two in active social interaction with each other (social behaviour; such as sniffing and following the partner, mounting and crawling under/over the partner) was measured and recorded on video tape.

Novel object recognition test

The novel object recognition test was conducted as described previously (Nagai *et al.* 2009), with minor

modifications. The experimental apparatus consisted of a Plexiglas box (30 × 30 × 35 cm), the floor covered in sawdust. Each mouse was individually habituated to the box, being allowed 10 min of exploration in the absence of objects for three consecutive days (habituation session). On day 4, two novel objects were symmetrically placed on the floor of the box and each animal was allowed to explore the box for 10 min (training session). The objects differed in shape and colour, but were similar in size. An animal was considered as exploring the object when its head was facing the object or it was touching or sniffing the object. A period of 24 h after the training session, the mice were returned to the same box with one of the familiar objects from the training session and one novel object for 5 min (retention session) and the time that they spent exploring each object was recorded on videotape. A preference index, the ratio of the amount of time that an animal spent exploring any one of the two objects (training session) or the novel object (retention session) over the total time spent exploring both objects was used as a measure of cognitive function. The novel object recognition test was conducted after the social interaction test in the same mice (EE experiment: $n = 11$–14 in each group; SB treatment experiment: $n = 12$–15 in each group).

Immunohistochemical analysis

Following the chronic PCP treatment (1 d after), the mice were anaesthetized with ethyl carbamate (1.2 g/kg) and transcardially perfused with phosphate buffered Sal, followed by 4% paraformaldehyde. The brains were removed, post-fixed in the same fixative for 2 d and soaked in 30% (w/v) sucrose. Coronal sections 20 μm thick were cut with a cryostat (HM 560 M; Carl Zeiss, Germany) and incubated with 0.3% Triton X-100 in 0.1 M Tris-HCl buffer (pH 7.6), followed by rabbit anti-acetyl-histone H3 (Lys^9) antibody (1:500; Millipore Corporation, USA) for 24 h at 4 °C. The sections were then washed and incubated with biotinylated goat anti-rabbit antibody (1:1000; Vector Laboratories, USA) at room temperature for 2 h. Finally, the sections were washed and processed with avidin-biotinylated horseradish peroxidase complex (Vector ABC kit; Vector Laboratories) and the reaction was visualized using diaminobenzidine. To quantify the number of cells positive for histone H3 acetylated at Lys^9 in the brain, we examined the sections under a light microscope (HB050; Carl Zeiss) and photographed them with Axio Vision (Carl Zeiss). Three sequential sections for the PFC, located according to the atlas of Franklin & Paxinos (1997), were examined for counting of the acetylated histone H3-positive cells. In each section, we defined a region of interest, measuring 360 × 360 μm, using Win Roof software (Mitani Corp., Japan). The average of three determinations was used for the statistical analysis.

Nuclear extraction and Western blot analysis

Following completion of the chronic PCP treatment (1 d after), the mice were killed by decapitation and the brains immediately removed. The PFC was rapidly dissected out and the dissected brain tissue was homogenized in an ice-cold hypotonic buffer [10 mM Tris–HCl (pH 7.6), 25 mM KCl, 10 mM NaCl, 1 mM $MgCl_2$, 0.1 mM EDTA, 1 mM NaF, ~10 mM of protease inhibitors (complete Mini; Roche Diagnostics, Germany)] and centrifuged at 100 *g* for 10 min at 4 °C. The pellet was suspended in lysis buffer [50 mM Hepes (pH 7.6), 150 mM NaCl, 0.5% Triton-X, 10% glycerol, ~10 mM of protease inhibitors], then centrifuged at 15700 *g* for 60 min at 4 °C. The pellet was further sonicated in RIPA buffer [50 mM Hepes (pH 7.6), 150 mM NaCl, 1 mM NaF, 1% Triton-X, 0.1% SDS, 1% DOC, ~10 mM of protease inhibitors]. The final suspension was subjected to Western blotting as the nuclear fraction. Samples were separated on a polyacrylamide gel and subsequently transferred to polyvinylidene difluoride membranes (Millipore Corporation). The membranes were blocked with 3% BSA, probed with a primary antibody and then incubated with a horseradish peroxidase-conjugated secondary antibody. The immune complexes were detected by ChemiDoc XRS (Bio-Rad Japan, Japan) based on chemiluminescence (ECL Plus Western blotting detection system; GE Healthcare, UK). The density of the bands was analysed by densitometry using the ATTO Densitograph Software Library Lane Analyzer (Atto, Japan). The primary polyclonal rabbit antibodies used were anti-histone deacetylase 5 (1:1000; Cell Signaling Technology, USA), anti-histone deacetylase 1 (1:1000; Cell Signaling Technology) and anti-Lamin AC (1:1000; Cell Signaling Technology). The secondary antibody, used at a dilution of 1:2000, was horseradish peroxidase-linked anti-rabbit IgG (KPL, USA).

Statistical analysis

Results were expressed as the means ± S.E. for each group. The differences between groups were analysed with a two-way or two-way repeated-measures analysis of variance (ANOVA), followed by the *post-hoc* Student–Newman–Keuls test. $p < 0.05$ was regarded as statistically significant.

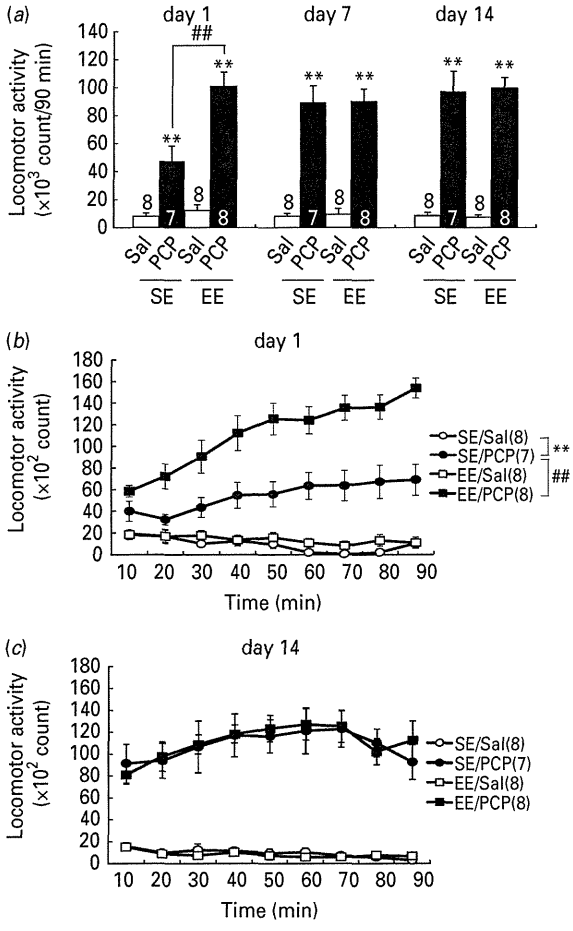

Fig. 2. Enriched environment (EE) exposure during adolescence enhanced hyperlocomotion induced by acute phencyclidine (PCP) treatment. Mice were treated with PCP for 14 consecutive days and locomotor activity was measured for 90 min on days 1, 7 and 14. Values indicate the means ± S.E. The number of mice is indicated on or within the columns for (*a*) and indicated in parentheses for (*b*) and (*c*). (*a*) Locomotor changes during the PCP treatment period. Results of two-way repeated-measures analysis of variance (ANOVA) were group: $F_{3,81}=47.09$, $p<0.01$; day: $F_{3,81}=4.661$, $p<0.01$; group × day interaction: $F_{9,81}=7.721$, $p<0.01$. ** $p<0.01$ compared to the results in the standard environment (SE)-exposed mice treated with saline (Sal) on the same day. ## $p<0.01$ compared to the results in the SE-exposed mice treated with PCP on day 1. Time-course of changes in the locomotor activity on day 1 (*b*) and day 14 (*c*). (*b*) Results of two-way repeated-measures ANOVA were group: $F_{3,216}=33.83$, $p<0.01$; time: $F_{8,216}=17.14$, $p<0.01$; group × time interaction: $F_{24,216}=16.02$, $p<0.01$. ** $p<0.01$ compared to the results in the SE-exposed mice treated with Sal at all-time points. ## $p<0.01$ compared to the results in the SE-exposed mice treated with PCP at 20–90 min. (*c*) Results of two-way repeated-measures ANOVA were group: $F_{3,216}=45.82$, $p<0.01$; time: $F_{8,216}=3.322$, $p<0.01$; group × time interaction: $F_{24,216}=2.043$, $p<0.01$. There were no differences between the SE-exposed and EE-exposed mice treated with PCP as determined by the *post-hoc* test.

Results

Exposure to EE decreased body weight

The schedule for the EE experiment is described in Fig. 1*a*. It has been reported that body weight after weaning was lower in the EE-exposed and exercised animals than in the SE-exposed and non-exercised animals (Meijer *et al.* 2007; Soares de Alencar Mota *et al.* 2008; Vieira *et al.* 2009). To confirm the effect of exposure of EE on body weight, the mice were weighed during the period of exposure to EE and PCP treatment. As shown in Fig. 1*c*, both the SE and EE groups of mice showed weight gain. The body weight of the latter, however, was lower (environment: $F_{1,120}=71.28$, $p<0.01$; age: $F_{4,120}=966$, $p<0.01$; environment × age: $F_{4,120}=9.865$, $p<0.01$, two-way repeated-measures ANOVA). During the PCP treatment period, body weight changed in both the SE- and EE-exposed group of mice (group: $F_{3,28}=5.444$, $p<0.01$; treatment period: $F_{1,28}=36.68$, $p<0.01$; group × treatment period, $F_{3,28}=13.56$, $p<0.01$, two-way repeated-measures ANOVA) (Fig. 1*d*). In the SE-exposed, but not the EE-exposed mice, repeated-dose PCP treatment decreased body weight ($p<0.01$ by *post-hoc* comparison).

Exposure to EE during adolescence enhanced acute PCP treatment-induced hyperlocomotion

Figure 2 shows the effect of exposure to EE during adolescence on the PCP-induced hyperlocomotion. On day 1, the SE group of mice treated with PCP showed hyperlocomotion compared to the animals treated with Sal (group: $F_{3,81}=47.09$, $p<0.01$; day: $F_{3,81}=4.661$, $p<0.01$; group × day interaction: $F_{9,81}=7.721$, $p<0.01$, two-way repeated-measures ANOVA and $p<0.01$ by *post-hoc* comparison) (Fig. 2*a*). The hyperlocomotion induced by acute PCP treatment was potentiated in the EE-exposed mice compared to that in the SE-exposed mice on day 1 ($p<0.01$ by *post-hoc* comparison) (Fig. 2*a*). The time course of changes in the locomotor activity from day 1 to day 14 is shown in Fig. 2*b*,*c*. The EE-exposed mice showed a greater degree of time-dependent PCP-induced hyperlocomotion than the SE-exposed mice on day 1 (group: $F_{3,216}=33.83$, $p<0.01$; time: $F_{8,216}=17.14$, $p<0.01$; group × time interaction: $F_{24,216}=16.02$, $p<0.01$, two-way repeated-measures ANOVA and $p<0.01$ by *post-hoc* comparison) (Fig. 2*b*). By day 14, there was no difference in the

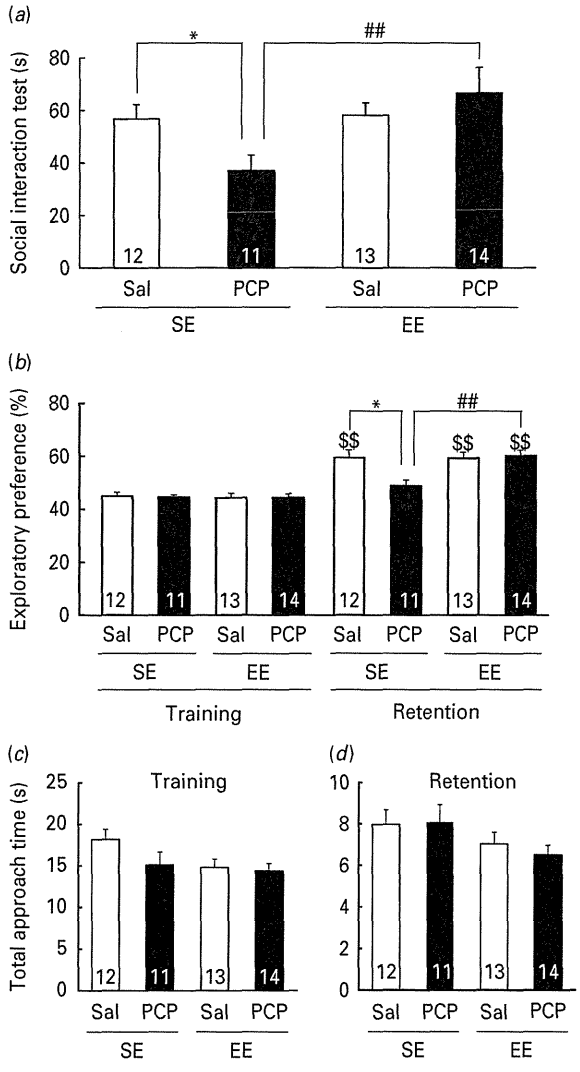

Fig. 3. Enriched environment (EE) exposure during adolescence prevented phencyclidine (PCP)-impaired sociality and objective recognition memory. (*a*) The time spent on social behaviour (sniffing, grooming, following, mounting and crawling) was measured for 600 s in the social interaction test. Values indicate the means ± S.E. The number of mice is indicated within the columns. Results of two-way analysis of variance (ANOVA) were environment: $F_{1,46}=6.368$, $p<0.05$; environment × drug treatment interaction: $F_{1,46}=5.447$, $p<0.05$. * $p<0.05$ compared to the results in the standard environment (SE)-exposed mice treated with saline (Sal). ## $p<0.01$ compared to the results in the SE-exposed mice treated with PCP. (*b*) Exploratory preference in the training and retention sessions in the novel object recognition test. The retention session (5 min) was carried out 24 h after the training session (10 min). Values indicate the means ± S.E. The number of mice is indicated within the columns. Results of two-way repeated-measures ANOVA were group: $F_{3,46}=2.327$, $p<0.05$; session: $F_{1,46}=95.46$, $p<0.01$; group × session interaction: $F_{3,46}=4.456$, $p<0.01$. * $p<0.05$ compared to the results in the SE-exposed mice treated with Sal in the retention session. ## $p<0.01$ compared to the results in the SE-exposed mice treated with PCP in the retention session. $$ $p<0.01$ compared to the findings during the training session in the same group. Total approach time for two objects in the training session (*c*) and retention session (*d*). Values indicate the means ± S.E. The number of mice is indicated within the columns.

degree of hyperlocomotion induced by PCP between the SE- and EE-exposed groups (Fig. 2*c*).

Exposure to EE during adolescence prevented PCP-impaired sociality and objective recognition memory

We examined, using the social interaction test, whether EE exposure during adolescence might prevent social impairment induced by repeated-dose PCP treatment (Fig. 3*a*). PCP treatment reduced the social interaction time in the SE-exposed mice, but not in the EE-exposed mice (environment: $F_{1,46}=6.368$, $p<0.05$; environment × drug treatment: $F_{1,46}=5.447$, $p<0.01$; two-way ANOVA and $p<0.05$, $p<0.01$ by *post-hoc* comparison). Next, using the novel object recognition test, we examined whether EE exposure might prevent the impairment of objective recognition memory induced by repeated-dose PCP treatment. PCP treatment significantly decreased the exploratory preference for a novel object in the retention session in the SE-exposed mice (group: $F_{3,46}=2.327$, $p<0.05$; session: $F_{1,46}=95.46$, $p<0.01$; group × session: $F_{3,46}=4.456$, $p<0.01$; two-way repeated-measures ANOVA and $p<0.05$ by *post-hoc* comparison) (Fig. 3*b*), suggesting impairment of objective recognition memory. No such impairment was observed in the EE-exposed mice ($p<0.01$ by *post-hoc* comparison) (Fig. 3*b*). The exploratory preference in the training session (Fig. 3*b*) and the total approach time for two objects in the training (Fig. 3*c*) and retention (Fig. 3*d*) sessions did not differ among the four groups.

Exposure to EE during adolescence prevented a decrease in the number of cells with histone H3 acetylated at Lys^9 and increase in HDAC5 protein expression induced by PCP in the PFC

Hypofrontality is a common pathological change in schizophrenia and PCP-treated animals (Molina *et al.* 2009; Mouri *et al.* 2007*b*; Murai *et al.* 2007; Pratt *et al.* 2008). Hypofrontality can alter transcription (or disturb protein synthesis), representing the cause, consequence or confounding effect of the disease process (Lehrmann *et al.* 2006; Lewis & Mirnics, 2006).