曽良一郎.	ドーパミン仮説. 現代精神医学事典	加藤敏, 神庭重信, 中谷陽二, 武田雅俊, 鹿島晴雄, 狩野力八郎, 市川宏伸編:773, 弘文堂, 東京	2011
曽良一郎.	モノアミン仮説. 現代精神医学事典	加藤敏, 神庭重信, 中谷陽二, 武田雅俊, 鹿島晴雄, 狩野力八郎, 市川宏伸編): 1021-1022, 弘文堂, 東京	2011
千葉大学大学院医学研究院 教授		伊豫 雅臣 (研究協力者 大宮宗一郎)	
Arakawa S et al.	Minocycline produced antidepressant-like effects on the learned helplessness rats with alterations in levels of monoamine in the amygdala and no changes in BDNF levels in the hippocampus at baseline.	Pharmacol Biochem Behav 100: 601-606. ㉜	2011
Tadokoro S, et al.	Fluvoxamine may prevent onset of psychosis: a case report of a patient at ultra-high risk of psychotic disorder.	Ann Gen Psychiatry 10:26	2011
Niitsu T, et al.	Associations of serum brain-derived neurotrophic factor with cognitive impairments and negative symptoms in schizophrenia.	Prog Neuropsychopharmacol. Biol Psychiatry 35(8): 1836-1840. ㉝	2011
Kobayashi H, et al.	Association analysis of the tryptophan hydroxylase 2 gene polymorphisms in patients with methamphetamine dependence / psychosis.	Curr Neuropharmacol 9(1): 176-82.	2011
Tsunoka T, et al.	No Association Between GRM3 and Japanese Methamphetamine-Induced Psychosis.	Curr Neuropharmacol 9(1): 160-162.	2011
Okahisa Y, et al.	Association between the regulator of G-protein signaling 9 gene and patients with Methamphetamine use disorder and Schizopherenia.	Curr Neuropharmacol 9(1): 190-194 ㉞	2011
Chen H, et al.	Protective effects of the antioxidant sulforaphane on behavioral changes and neurotoxicity in mice after the administration of methamphetamine.	Psychopharmacology (Berl) ㉟	In press
Fujita Y, et al.	The antibiotic minocycline prevents methamphetamine-induced rewarding effects in mice.	Pharmacol Biochem Behav. 101(2):303-306 ㊱	2012
東京都医学総合研究所 ・参事研究員		池田 和隆	
Sugaya N, et al.	Influence of GIRK channel inhibition on relapse risk in Japanese alcohol-dependent inpatients.	Jpn. J. Neuropsychopharmacol	In press
Ogai Y, et al.	Assessment of craving to predict relapse in patients with substance abuse/dependence.In: Advances in Psychology Research. Vol. 88.	Nova Science Publishers, New York	In press.

Berrocoso E, et al.	Active behaviours produced by antidepressants and opioids in the mouse tail suspension test.	Int J Neuropsychopharmacol. 5:1-12.	2012
井手聡一郎, 南雅文, 池田和隆	痛み感受性の遺伝的要因.	ペインクリニック. 33(1):67-74.	2012
Kobayashi T, et al.	Inhibition of G protein-activated inwardly rectifying K+ channels by different classes of antidepressants.	PLoS ONE. 6:e28208. ㊲	2011
Sugaya N, et al.	Family dysfunction differentially affects alcohol dependence and drug dependence: a view from the Addiction Severity Index in Japan.	Int J Environ Res Public Health. 8:3922-3937. ㊳	2011
Fukuda K, et al.	Diversity of opioid requirements for postoperative pain control following oral surgery - Is it affected by polymorphism of the mu-opioid receptor?	Anesth Prog. 57:145-149.	2011
Han W, et al.	Inhibitory role of inducible cAMP early repressor (ICER) in methamphetamine-induced locomotor sensitization.	PLoS ONE. 6:e21637. ㊴	2011
Ide S, et al.	(-)-Pentazocine induces visceral chemical antinociception, but not thermal, mechanical, or somatic chemical antinociception, in mu-opioid receptor knockout mice.	Molecular Pain. 7:23.	2011
Kobayashi T, et al.	Inhibition of G protein-activated inwardly rectifying K+ channels by phencyclidine.	Curr Neuropharmacol. 9(1):244-246.	2011
Takamatsu Y, et al.	The selective serotonin reuptake inhibitor paroxetine, but not fluvoxamine, decreases methamphetamine conditioned place preference in mice.	Curr Neuropharmacol. 9(1):68-72. ㊵	2011
Hagino Y, et al.	Effects of MDMA on extracellular dopamine and serotonin levels in mice lacking dopamine and/or serotonin transporters.	Curr Neuropharmacol. 9(1):91-95.	2011
Takamatsu Y, et al.	Enhanced hyperthermia induced by MDMA in parkin knockout mice.	Curr Neuropharmacol. 9(1):96-99.	2011
Nishizawa D, et al.	Identification of selective agonists and antagonists to G protein-activated inwardly rectifying potassium channels: candidate medicines for drug dependence and pain.	Curr Neuropharmacol. 9(1):113-117.	2011
Kasai S, et al.	Quantitative detection of mu opioid receptor: Western blot analyses using mu opioid receptor knockout mice.	Curr Neuropharmacol. 9(1):219-222.	2011
Yamamoto H, et al.	MOP reduction during long-term methamphetamine withdrawal was restored by chronic post-treatment with fluoxetine.	Curr Neuropharmacol. 9(1):73-78.	2011

Aoki J, et al.	Association study on catechol-O-methyltransferase (COMT) Val158Met gene polymorphism and NEO-FFI.	Psychiatry Res. 187:312-313.	2011
Kasai S, et al.	Pharmacogenomics of the human mu-opioid receptor.	Pharmacogenomics. 12:1305-1320. ㊶	2011
菅谷渚, 池田和隆	報酬系におけるGIRKチャネルの役割.	日本生物学的精神医学会誌. 22(4):263-268.	2011
笠井慎也, ほか	野生由来近交系マウス系統におけるOprm1 遺伝子多型とモルヒネ感受性の関連性.	日本神経精神薬理学雑誌. 31:87-88.	2011
池田和隆	依存性薬物作用の解明が拓く新しい精神医学.	精神医学. 53(12):1189-1194.	2011
池田和隆	脳内報酬系の分子メカニズム.	日本神経精神薬理学雑誌 31:263-266.	2011
Han W, et al.	Reduced locomotor sensitization induced by methamphetamine and altered gene expression in ICER overexpressing mice.	日本神経精神薬理学雑誌. 31:79-80.	2011
Ogai Y,et al.	Influence of GIRK channel inhibition on alcohol abstinence and relapse risk in Japanese alcohol-dependent outpatients.	日本神経精神薬理学雑誌. 31:95-96.	2011
特許	池田和隆, 西澤大輔, 福田謙一 各種鎮痛関連遺伝子解析による薬物感受性の評価方法 特願 2011-288940 2011年12月 28日【出願】		
	池田和隆, 笠井慎也, 林田眞和, 樋口進 POMC遺伝子解析による薬物感受性の評価方法 European Patent Office, 109055 2011年10月17日【成立】 (20080425 国内PCT出願:PCT-JP2008-058083) (20091023 国内特願:2009-511918)		
	池田和隆, 西澤大輔, 福田謙一 サイクリックAMP応答配列結合タンパク質遺伝子解析による薬物感受性および疾患脆弱性の評価方法 特願 2011-217104 2011年9月30日【出願】		
	池田和隆, 西澤大輔, 福田謙一 アドレナリン受容体遺伝子解析による薬物感受性および疾患脆弱性の評価 特願 2011-191861 2011年9月2日【出願】		

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 13 000 *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

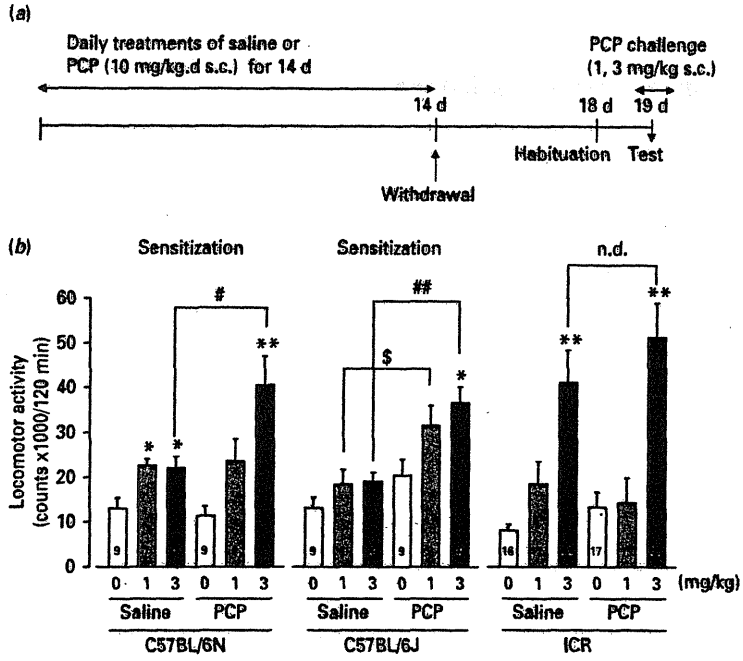

Fig. 1. Supersensitivity in hyperlocomotion induced by low doses of phencyclidine (PCP) was observed in C57BL/6N and C57BL/6J but not ICR strains 5 d after withdrawal from chronic PCP treatment. (*a*) Experimental design. One day after habituation, a locomotor activity test was performed 5 d after cessation 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,

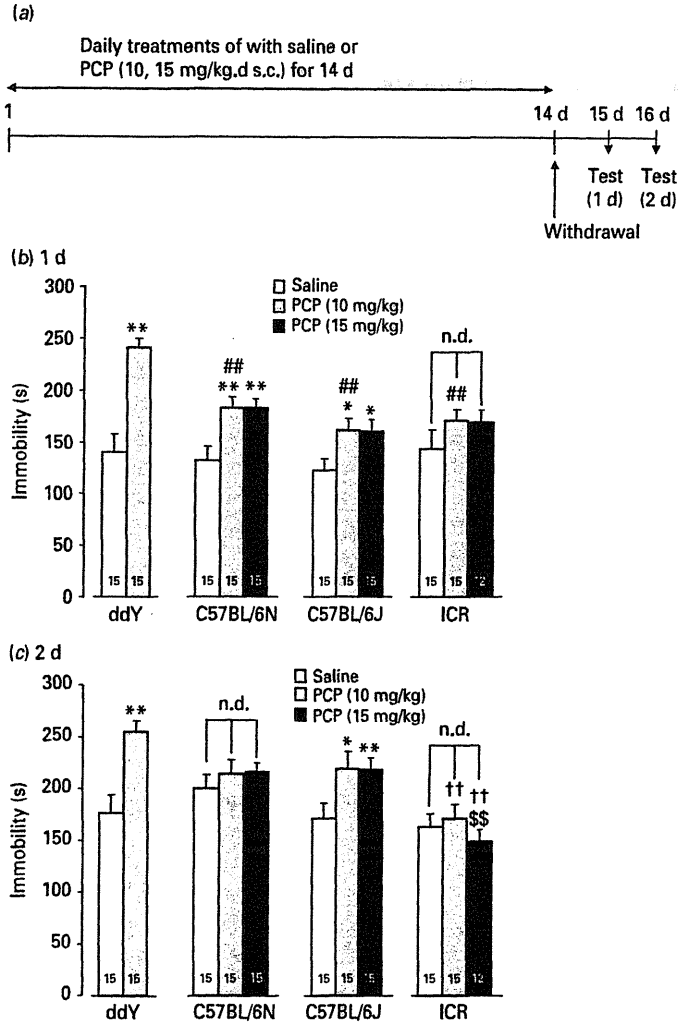

Fig. 2. Enhanced immobility was observed in ddY, C57BL/6N, and C57BL/6J but not ICR strains in the forced swim test 1 d after withdrawal from chronic phencyclidine (PCP) treatment. (*a*) Experimental design. Mice were sequentially subjected to forced swim tests 1 and 2 d after the final PCP treatment. (*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.

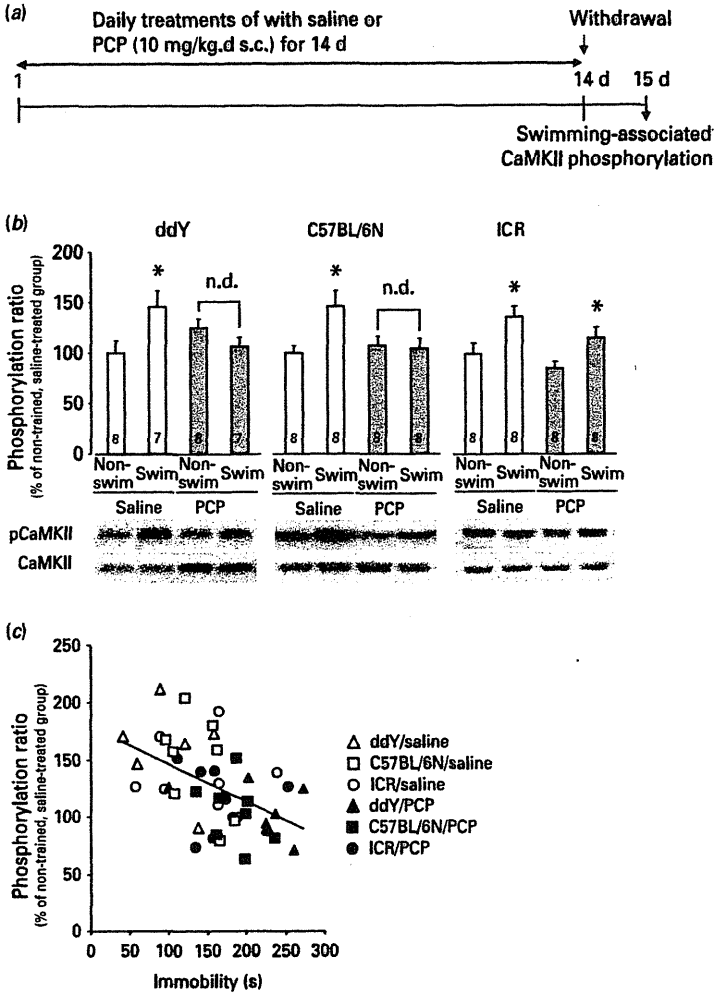

Fig. 3. Impairment of swimming-associated CaMKII phosphorylation in the frontal cortex of mice showing enhanced immobility in the forced swim test after withdrawal from chronic phencyclidine (PCP) treatment. (*a*) Experimental design. A forced swim test was performed 1 d after cessation of chronic PCP 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

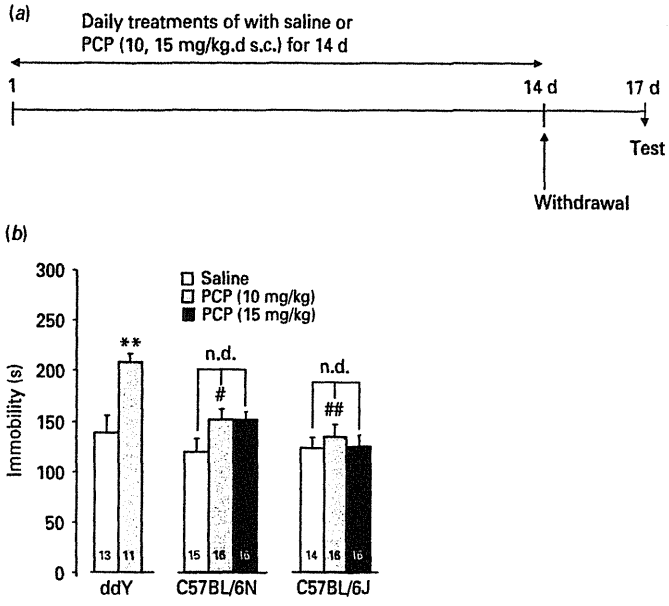

Fig. 4. Enhancement of immobility in the forced swim test was observed in ddY but not C57BL/6N and C57BL/6J strains 3 d after withdrawal from chronic phencyclidine (PCP) treatment. (*a*) Experimental design. Mice were individually subjected to a forced swim test 3 d after the final PCP treatment, and 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: $F_{3,57}=12.37$,

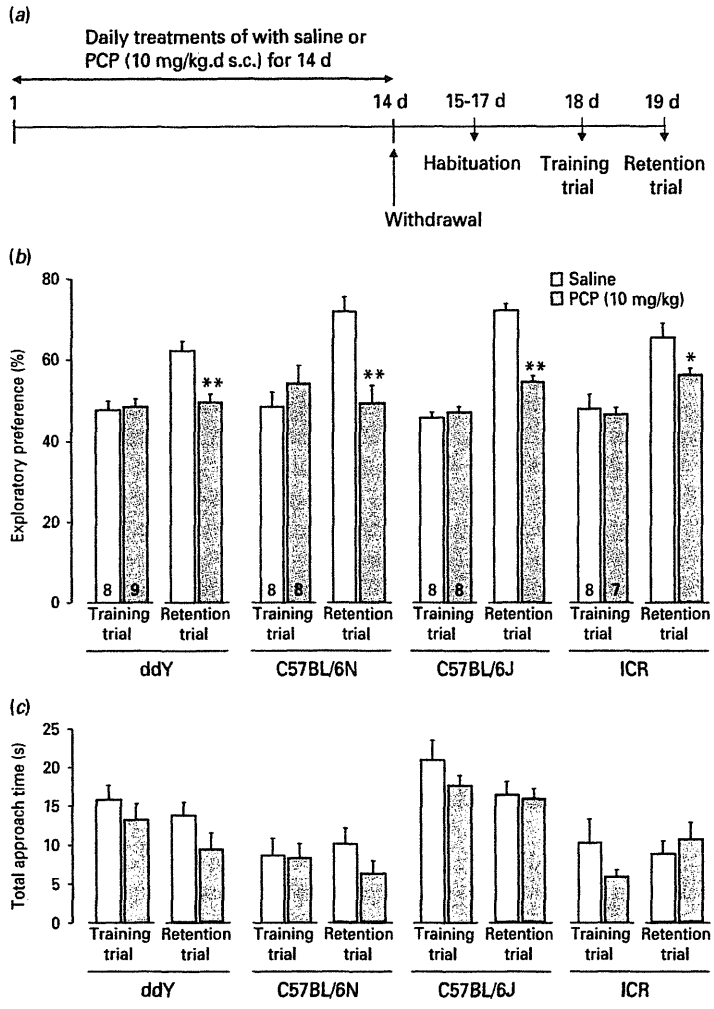

Fig. 5. No strain difference in the impairment of object recognition memory in the novel object recognition test after withdrawal from chronic phencyclidine (PCP) administration. (*a*) Experimental design. Mice were habituated to the apparatus for 3 d and then subjected to a novel object recognition 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.

Behavioural Brain Research 220 (2011) 132–139

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Prenatal exposure to PCP produces behavioral deficits accompanied by the overexpression of GLAST in the prefrontal cortex of postpubertal mice

Lingling Lu[a,b], Takayoshi Mamiya[a], Ping Lu[a,b], Kazuya Toriumi[a], Akihiro Mouri[a,c], Masayuki Hiramatsu[a], Li-Bo Zou[b], Toshitaka Nabeshima[a,d,*]

[a] Department of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Meijo University, Nagoya 468-8503, Japan
[b] Department of Pharmacology, School of Life Science and Biopharmaceutics, Shenyang Pharmaceutical University, Shenyang 110016, China
[c] Department of Neuropsychopharmacology and Hospital Pharmacy, Nagoya University Graduate School of Medicine, Nagoya 466-8560, Japan
[d] Japanese Drug Organization for Appropriate Use and Research, Nagoya 468-0069, Japan

ARTICLE INFO

Article history:
Received 12 November 2010
Received in revised form 13 January 2011
Accepted 19 January 2011

Keywords:
Prenatal PCP
Behavior
GLAST
Glutamate transmission
Prefrontal cortex
Mice

ABSTRACT

Altered glutamatergic neurotransmission in the prefrontal cortex (PFC) has been implicated in a myriad of neuropsychiatric disorders. We previously reported that prenatal exposure to PCP produced long-lasting behavioral deficits, accompanied by the abnormal expression and dysfunction of NMDA receptors. In addition, these behavioral changes were attenuated by clozapine treatment. However, whether the prenatal exposure adversely affects pre-synaptic glutamatergic neurotransmission in postpubertal mice remains unknown. In the present study, we investigated the involvement of prefrontal glutamatergic neurotransmission in the impairment of cognitive and emotional behavior after prenatal PCP treatment (5 mg/kg/day) from E6 to E18 in mice. The PCP-treated mice showed an impairment of recognition memory in a novel object recognition test and enhancement of immobility in a forced swimming test at 8 weeks of age. Moreover, the prenatal treatment reduced the extracellular glutamate level, but increased the expression of a glial glutamate transporter (GLAST) in the PFC. The microinjection of DL-threo-β-benzyloxyaspartate (DL-TBOA, 10 nmol/site/bilaterally), a potent blocker of glutamate transporters, reversed these behavioral deficits by enhancing the prefrontal glutamatergic neurotransmission. Taken together, prenatal exposure to PCP produced impairments of long-term memory and emotional function which are associated with abnormalities of pre-synaptic glutamate transmission in the PFC of postpubertal mice. These findings suggest the prenatal inhibition of NMDA receptor function to contribute partly to the pathophysiology of neurodevelopment-related disorders, such as schizophrenia.

1. Introduction

Disruption of the brain's development at an early stage can potentially alter neural networks and may increase the risk for neuropsychiatric disorders in later life. According to the neurodevelopmental hypothesis, disruption of the developing brain predisposes the neural systems to long-lasting structural and functional abnormalities, leading to the emergence of psychopathological behavior in adulthood [3].

NMDA receptor plays a critical role in neuronal development [10]. The stimulation of NMDA receptors during development is critical for the survival, differentiation and migration of immature neurons [4,20], controls structure and plasticity [40], and establishes normal neural networks in the developing brain [12]. On the other hand, pharmacological inhibition of NMDA receptors at an early stage disturbs neural function in development [6,13,22].

The blockade of NMDA receptors with phencyclidine (PCP), a noncompetitive antagonist, produces a transient state of psychosis and schizophrenia-like deficits in normal subjects and exacerbates several symptoms in schizophrenia patients [18]. Moreover, PCP elicited a prolonged recrudescence of the acute psychotic state in patients with stable chronic schizophrenia, suggesting that a similar mechanism is compromised [21]. These observations, along with the finding of reduced glutamate levels in the cerebrospinal fluid of schizophrenic patients [19], form the basis of the glutamatergic hypofunction hypothesis of schizophrenia.

According to this hypothesis, PCP is widely used to produce abnormal behavior and biochemical changes resembling the positive symptoms, negative symptoms, and cognitive deficits of patients with schizophrenia [32,33,38]. Although a series of schizophrenia-like symptoms are observed in PCP-treated adult

* Corresponding author at: Department of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Meijo University, 150 Yagotoyama, Tenpaku-ku, Nagoya 468-8503, Japan. Tel.: +81 52 839 2735; fax: +81 52 839 2738.
E-mail addresses: tnabeshi@meijo-u.ac.jp, tnabeshi@ccmfs.meijo-u.ac (T. Nabeshima).

0166-4328/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2011.01.035

rodents, this animal model is unlikely to completely resemble the pathogenesis of schizophrenia, since at least in some cases, the pathologic abnormalities occur during development and are initiated by prenatal insults [35,39]. Therefore, it is necessary to consider the process by which the symptoms of schizophrenia develop. Based on the neurodevelopment hypothesis of psychiatric disorders, several studies have modified the classic "PCP-based animal model", with treatment using NMDA antagonists during the early development of the brain [1,12,44,49,50]. Moreover, one recent study has revealed that postnatal, but not adulthood, NMDA receptor ablation in the corticolimbic interneurons confers schizophrenia-like phenotypes in adult mice [5].

Our previous study confirmed that prenatal exposure to PCP (20 mg/kg) produced a cognitive deficit and hypersensitivity to PCP in terms of locomotor activity, which was associated with abnormal expression of the NMDA receptor [27]. Furthermore, these behavioral changes were attenuated by clozapine [27], an atypical antipsychotic that enhanced the function of glutamatergic transmission [9,30] and inhibited the up-regulation of glutamate transporters [29,48]. These findings suggest that the pre-synaptic glutamatergic system is involved in the behavioral deficits induced by prenatal PCP treatment. However, little attempt has been made to investigate the role of pre-synaptic glutamate transmission in this model. In this study, we evaluated the long-term effects of prenatal exposure to PCP on the prefrontal glutamatergic neurotransmission in mice.

2. Materials and methods

2.1. Animals

ICR female and male mice (8 weeks old) were obtained from Japan SLC (Shizuoka, Japan) and maintained on a 12/12 h, light/dark cycle (lights on from 08:00 to 20:00) with free access to food (CE2; Clea Japan Inc., Tokyo, Japan) and water. The mice were mated one pair per cage. Females were checked in the next morning and those with an embolus in their vaginas were considered pregnant [embryonic day 0 (E 0)]. The dams were randomly divided into saline- (SAL) and PCP-treated groups. All were housed individually till parturition. There were no maternal deaths and resorption or stillbirths caused by exposure to PCP in this study. At birth [postnatal day 0 (PD 0)], pups were culled to 8–10 per litter with a balance of males and females as possible. Maternal care behavior during feeding was monitored till weaning on PD 21. After weaning, pups given the same prenatal treatment were mixed by gender, and then randomly assigned into groups to do behavioral tests. Each behavioral test was involved 2–3 litters each time and repeated more than 3 times by using different mice to reduce the influence of litters. Moreover, a balanced number of males and females were used in each experiment, since no significant differences were observed between genders in our preliminary study [27].

The experiments with offspring were started at the age of 8 weeks and carried out in a sound-attenuated and air-conditioned room (23 ± 1 °C, 50 ± 5% humidity). The mice were habituated to the room for more than 40 min before behavioral experiments. All the behavioral tests were recorded with a digital versatile disc camera to analyze 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).

2.2. Drugs

PCP hydrochloride was synthesized according to the method of [28] and checked for purity. PCP was dissolved in saline. DL-threo-β-benzyloxyaspartate (DL-TBOA, Tocris, MO) was prepared as a stock solution of 100 mM in 50% dimethyl sulfoxide (DMSO) and 100 mM NaOH, and diluted with artificial cerebrospinal fluid (aCSF) before the behavioral test, or with Ringer's solution before the microdialysis analysis [34].

2.3. Drug treatment

The dams were administered SAL or PCP (5 mg/kg, s.c.) once daily at 18:00 from E 6 to E 18, the middle and late stages of pregnancy, covering the entire period of neurodevelopment in the prenatal brain from neurulation to corticogenesis [45]. The injection by s.c. was performed gently as possible to minimize potential stress-related influences on dams. The dose of PCP (5 mg/kg) was selected according to one study [44], since it was not toxic enough to affect the sensitization of dopaminergic system or tolerance of serotonergic ataxic behaviors in adults [25].

Under light anesthesia with diethyl ether, mice received an microinjection of DL-TBOA (1 or 10 nmol/μl/site; bilaterally) into the PFC [Anteroposterior (AP): +1.7 mm from Bregma; Mediolateral (ML): ±0.5 mm from Bregma; Dorsoventral (DV): +2 mm from the skull] according to the mouse brain atlas of [15], 30 min before each behavioral test [34]. DL-TBOA was injected over a period of 30 s, and the injector was left in the place for 1 min to allow diffusion. For the analysis of microdialysis, according to the effective dose observed in the behavioral tests, DL-TBOA (1 mM) was administered through the dialysis probe at a rate of 1 μl/min for a total of 30 min.

2.4. Novel object recognition test

The novel object recognition test was performed as described previously [27]. 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 days (habituation session). During the training session, two objects (a red painted triangular prism and a yellow painted cube) were symmetrically fixed to the floor of the box, 8 cm from the walls, and each animal was allowed to explore in 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 less than 2 cm and/or touching it with the 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 placed back into 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 the objects, was used to assess cognitive function.

2.5. Forced swimming test

The forced swimming test was done according to a previous report [34], with a minor modification. Mice were placed in a transparent glass cylinder (20 cm high, 15 cm in diameter), which contained water at 22 °C to a depth of 11.5 cm, and forced to swim for 6 min. The duration of swimming was measured by using a SCANET MV-10 AQ apparatus (Melquest Co. Ltd., Toyama, Japan). Immobility time was calculated as: immobility time (s) = 360 − swimming time.

2.6. Microdialysis analysis

Different mice from behavioral tests above were used for the following microdialysis analysis. Mice were anesthetized with pentobarbital-Na (50 mg/kg, i.p.) and fixed in a stereotaxic apparatus (David Kopf Instruments, CA). A dialysis probe (D-I-6-01; EICOM, Kyoto, Japan) was implanted into the PFC [AP: +1.7, ML: −0.05 from bregma, DV: −2 mm from the skull]. Twenty-four hours later, Ringer's solution (147 mM, NaCl, 4 mM KCl, and 2.3 mM $CaCl_2$) was perfused at a flow rate of 1 μl/min. Dialysate was collected every 10 min and the amount of glutamate was determined by an HPLC system (HTEC-500, EICOM) with electrochemical detection (ECD). For depolarization, 100 mM KCl-containing Ringer's solution (51 mM NaCl, 100 mM KCl, and 2.3 mM $CaCl_2$) was delivered through the dialysis probe for 30 min. Then, dialysate was collected for 90 min with Ringer's solution. For the rescue with DL-TBOA, after the collection of baseline fractions, 10 nmol of DL-TBOA dissolved in 10 μl of Ringer's solution was injected through the probe through the microinjection tube for 30 min.

2.7. Preparation of brain slice and staining

Histological procedures were performed as previously described with a minor modification [34]. Mice were anesthetized with chloral hydrate (200 mg/kg i.p.) and perfused transcardially with ice-cold phosphate-buffered saline (PBS), followed by 4% paraformaldehyde in PBS and then soaked in 10–30% (w/v) sucrose in PBS. Coronal sections 20 μm thick were cut with a cryostat (CM 1850; Leica, Germany). Cresyl violet staining was performed and the areas of brains and ventricles, and the number of neurons with a visible nucleus in the layers II/III of the prelimbic area were counted using computer-based image analysis system (WinRoof, Mitani, Japan). Images were acquired with a microscope (BZ-9000, Keyence, JP).

2.8. Western blotting analysis

Western blotting was performed as described previously with a minor modification [34]. The PFC including the cingulate and prelimbic area (Bregma +2.96 to Bregma +1.34) was rapidly dissected out, frozen, and stored at −80 °C prior to assays. The brain samples were homogenized in ice-cold buffer (50 mM Tris–HCl, pH 7.5; 150 mM NaCl; 10 mM NaF; 10 mM EDTA, 1% NP-40; 1 mM sodium orthovanadate; 10 mM sodium pyrophosphate; 0.5 mM DTT; 0.2 mM PMSF; 4 μg/ml pepstatin, 4 μg/ml aprotinin, and 4 μg/ml leupeptin). The lysate was centrifuged at 8000 × g for 10 min at 4 °C, and the suspension of precipitate was used.

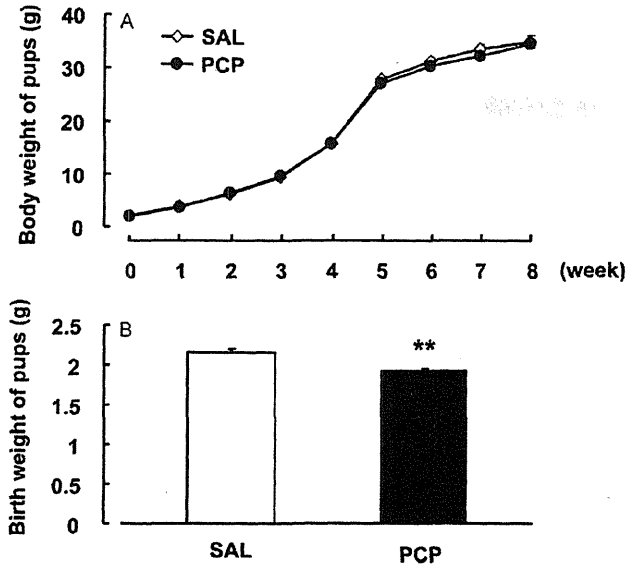

Fig. 1. Effects of prenatal PCP treatment on body weight of pups. The body weight of pups during the developing period from birth to 8 weeks old (A) (repeated one-way ANOVA with Bonferroni's test); the birth weight of pups at PD1 (B) (Student's *t*-test). $^{**}P<0.01$ compared with the prenatal SAL-treated group. Data are expressed as the mean ± S.E.M for 36–38 mice. SAL, saline; PCP, phencyclidine.

The protein concentrations were determined using a Pierce BCA Protein Assay Kit (Thermo Fishers, CA, USA). Samples were boiled at 95 °C for 5 min in 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, MA, USA). The membranes were blocked with a Detector Block Kit (Kirkegaard and Perry Laboratories, MD, USA) and probed with primary anti-GLAST, anti-GLT-1 (1:1000; Upstate Biotechnology, NY, USA), and anti-GFAP (1:1000; Upstate Biotechnology) antibodies. 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 and Perry Laboratories). Immunoreactive complexes on the membrane were detected using Western blotting detection reagents (GE Healthcare Biosciences, NJ, USA). The intensity of bands was detected by densitometry using ChemiDoc system and Quantity One Software (Bio-Rad, Hercules, CA, USA). After the GLAST, GLT-1 and GFAP proteins were 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 the expression of β-actin was detected with a primary anti-actin antibody (1:1000; Santa Cruz Biotechnology, CA, USA) as described above.

2.9. Statistical analysis

All data were expressed as the mean ± S.E.M. Significant differences between two groups were determined with Student's *t*-test. That among three groups or more was determined using a one- or two-way analysis of variance (ANOVA), or repeated one- or two way ANOVA, respectively, followed by Bonferroni's test ($P<0.05$).

3. Results

3.1. Effect of prenatal PCP treatment on body weight during development

To confirm the effects of prenatal PCP administration on development, the body weight of pups was observed throughout the development. As shown in Fig. 1, there were no significant differences between SAL- or PCP-treated offspring from birth to the age of 8 weeks old, when the behavioral tests were carried out ($F_{group\,(1,576)}=1.58$, $P>0.05$; $F_{week\,(8,576)}=2584.08$, $P<0.01$; $F_{group\times week\,(8,576)}=1.24$, $P>0.05$; repeated one-way ANOVA; Fig. 1A). These results suggested that prenatal PCP treatment did not affect the growth of body weight in pups, although there is a significant decrease in the weight at birth (SAL-treated mice, 2.15 ± 0.04 g; PCP-treated mice, 1.93 ± 0.03 g; $P<0.01$, $n=36$–38; Student's *t*-test; Fig. 1B).

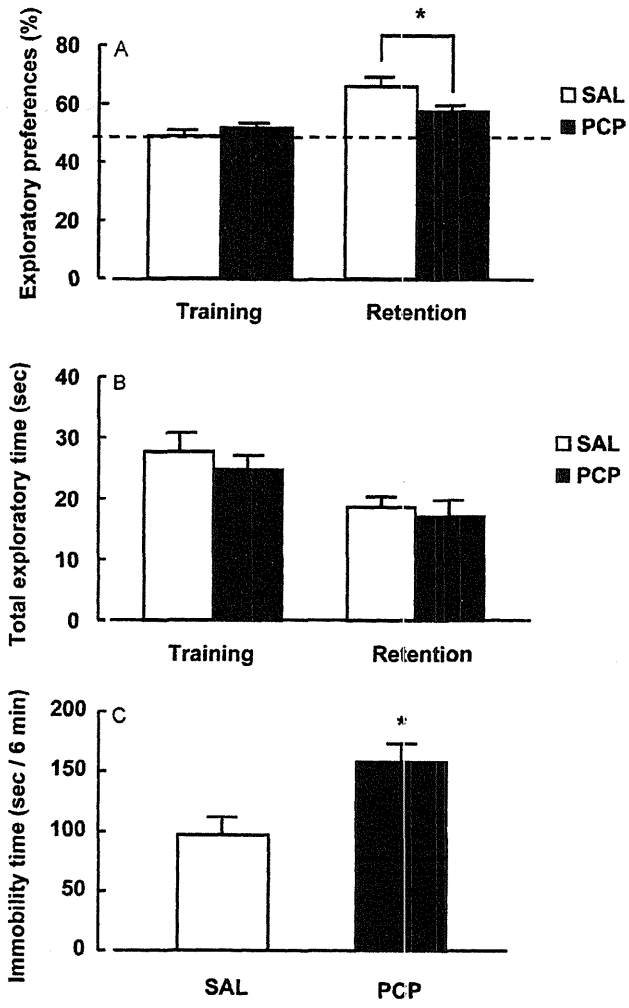

Fig. 2. Influences of prenatal PCP treatment on cognitive and emotional behavior in offspring at 8 weeks old. Exploratory preference (%) (A); and total time spent exploring the objects (s) (B) in training and retention sessions of the novel object recognition test. In the forced swimming test, immobility time (s) was assessed for 6 min (C). $^{*}P<0.05$ compared with the prenatal SAL-treated group. Data are expressed as the mean ± S.E.M. for 10–14 mice in each group (Student's *t*-test). SAL, saline; PCP, phencyclidine.

3.2. Abnormal cognitive and emotional behavior in prenatal PCP-treated mice

To investigate effects of prenatal PCP treatment on cognitive function, we performed a novel object recognition test. In the training session, the prenatal SAL- or PCP-treated mice spent almost equal amounts of time exploring either of the two objects, and there was no biased exploratory preference in each group of mice (SAL-treated mice, 49.05 ± 1.82%; PCP-treated mice, 51.76 ± 1.49%; $P>0.05$, $n=10$–14; Student's *t*-test; Fig. 2A). In addition, the total time spent on the exploration of objects in the training session did not differ between the two groups (SAL-treated mice, 27.65 ± 3.05 s; PCP-treated mice, 24.89 ± 2.38 s; $P>0.05$, $n=10$–14; Student's *t*-test; Fig. 2B). In the retention session, the PCP-treated mice showed a reduced level of exploratory preference for the novel objects compared with the SAL-treated group (SAL-treated mice, 65.95 ± 3.14%; PCP-treated mice, 57.20 ± 2.33%; $P<0.05$,

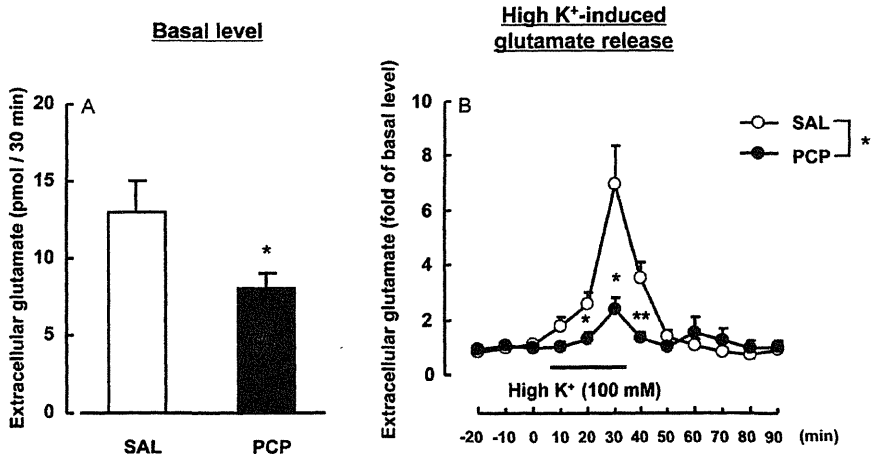

Fig. 3. Basal extracellular glutamate levels and high K^+-evoked glutamate release in the prefrontal cortex of prenatal PCP-treated mice. Basal extracellular levels of glutamate (A) and high K^+ (100 mM)-evoked glutamate release (B) in the prefrontal cortex of prenatal SAL- or PCP-treated mice were determined by microdialysis. Data are expressed as the mean ± S.E.M. for 7 mice in each group. *$P<0.05$, **$P<0.01$ compared with the prenatal SAL-treated group (Student's *t*-test or repeated one-way ANOVA with Bonferroni's test).

$n=10$–14; Student's *t*-test; Fig. 2A). However, no significant difference was observed in the total exploration time (SAL-treated mice, 18.74 ± 1.72 s; PCP-treated mice, 17.08 ± 2.97 s; $P>0.05$, $n=10$–14; Student's *t*-test; Fig. 2B).

To investigate effects of prenatal exposure to PCP on emotional behavior, we performed the forced swimming test. The PCP-treated mice showed significantly prolonged immobility throughout the 6-min test, compared with the SAL-treated mice (SAL-treated mice, 96.8 ± 13.48 s; PCP-treated mice, 157.47 ± 15.69 s; $P<0.05$, $n=10$–14; Student's *t*-test; Fig. 2C), which implied that emotional deficits were induced by the prenatal exposure.

3.3. Reduced glutamate release in the prefrontal cortex of prenatal PCP-treated mice

To investigate whether pre-synaptic glutamatergic neurotransmission was adversely affected by prenatal exposure to PCP, we examined extracellular glutamate levels in the PFC using microdialysis. When the extracellular levels reached a steady state without any treatment, the basal release of glutamate was monitored for 30 min. The prenatal PCP-treated mice showed a dramatically reduced level of extracellular glutamate in the PFC, compared with the SAL-treated mice (SAL-treated mice, 12.97 ± 1.96 pmol; PCP-treated mice, 8.06 ± 0.95 pmol; $P<0.05$, $n=7$; Student's *t*-test; Fig. 3A). Next, we evaluated the glutamate release induced by high potassium (high K^+, 100 mM). The high K^+ treatment increased the release of extracellular glutamate in both the SAL- and PCP-treated mice. However, the increase was significantly lower in the PCP-treated mice ($F_{\text{treatment}(1,12)}=20.63$, $P<0.01$; $F_{\text{time}(4,12)}=11.11$, $P<0.01$; $F_{\text{treatment}\times\text{time}(4,12)}=3.93$, $P<0.01$; repeated one-way ANOVA for 10–50 min; Fig. 3B).

3.4. Overexpression of glutamate transporters in the prefrontal cortex of prenatal PCP-treated mice

To further examine whether the decrease in the extracellular glutamate level was due to changes to glutamate transporters, we investigated the protein expression of GLAST and GLT-1 in the PFC by Western blotting. The level of GLAST protein was significantly higher in the PCP-treated mice than SAL-treated mice (SAL-treated mice, 100 ± 10.24%; PCP-treated mice, 135.18 ± 9.98%; $P<0.05$, $n=6$–7; Student's *t*-test; Fig. 4A). However, we did not observe a significant difference in GLT-1 expression between the groups (SAL-treated mice, 100 ± 12.49%; PCP-treated mice, 121.98 ± 12.29%; $P>0.05$, $n=6$–7; Student's *t*-test; Fig. 4B). We further examined the level of GFAP expression, a marker of glial cell, by Western blotting. There was no significant difference in GFAP expression between the prenatal SAL- and PCP-treated mice (SAL-treated mice, 100 ± 5.19%; PCP-treated mice, 104.38 ± 6.72%; $P>0.05$, $n=6$–7; Student's *t*-test; Fig. 4C), suggesting that the increased expression of GLAST was not directly due to increasing numbers of glial cells in the PFC of mice.

3.5. The neuronal number in the prefrontal cortex of prenatal-PCP treated mice

To further investigate whether the reduced glutamate release was due to the decrease of number of neurons in adult mice, we examined morphological changes of neurons in the PFC by Cresyl violet staining. As shown in the results, there was only a tendency to decrease in the number of neurons in the layer II/III of the PFC at PD 56, but not significant difference regardless of prenatal PCP treatment (SAL-treated mice, 2247 ± 117; PCP-treated mice, 1925 ± 116; $P=0.08$, $n=6$; Student's *t*-test; Supplementary Fig. 1A and B). It suggested that the decrease in extracellular glutamate level might be not due to the changes of number of neurons in adult.

3.6. Effects of a potent glutamate transporter inhibitor on behavioral deficits and the impairment of glutamate release in prenatal PCP-treated mice

To further investigate whether the prenatal PCP-induced behavioral changes and glutamatergic dysfunction were associated with the overexpression of glutamate transporters, we examined the effects of a potent inhibitor of glutamate transporters, DL-TBOA. In the PCP-treated mice, DL-TBOA (10 nmol) attenuated the impairment of recognition memory in the novel recognition test. There was no biased exploratory preference ($F_{\text{group}(1,40)}=0.17$, $P>0.05$; $F_{\text{treatment}(2,40)}=1.29$, $P>0.05$; $F_{\text{group}\times\text{treatment}(2,40)}=0.16$, $P>0.05$; two-way ANOVA; Fig. 5A), and no difference in total exploration time between the two groups in the training session ($F_{\text{group}(1,40)}=0.61$, $P>0.05$; $F_{\text{treatment}(2,40)}=2.71$, $P>0.05$; $F_{\text{group}\times\text{treatment}(2,40)}=0.57$, $P>0.05$; two-way ANOVA; Fig. 5B). In the retention session, the impairment of recognition memory in the PCP-treated mice was significantly improved by the