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

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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 Lys9 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 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. 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.

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...
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. 1b) 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
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.
modifications. The experimental apparatus consisted of a Plexiglas box (30 x 30 x 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 carbaminate (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, 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 MgCl₂, 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 fluoride 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.
Results

Exposure to EE decreased body weight

The schedule for the EE experiment is described in Fig. 1a. 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. 1c, both the SE and EE groups of mice showed weight gain. The body weight of the latter, however, was lower (environment: $F_{1,28} = 71.28, p < 0.01$; age: $F_{4,120} = 96.6$, $p < 0.01$; 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. 1d). 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: $F_{3,30} = 4.709$, $p < 0.01$; day: $F_{3,30} = 4.661$, $p < 0.01$; group × day interaction: $F_{9,90} = 7.721$, $p < 0.01$. **$p < 0.01$ 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 analysis of variance (ANOVA) were group: $F_{3,30} = 47.09$, $p < 0.01$; day: $F_{3,30} = 4.661$, $p < 0.01$; group × day interaction: $F_{9,90} = 7.721$, $p < 0.01$. **$p < 0.01$ 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 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: $F_{3,120} = 45.82$, $p < 0.01$; time: $F_{4,120} = 3.322$, $p < 0.01$; group × time interaction: $F_{12,480} = 2.043$, $p < 0.01$. There were no differences between the SE-exposed and EE-exposed mice treated with PCP as determined by the post-hoc test.
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. 3a). PCP treatment reduced the social interaction time in the SE-exposed mice, but not in the EE-exposed mice (environment: $F_{1,44}=6.368$, $p<0.05$; environment $\times$ drug treatment: $F_{1,44}=5.447$, $p<0.01$; 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: $F_{3,44}=2.327$, $p<0.05$; session: $F_{3,44}=95.46$, $p<0.01$; group $\times$ session: $F_{3,44}=4.456$, $p<0.01$; two-way repeated-measures ANOVA and $p<0.05$ by post-hoc comparison) (Fig. 3b), suggesting impairment of objective recognition memory. No such impairment was observed in the EE-exposed mice ($p<0.01$ by post-hoc comparison) (Fig. 3b). The exploratory preference in the training session (Fig. 3b) and the total approach time for two objects in the training (Fig. 3c) and retention (Fig. 3d) 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$^3$ 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. 2007b; 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).
Recently, it was reported that the levels of an ‘open’ chromatin marker, the acetylated Lys$^9$/14 of histone H3, were decreased in cultures of lymphocytes obtained from schizophrenia subjects (Gavin et al. 2008), whereas the levels of a ‘closed’ chromatin mark, the dimethylated Lys$^9$ of histone H3, were increased (Gavin et al. 2009). To investigate whether EE exposure or PCP treatment might affect chromatin modifications, we investigated the number of cells with a histone H3 acetylated at Lys$^9$ in the PFC (Fig. 4a). As shown in Fig. 4a, the numbers were markedly decreased in the SE-exposed mice treated repeatedly with PCP (environment: $F_{1,12}=11.59$, $p<0.05$; two-way ANOVA and $p<0.05$ by post-hoc comparison). In contrast, the EE-exposed mice treated with PCP showed no such decrease in the number of these cells ($p<0.05$ by post-hoc comparison).

Furthermore, to investigate whether the protein expression levels of HDACs might be altered by EE exposure or PCP treatment, we performed Western blotting to examine the changes in the expression level of HDAC5 and HDAC1 in the PFC of the mice (Fig. 4b,c). The HDAC5 level increased with PCP treatment in the SE-exposed mice (environment: $F_{1,20}=7.863$, $p<0.05$; environment x drug treatment interaction: $F_{1,20}=10.26$, $p<0.01$; two-way ANOVA and $p<0.05$ by post-hoc comparison), whereas no such increase in the HDAC5 protein expression induced by PCP was observed in the EE-exposed mice (Fig. 4b). There were no differences in the HDAC1 protein expression levels among the four groups (Fig. 4c).

**Inhibition of HDACs during adolescence prevented PCP-impaired sociality and objective recognition memory**

To investigate whether the inhibition of HDACs during adolescence prevents PCP-induced abnormal

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**Fig. 4.** Enriched environment (EE) exposure during adolescence prevented a decrease in the number of cells positive for histone H3 acetylated at Lys$^9$ and increase in the histone deacetylase (HDAC)5 protein expression induced by phencyclidine (PCP) in the prefrontal cortex (PFC). Mice were killed 24 h after the last PCP treatment. (a) Representative photomicrographs of immunostaining and quantification of the changes in the number of cells with histone H3 acetylated at Lys$^9$ in the PFC. Three sequential sections for the PFC were examined for counting the number of positive cells. Values indicate the means $\pm$ s.e. The number of mice is indicated within the columns. Results of two-way analysis of variance (ANOVA) were environment: $F_{1,12}=11.59$, $p<0.01$. $^*p<0.05$ compared to the results in the standard environment (SE)-exposed mice treated with saline (Sal). $^#p<0.05$ compared to the results in the SE-exposed mice treated with PCP. Scale bar: 50 μm. HDAC5 (b) and HDAC1 (c) expression levels in the nuclear fraction of the PFC. The value was corrected by the level of Lamin AC. Values indicate the means $\pm$ s.e. The number of mice is indicated within the columns. Results of two-way ANOVA were environment: $F_{1,18}=7.863$, $p<0.05$; environment x drug treatment interaction: $F_{1,18}=10.26$, $p<0.01$ for (b). $^*p<0.05$ compared to the results in the SE-exposed mice treated with Sal. $^{##}p<0.01$ compared to the results of the SE-exposed mice treated with PCP.
behaviour, we administered a non-selective HDAC inhibitor, SB (1 g/kg.d i.p.), to mice for 4 wk after weaning (Fig. 5a). SB treatment during adolescence did not affect the gain in body weight (Fig. 5b). Similar to the effect of EE exposure, SB treatment potentiated the hyperlocomotion induced by acute PCP treatment on day 1 (group: $F_{3,54} = 88.34, p < 0.01$; day: $F_{2,54} = 35.08, p < 0.01$; group × day interaction: $F_{6,54} = 12.75, p < 0.01$; two-way repeated-measures ANOVA and $p < 0.05$ by post-hoc comparison) (Fig. 5c). SB treatment...
during adolescence prevented PCP-induced impairments in the social interaction test (SB treatment × PCP treatment interaction: $F_{1,50} = 6.698$, $p < 0.05$; two-way ANOVA and $p < 0.05$ by post-hoc comparison) (Fig. 5d). In addition, SB treatment also prevented PCP-induced impairments in the novel object recognition test (group: $F_{3,50} = 3.802$, $p < 0.01$; session: $F_{1,50} = 69.24$, $p < 0.01$; group × session interaction: $F_{3,50} = 4.652$, $p < 0.01$; two-way repeated-measures ANOVA and $p < 0.05$, $p < 0.01$ by post-hoc comparison) (Fig. 5e).

**Inhibition of HDACs during adolescence prevented a decrease in the number of cells with histone H3 acetylated at Lys⁹ and increase in the HDAC5 protein expression induced by PCP in the PFC**

Finally, to investigate whether the inhibition of HDACs during adolescence might prevent a PCP-induced decrease in the number of cells with a histone H3 acetylated at Lys⁹ and increase in the HDAC protein expressions in the PFC, we administered SB (1 g/kg.d i.p.) for 4 wk to the mice after weaning, as shown in the schedule of Fig. 5a. As shown in Fig. 6a, the numbers of cells with histone H3 acetylated at Lys⁹ were markedly decreased by PCP treatment in the Sal-treated mice (SB treatment: $F_{1,50} = 4.363$, $p < 0.05$; SB treatment × PCP treatment interaction: $F_{1,50} = 6.551$, $p < 0.05$; two-way ANOVA and $p < 0.05$ by post-hoc comparison). In contrast, this decrease induced by PCP treatment was not observed in the SB-treated mice ($p < 0.05$ by post-hoc comparison). Furthermore, the HDAC5 protein expression level was increased by PCP treatment in the Sal-treated mice (PCP treatment: $F_{1,50} = 11.97$, $p < 0.01$; two-way ANOVA and $p < 0.05$ by post-hoc comparison), whereas no such increase of the HDAC5 protein expression induced by PCP treatment was observed in the SB-treated mice (Fig. 6b).

**Discussion**

In this study, we showed that exposure to an EE during adolescence prevented the impairments of sociality and recognition memory and also occurrence of the epigenetic abnormalities induced by chronic PCP treatment. SB treatment during adolescence also prevented the aforementioned behavioural impairments. Our results suggest that the effect of EE exposure of preventing PCP-induced abnormal behaviour is mediated by changes in the histone acetylation levels in the brain.

Pharmacological blockade of NMDA receptors with non-selective NMDA receptor antagonists such as PCP and MK-801 has been demonstrated to induce...
temporal psychosis in normal human subjects and to provoke schizophrenia-like symptoms (Abi-Saab et al. 1998). It has been suggested that the impairments of sociality and cognitive functions induced by chronic PCP treatment may be mediated by NMDA receptor dysfunction (Mouri et al. 2007b; Qiao et al. 2007). Indeed, repeated-dose PCP treatment has been shown to disrupt activation of CaMKII mediated by NMDA receptors, and the impairment of latent learning and emotional behaviour in PCP-treated mice has been shown to be attributable to dysfunctional NMDA–CaMKII signalling (Mouri et al. 2007b; Murai et al. 2007). The NMDA receptor plays a crucial role in synaptic plasticity and induction of long-term potentiation in many regions of the brain, including the PFC (Bliss & Collingridge, 1993; Zhao et al. 2005).

Exposure to an EE was shown to enhance cortical plasticity (Duffy et al. 2001) and to increase NR2A and NR2B NMDA receptor subunit expression in the forebrain (Tang et al. 2001). The cellular mechanism of NMDA receptor-mediated enhancement of cortical plasticity was shown to be dependent on the NR2B:NR2A ratio in the PFC (Zhao et al. 2005). Therefore, our finding that EE exposure prevented impairments of social behaviour and recognition memory could be explained by an enhancement of the glutamatergic activities through transcriptional activation in the EE-exposed animals.

The EE-exposed mice in our study did display hypersensitivity to acute PCP treatment. However, decreased sensitivity to NMDA receptor antagonists has been reported in NR1 (Belforte et al. 2010) and GluR1 knockout mice (Wiedholz et al. 2008), which exhibit hypoglutamatergic neurotransmission. Increases in GluR1, NR2A and NR2B expressions were observed 3 h after the start of exposure to an EE, to reach substantial levels by 2 wk (Tang et al. 2001), indicating that glutamatergic activity is enhanced by exposure to an EE. Thus, the hypersensitivity of the locomotor-stimulant response to PCP in the EE-exposed mice could be caused by facilitation of the glutamatergic activity. Although SB treatment also potentiated locomotor activity induced by PCP treatment, the degree of increase of the locomotor activity in the EE-exposed mice (about two-fold higher compared to that in the SE-exposed mice) was higher than that in the SB-treated mice (about 1.25-fold higher compared to that in the Sal-treated mice). EE exposure affects not only transcriptional activation, such as histone acetylation, but also release of neurotransmitters (Segovia et al. 2009). Consistent with this notion, EE-exposed mice have been shown to exhibit elevated extracellular dopamine levels and enhancedamphetamine-induced dopamine release in the nucleus accumbens (Segovia et al. 2010). The enhancement of hyperlocomotion in the EE-exposed mice might also arise from the effect of EE exposure on the release of neurotransmitters, such as dopamine, in addition to its effect on histone modification.

Chromatin remodelling has been implicated in the development of several chronic psychiatric conditions, as a potential mechanism underlying long-lasting changes in gene expressions and behaviour caused by environmental stimuli (Tsankova et al. 2007). In schizophrenia subjects, the amount of histone H3 acetylated at Lys9 in lymphocyte cultures is decreased (Gavin et al. 2008). Administration of valproic acid, which has an inhibitory effect on HDAC, produces significantly smaller increases in the amount of acetylated histone H3 in schizophrenia subjects (Sharma et al. 2006). These results suggest that schizophrenia is associated with ‘rigid’ chromatin associated with the decrease in the acetylation of Lys9 of histone H3. We demonstrated that chronic PCP treatment decreased the amount of acetylated histone H3 in the PFC, which was blocked by EE exposure during adolescence. Our findings suggest that chronic PCP-treated animals have epigenetic abnormalities similar to those in schizophrenia subjects and that EE could prevent these epigenetic changes.

In the chromatin remodelling, HDACs repress transcription by deacetylating nucleosomal histones and other components of the transcriptional machinery. HDAC5 and histone acetylation have been suggested to have important roles in the development of psychiatric disorders and in the execution of fundamental brain functions (Renthal et al. 2007; Tsankova et al. 2006). HDAC5 decreases the levels of histone H3 acetylated at Lys9 in the mouse brain (Tsankova et al. 2006). We demonstrated that HDAC5, but not HDAC1 expression, was increased in the nuclear fraction of the PFC of PCP-treated mice, which could be prevented by EE exposure during adolescence. Furthermore, administration of SB, an HDAC inhibitor, during adolescence also prevented PCP-induced behavioural abnormalities. It is suggested that these changes in HDAC5 expression may contribute to the changes in the acetylated histone H3 levels and be associated with behavioural changes.

Activation of HDAC5 is regulated through phosphorylation via neural activity-dependent mechanisms (Chawla et al. 2003). Activation of synaptic NMDA receptors induces translocation of HDAC5 to the cytoplasm through its phosphorylation by CaMKII, which is influenced by NMDA receptor
signalling (Renthal et al. 2007). Chronic PCP treatment decreases NMDA-stimulated and behaviour-associated phosphorylation of CaMKII in the PFC (Mouri et al. 2007b; Murai et al. 2007). The PCP-induced increase in nuclear HDAC5 expression was attributable to a disruption of NMDA receptor-mediated CaMKII activity and was prevented by EE exposure through the potentiation of glutamatergic activity.

In our study, SB treatment mimicked the effect of EE exposure during adolescence on PCP-induced behavioural and biochemical abnormalities. This result suggests that histone modification during adolescence powerfully affects PCP-induced behavioural changes. SB is a hydroxamate-based HDAC inhibitor and might affect brain function mainly through the inhibition of class I HDACs, including HDAC1, HDAC2, HDAC3 and HDAC8 (Kazantsev & Thompson, 2008), but not HDAC5. Therefore, SB treatment might prevent PCP-induced behavioural abnormalities through inhibition of HDACs except HDAC5. It remains to be clarified whether other members of the HDAC family might also have a role. Further, SB treatment in adolescence prevented not only the deacetylation of Lys of histone H3, but also the increase of HDAC5 expression induced by PCP. Since suppression of the increase of HDAC5 expression induced by PCP treatment was involved in the effect of SB, the mechanism underlying this suppression by SB treatment must be clarified in the future.

In conclusion, our results suggest that EE during adolescence prevents the onset and/or development of schizophrenia through modification of the epigenetic machinery. Raine et al. (2003) reported that early enrichment programmes such as nutrition, education and physical exercise enrichment programmes were associated with lower levels of antisocial behaviour and schizotypal personality in adulthood in humans. Our observations support this clinical finding and suggest the involvement of epigenetic changes in the effect of enrichment of the environment, which might therefore serve as a novel prophylaxis strategy against schizophrenia.

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Statement of Interest

None.

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