

Nicotine Ameliorates Emotional and Cognitive Impairments Induced by Neonatal PolyI:C Treatment in Mice

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Abstract: Environmental factors such as maternal and neonatal infection are potentially associated with the pathogenesis of various psychiatric disorders, including schizophrenia. Polyriboinosinic-polyribocytidilic acid (polyI:C) is a synthetic analogue of double-stranded RNA that induces strong innate immune responses. We have recently developed the mouse model of neurodevelopmental psychiatry disorders that exhibits emotional and cognitive impairments in adulthood following neonatal polyI:C treatment. In this study, we examined whether nicotine ameliorates emotional and cognitive impairments in the neonatal polyI:C model because recent studies have indicated the therapeutic benefits of nicotine in schizophrenia. Neonatal ICR mice were repeatedly injected with polyI:C (5 mg/kg, s.c.) for 5 days (postnatal days 2 to 6). At postnatal 10 weeks, emotional functions were analyzed in open field and social interaction tests. Cognitive function was analyzed in novel object recognition and prepulse inhibition (PPI) tests. PolyI:C-treated mice showed an increase in anxiety-like behaviors and impairments in social behaviors, object recognition memory, and PPI, compared with the vehicle-treated control group. Nicotine (0.15 and 0.5 mg/kg, s.c.) dose-dependently improved polyI:C-induced impairments of emotional and cognitive behaviors, but had no effect on PPI deficit. The ameliorating effect of nicotine was antagonized by pretreatment with dihydro- β -erythroidine or methyllycaconitine. These results suggest that nicotine ameliorates emotional and cognitive impairments of the present polyI:C model through nicotinic acetylcholine receptors.

Keywords: Cognition, emotion, nicotine, polyI:C, schizophrenia, nicotinic acetylcholine receptors.

INTRODUCTION

Schizophrenia is a chronic psychiatric disorder characterized by positive and negative symptoms and impaired cognitive function, which affects approximately 1% of the general population [1]. Both genetic factors and environmental insults, including prenatal infection and perinatal complication, are involved in the development of schizophrenia [2]. Recent immunologic, epidemiologic, and neuropsychiatric studies suggest infectious etiologies of several major neuropsychiatric diseases [3]. Infectious organisms that have been implicated in schizophrenia etiology include rubella, influenza, herpes simplex virus, cytomegalovirus, poliovirus, and toxoplasma gondii [4].

Toll-like receptors (TLRs) constitute several families of the pattern-recognition receptors that sense nucleic acids derived from viruses and trigger antiviral innate immune

responses [5]. The TLR family consists of more than 13 members in mammals, each detecting different pathogen-associated molecular ligands [5]. In particular, TLR3 recognizes viral double-stranded RNA and host cell mRNA and in turn initiates inflammatory responses [6]. Polyriboinosinic-polyribocytidilic acid (polyI:C) is a synthetic analogue of double-stranded RNA that leads to the pronounced but time-limited production of pro-inflammatory cytokines after it binds to and activates TLR3 [7]. Maternal immune activation by polyI:C exposure in rodents is known to precipitate a wide spectrum of behavioral, cognitive, and pharmacological abnormalities in adult offspring [8-12]. Recently, we have reported that neonatal injection of polyI:C in mice results in schizophrenia-like behavioral alterations, such as emotional and cognitive impairments and dysfunction of glutamatergic neurotransmission in adulthood [13]. We have also proposed a possible interaction of genetic and environmental factors by injecting polyI:C into transgenic mice that express a dominant-negative form of human *disrupted-in-schizophrenia 1*, which is one of the susceptibility genes for schizophrenia [14]. Therefore, polyI:C-treated mice are the useful animal model for schizophrenia that is supported by epidemiological findings.

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Tobacco smoking is frequent among schizophrenia patients [15, 16]. It has been reported that while 25-30% of the population smoke tobacco regularly, 70-90% of chronic schizophrenic patients are tobacco smokers [17, 18]. Nicotine is a potent cholinergic receptor agonist that is inhaled during tobacco smoking. Interestingly, both the expression and function of nicotinic acetylcholine receptors (nAChRs) are down-regulated in the brains of patients with schizophrenia [19, 20]. The enzyme activity of choline acetyltransferase, a biosynthetic enzyme for acetylcholine production is decreased and correlated with poor cognitive function in schizophrenia [21]. It is suggested that the high rate of tobacco smoking among schizophrenia patients represents an attempt to self-medicate, that is, to correct for some disease-associated abnormalities of cholinergic (nicotinic) neurotransmission. Thus, the therapeutic effects of nicotine in schizophrenia have received much interest in recent years.

In this study, to examine whether nicotine ameliorates emotional and cognitive impairments in adult mice that challenged with polyI:C as neonates, neurobehavioral effects of nicotine were analyzed in open field, social interaction, novel object recognition, and prepulse inhibition tests.

MATERIALS AND METHODS

Animals

Timed pregnant ICR mice were obtained from Japan SLC Inc. (Hamamatsu, Japan) and maintained under standard specific pathogen-free environmental conditions. Pregnant females were monitored for the parturition date, which was taken as postnatal day (PD) 0. They were housed under a standard 12-h light/dark cycle (lights on at 9:00) at a constant temperature of $23 \pm 1^\circ\text{C}$, with free access to food and water throughout the experiments. We used male mice exclusively to minimize any potential variability due to sex-specific effects in behavioral performance. The animals were handled in accordance with the guidelines established by the Institutional Animal Care and Use Committee of Nagoya University, the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society, and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Drugs and Treatment

PolyI:C, (-)-nicotine, dihydro- β -erythroidine [DH β E, an antagonist for $\alpha 4\beta 2$ subunit-containing nAChR ($\alpha 4\beta 2$ nAChR)], and methyllycaconitine [MLA, a selective antagonist for $\alpha 7$ subunit-containing nAChR ($\alpha 7$ nAChR)] were purchased from Sigma-Aldrich (St Louis, MO, USA) and dissolved in saline. All litters were randomly divided into saline and polyI:C-treated groups. From PD 2 to 6, mice were subcutaneously injected daily with either pyrogen-free saline or polyI:C at a dose of 5 mg/kg. Animals were weaned at PD 21, and divided by gender at PD 28. Both groups were derived from multiple litters to preclude possible differences in individual maternal behavior as a mitigating factor in any subsequent long-lasting changes induced in the offspring. Behavioral analyses were started at 10-12 weeks of age in the following order: prepulse inhibition (PPI), open field, novel object recognition test, and social interaction test for studying the effects of nicotine (Figs. 1-4); open field, novel

object recognition test, and social interaction test for studying the effects of nAChR antagonists (Figs. 5-7). Nicotine (0.15 or 0.5 mg/kg), DH β E (2.0 mg/kg), or MLA (5 mg/kg) was subcutaneously administered 15 min, 25 min, or 25 min, respectively, before behavioral tests. The dose of each drug was in accordance with previous reports [22, 23].

Open Field Test

Mice were placed at the center of an arena and allowed to explore an open field (diameter: 60 cm, height: 35 cm) for 5 min, while their activity was measured automatically using the ethovision automated tracking program (BrainScience Idea Co. Ltd., Osaka, Japan) [24, 25]. The open field was divided into an inner circle (diameter: 40 cm) and an outer area surrounding the inner circle. The movement of mice was measured *via* a camera mounted above the open field. Measurements included distance and time spent in the inner and outer sections.

Social Interaction Test

We used the experimental paradigm described by Tremolizzo *et al.* [26] to measure social behavior (e.g., social interaction, aggression, and escape behavior). PolyI:C-treated or vehicle-treated control mice were individually housed in cages (29 \times 18 \times 12 cm) for 2 days before the trial. We used 10-12-week-old male ICR mice that had not shown aggressive behavior as intruders. In the first trial (5 min duration), an intruder mouse was introduced into the resident's home cage. The duration of social interaction (close following, inspection, anogenital sniffing, and other social body contacts except aggressive behavior), aggression (attacking/biting and tail rattling), and escape behavior were analyzed. Four trials, with an inter-trial interval of 30 min, were used to analyze social behavior using the same intruder mouse.

Novel Object Recognition Test

A novel object recognition test was carried out as described previously [27]. Mice were individually habituated to an open-box (30 \times 30 \times 35 high cm) for 3 days. During the training session, two novel objects were placed in the open field and the animals were allowed to explore for 10 min. The time spent exploring each object was recorded. During retention sessions, the animals were placed back into the same box 24 h after the training session, one of the familiar objects used during training was replaced by a novel object, and the mice were allowed to explore the two objects freely for 5 min. The preference index in the retention session, the ratio of the amount of time spent exploring the novel object to the total time spent exploring both objects, was used to measure cognitive function. In the training session, the preference index was calculated as the ratio of time spent exploring the object that was replaced by a novel object in the retention session to the total exploration time.

PPI Test

The PPI test was carried out as described previously [28, 29]. After the animals were placed in the chamber (San Diego Instruments, San Diego, California), they were allowed to habituate for 10 min, during which time they

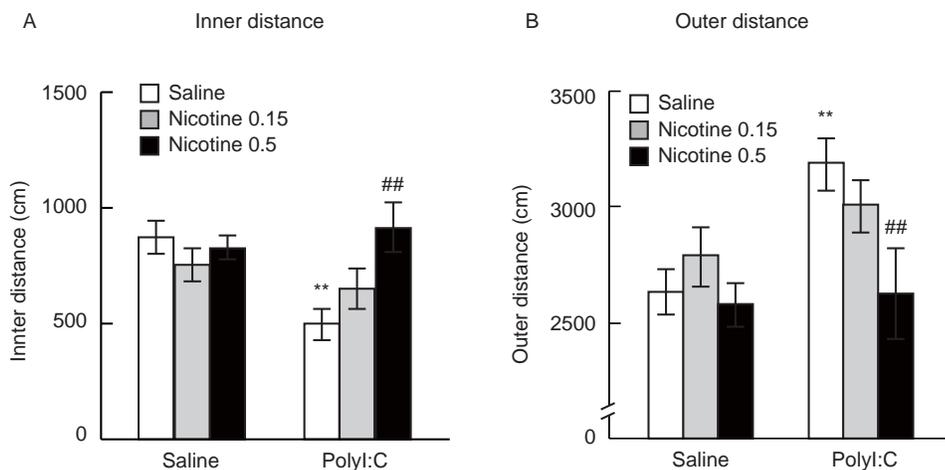


Fig. (1). Effect of nicotine on emotional deficits in open field test in polyI:C-treated mice. Nicotine (0.15 and 0.5 mg/kg, s.c.) was administered 15 min before the behavioral test. Individual mice were allowed to explore the open field freely for 5 min. (A and B) Distance traveled in (A) inner and (B) outer sectors. Values indicated the mean \pm S.E. ($n=8-10$). ** $p<0.01$ vs. saline-treated control group (Fisher's LSD test). ## $p<0.01$ vs. polyI:C-treated control group (Fisher's LSD test).

were subjected to 65 dB background white noise. The animals then received 10 startle trials, 10 no-stimulus trials, and 40 PPI trials. The intertrial interval was between 10 and 20 sec and the total session lasted 17 min. The startle trial consisted of a single 120 dB white noise burst lasting 40 msec. PPI trials consisted of a prepulse (20 msec burst of white noise at 69, 73, 77, or 81 dB intensity) followed, 100 msec later, by the startle stimulus (120 dB, 40 msec white noise). Each of the four prepulse trials (69, 73, 77, or 81 dB) was carried out 10 times. Sixty different trials were presented pseudo-randomly, ensuring that each trial was carried out 10 times and that no two consecutive trials were identical. The resulting movement of the animal in the startle chamber was measured for 100 msec after startle stimulus onset (sampling frequency 1 kHz), rectified, amplified, and fed into a computer, which calculated the maximal response over the 100 msec. Basal startle amplitude was determined as the mean amplitude of the 10 startle trials. PPI was calculated according to the formula: $100 \times [1 - (PPx/P120)]$ %, in which PPx was the mean amplitude of the 10 PPI trials (PP69, PP73, PP75, or PP80) and P120 was the basal startle amplitude.

Statistical Analysis

Data are expressed as the mean \pm S.E. Statistical significance was determined using analysis of variance (ANOVA) with two-way (Figs. 1-3 and 4B) or three-way (Fig. 4A), followed by the Fisher's LSD test when F ratios were significant ($p<0.05$). In Figs. (5-7), two-tailed Student's t-test was used between the saline-treated polyI:C and control (saline + saline) groups while two-way ANOVA was performed among polyI:C-treated groups.

RESULTS

Effects of Nicotine on Emotional Deficits in PolyI:C-Treated Mice in Open Field Test

To investigate the effects of nicotine on emotional deficits in polyI:C-treated mice in adulthood, an open field test

was carried out at the age of 10-12 weeks, in which the conflict between the drive to explore a new environment and a natural aversion to illuminated open areas was used to examine both anxiety and motor activity [25]. Two-way ANOVA revealed significant effects of polyI:C and nicotine in distance traveled in inner sectors (polyI:C, $F(1,47)=4.24$, $p<0.05$; nicotine, $F(2, 47)=3.45$, $p<0.05$; polyI:C \times nicotine interaction, $F(2,47)=4.73$, $p<0.05$, Fig. 1A) and outer sectors (polyI:C, $F(1,47)=4.17$, $p<0.05$; nicotine, $F(2, 47)=5.10$, $p<0.01$; polyI:C \times nicotine interaction, $F(2,47)=2.50$, $p=0.09$, Fig. 1B) of the open field. The distance traveled in the inner sector of the open field was significantly decreased while the distance traveled in the outer sector was significantly increased in polyI:C-treated mice compared with vehicle-treated control mice ($p<0.01$, Fig. 1A and B). Nicotine dose-dependently and significantly (0.5 mg/kg) increased the distance traveled in the inner sector ($p<0.01$, Fig. 1A), and decreased the distance traveled in the outer sector in polyI:C-treated mice ($p<0.01$, Fig. 1B). Likewise, nicotine (0.5 mg/kg) treatment tended to ameliorate the changes in time spent in inner and outer sectors of the open field in polyI:C-treated mice (data not shown). Nicotine itself had no effect on performance in the saline-treated control group (Fig. 1), suggesting that nicotine has no effect on motor function in mice. These results suggest that nicotine ameliorates polyI:C-induced emotional deficits in adults.

Effects of Nicotine on Deficits of Social Behavior in PolyI:C-Treated Mice

Social interaction in polyI:C-treated mice was investigated at the age of 10-12 weeks. In saline-treated control mice, repeated exposure to an unfamiliar intruder mouse (4 trials) caused a gradual decrease in social interaction time. The polyI:C-treated mice exhibited a marked reduction in the social interaction time in all 4 trials compared with saline-treated control mice (data not shown). Therefore, total social interaction time was evaluated in the following analysis. Two-way ANOVA revealed significant effects of polyI:C and nicotine in social interaction (polyI:C, $F(1,47)=25.27$,

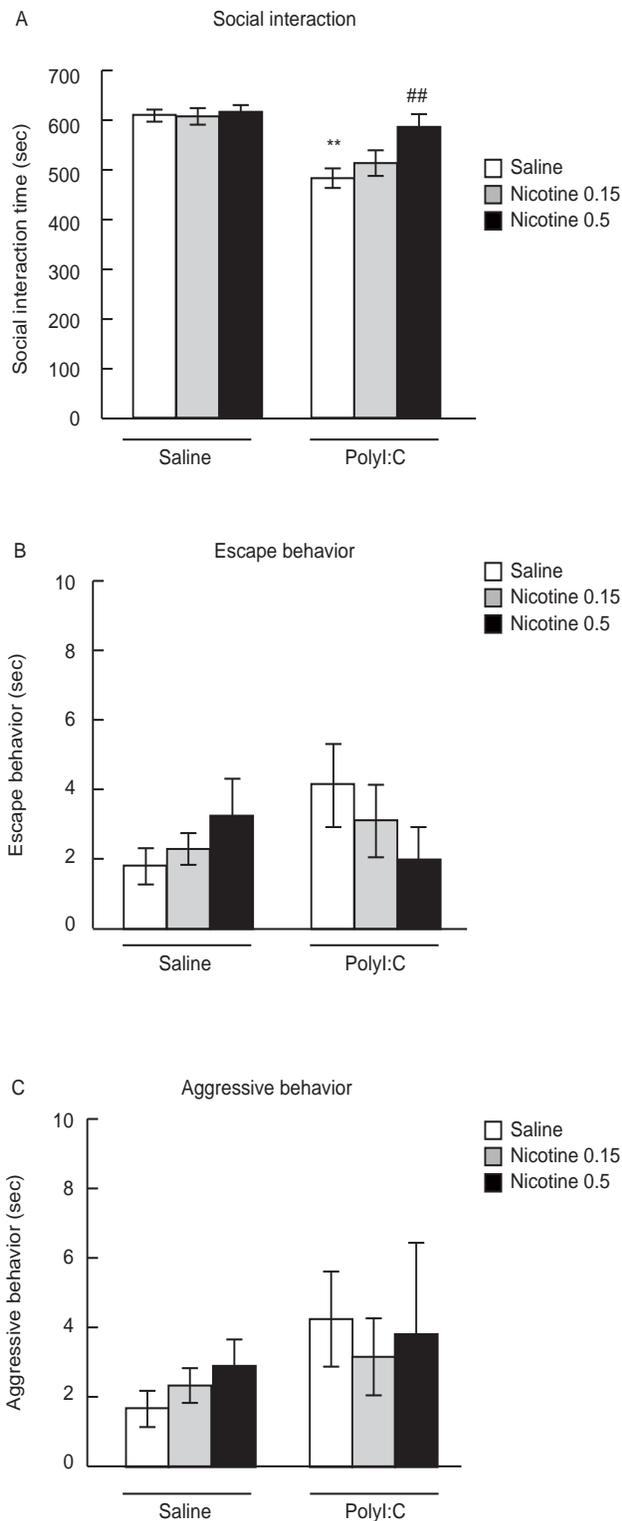


Fig. (2). Effect of nicotine on deficits of social behaviors in social interaction test in polyI:C-treated mice. **(A)** Social interaction, **(B)** escape behavior, and **(C)** aggressive behavior. Nicotine (0.15 and 0.5 mg/kg, s.c.) was administered 15 min before the behavioral test. Values indicate the mean \pm S.E. ($n=8-10$). ** $p<0.01$ vs. saline-treated control group (Fisher's LSD test). ## $p<0.01$ vs. polyI:C-treated control group (Fisher's LSD test).

$p<0.01$; nicotine, $F(2,47)=3.82$, $p<0.05$; polyI:C \times nicotine interaction, $F(2,47)=3.06$, $p=0.06$, Fig. 2A). These treatments had no effects on escape (polyI:C, $F(1,47)=0.68$, $p=0.41$; nicotine, $F(2,47)=0.08$, $p=0.92$; polyI:C \times nicotine interaction, $F(2,47)=1.98$, $p=0.15$, Fig. 2B) or aggressive behavior (polyI:C, $F(1,47)=1.56$, $p=0.22$; nicotine, $F(2,47)=0.09$, $p=0.92$; polyI:C \times nicotine interaction, $F(2,47)=0.26$, $p=0.77$, Fig. 2C). A single treatment with nicotine (0.5 mg/kg) significantly improved deficits of social interaction in polyI:C-treated mice ($p<0.01$, Fig. 2A) without affecting escape (Fig. 2B) or aggressive behavior (Fig. 2C). In saline-treated mice, nicotine (0.15 and 0.5 mg/kg) had no effect on social interaction (Fig. 2A), escape (Fig. 2B), or aggressive behavior (Fig. 2C). These results suggest that the ameliorating effect of nicotine on social interaction deficit is not due to locomotor stimulation by the drugs or to changes in escape or aggressive behavior of polyI:C-treated mice.

Effects of Nicotine on Deficits of Object Recognition Memory in PolyI:C-Treated Mice

To examine the effects of nicotine treatment on neonatal polyI:C treatment-induced memory impairment in adults, a novel object recognition test was carried out at the age of 10-12 weeks. During the training session, both polyI:C-treated and saline-treated control mice spent equal amounts of time exploring either one of two objects (two-way ANOVA analysis; polyI:C, $F(1,42)=1.48$, $p=0.23$; nicotine $F(2,42)=2.62$, $p=0.09$; polyI:C \times nicotine interaction, $F(2,42)=0.04$, $p=0.96$, Fig. 3B), and there was no biased exploratory preference in either group (two-way ANOVA analysis; polyI:C, $F(1,42)=0.01$, $p=0.95$; nicotine $F(2,42)=0.28$, $p=0.76$; polyI:C \times nicotine interaction, $F(2,42)=0.05$, $p=0.95$, Fig. 3A), suggesting no differences in motivation and curiosity about novel objects, and motor function between polyI:C-treated and saline-treated control mice. The retention session was carried out 24 h after the training session. Two-way ANOVA indicated that significant effects of polyI:C and nicotine were detected in the level of exploratory preference to the novel object (polyI:C, $F(1,42)=51.72$, $p<0.01$; nicotine $F(2,42)=11.07$, $p<0.01$; polyI:C \times nicotine interaction, $F(2,42)=8.70$, $p<0.01$, Fig. 3A). The level of exploratory preference to the novel object was significantly decreased in polyI:C-treated mice compared with saline-treated control mice ($p<0.01$, Fig. 3A). Total exploration time in the retention session did not differ among the groups (two-way ANOVA analysis; polyI:C, $F(1,42)=3.70$, $p=0.06$; nicotine $F(2,42)=0.35$, $p=0.71$; polyI:C \times nicotine interaction, $F(2,42)=0.08$, $p=0.92$, Fig. 3B), indicating that polyI:C-treated mice are unable to discriminate novel and familiar objects. These results suggest that polyI:C-treated mice have impaired recognition memory in adulthood. A single treatment with nicotine (0.5 mg/kg) significantly improved cognitive impairment in polyI:C-treated mice during the retention session ($p<0.01$, Fig. 3A) when nicotine was administered 15 min before the training session. Nicotine had no effect on the level of exploratory preference to the novel object in the training session in polyI:C-treated mice (Fig. 3A). The total exploration time in polyI:C-treated mice was not affected by nicotine in either training or retention session (Fig. 3B). In saline-treated mice, nicotine had no effect on the level of exploratory preference or total exploration time throughout the experiment (Fig. 3A and B).

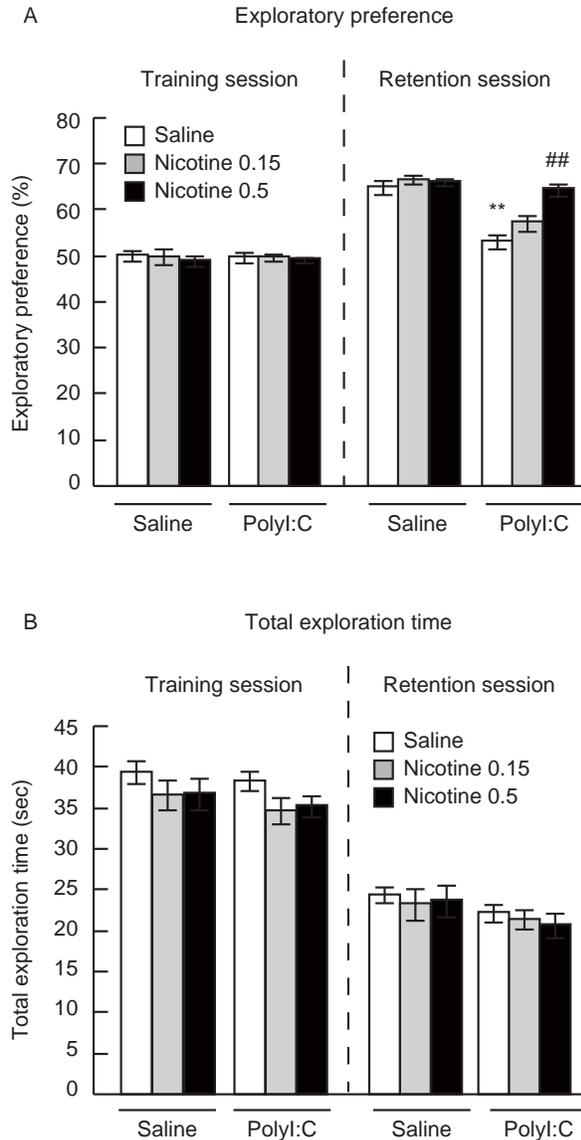


Fig. (3). Effect of nicotine on deficits of object recognition memory in novel object recognition test in polyI:C-treated mice. (A) Exploratory preference. (B) Total exploration time. Nicotine (0.15 and 0.5 mg/kg, s.c.) was administered 15 min before the training session. The retention session was carried out 24 h after the training session. Values indicate the mean \pm S.E. (n=8). **p<0.01 vs. saline-treated control group (Fisher's LSD test). ## p<0.01 vs. polyI:C-treated control group (Fisher's LSD test).

Effects of Nicotine on PPI Deficits of Startle Response in PolyI:C-Treated Mice

The PPI test was carried out at the age of 10-12 weeks to assess the sensorimotor gating function in polyI:C-treated mice. Three-way ANOVA revealed that significant effects of polyI:C, but no significant effects of nicotine was detected in PPI test (polyI:C, $F(1,42)=72.16$, $p<0.01$; nicotine, $F(2,42)=0.21$, $p=0.81$; polyI:C \times nicotine interaction, $F(2,42)=0.14$, $p=0.87$; prepulse, $F(3,126)=55.26$, $p<0.01$; polyI:C \times prepulse interaction, $F(3,126)=16.36$, $p<0.01$; nicotine \times

prepulse interaction, $F(3,126)=0.18$, $p=0.98$; polyI:C \times nicotine \times prepulse interaction, $F(3,126)=0.67$, $p=0.67$). PolyI:C-treated mice showed a marked impairment of PPI compared with the saline-treated control group at the prepulse intensities (69, 73, 77 and 81 dB) ($p<0.05$ or $p<0.01$, Fig. 4A). Single treatment with nicotine (0.15 and 0.5 mg/kg) had no effect on PPI deficits (Fig. 4A) or acoustic startle amplitude in the polyI:C-treated group nor in the saline-treated control group (two-way ANOVA analysis; polyI:C, $F(1,42)=0.06$, $p=0.80$; nicotine, $F(2,42)=0.05$, $p=0.95$; polyI:C \times nicotine, $F(2,42)=0.11$, $p=0.89$, Fig. 4B).

Effects of nAChR Antagonists on Ameliorative Effect of Nicotine Against Emotional and Cognitive Deficits in PolyI:C-Treated Mice

To clarify the subtype of nAChRs involved in the ameliorative effect of nicotine on the emotional and cognitive deficits in polyI:C-treated mice, the mice were pretreated with a selective antagonist for $\alpha 7$ nAChR, MLA, or an antagonist for $\alpha 4\beta 2$ nAChR, DH β E, before nicotine treatment.

In the open field test, two-way ANOVA revealed significant differences in distance traveled in inner sectors (nicotine, $F(1,50)=0.01$, $p=0.93$; nAChR antagonists, $F(2,50)=0.20$, $p=0.82$; nicotine \times nAChR antagonists interaction, $F(2,50)=5.91$, $p<0.01$, Fig. 5A) and outer sectors (nicotine, $F(1,50)=15.96$, $p<0.01$; nAChR antagonists, $F(2,50)=6.83$, $p<0.01$; nicotine \times nAChR antagonists interaction, $F(2,50)=0.76$, $p=0.47$, Fig. 5B). The ameliorating effect of nicotine on anxiety-like behavioral changes in polyI:C-treated mice was completely blocked by pretreatment with either MLA or DH β E ($p<0.05$, Fig. 5A and B). Treatment with MLA or DH β E alone did not affect the change in distance traveled in the inner or outer sector for saline-treated polyI:C mice (Fig. 5A and B). Similar results were observed in the change in time spent in each sector (data not shown).

In the social interaction test, pretreatment with MLA or DH β E significantly attenuated the ameliorating effect of nicotine in polyI:C-treated mice although the same treatment failed to affect saline-treated polyI:C mice (nicotine, $F(1,50)=8.94$, $p<0.01$; nAChR antagonists, $F(2,50)=4.80$, $p<0.05$; nicotine \times nAChR antagonists interaction, $F(2,50)=2.63$, $p=0.08$, Fig. 6A). Furthermore, nAChR antagonists had no effect on escape (nicotine, $F(1,50)=0.05$, $p=0.82$; nAChR antagonists, $F(2,50)=0.01$, $p=0.99$; nicotine \times nAChR antagonists interaction, $F(2,50)=0.23$, $p=0.80$, Fig. 6B) or aggressive behaviors (nicotine, $F(1,50)=0.54$, $p=0.47$; nAChR antagonists, $F(2,50)=0.07$, $p=0.93$; nicotine \times nAChR antagonists interaction, $F(2,50)=0.12$, $p=0.89$, Fig. 6C) in either saline-treated or nicotine-treated polyI:C mice.

In the novel object recognition test, MLA and DH β E significantly and completely blocked the ameliorating effect of nicotine on the impairment of object recognition memory in polyI:C-treated mice (nicotine, $F(1,50)=10.85$, $p<0.01$; nAChR antagonists, $F(2,50)=3.66$, $p<0.05$; nicotine \times nAChR antagonists interaction, $F(2,50)=5.65$, $p<0.01$, Fig. 7A). Treatment with MLA or DH β E did not affect the exploratory preference in saline-treated mice or the total

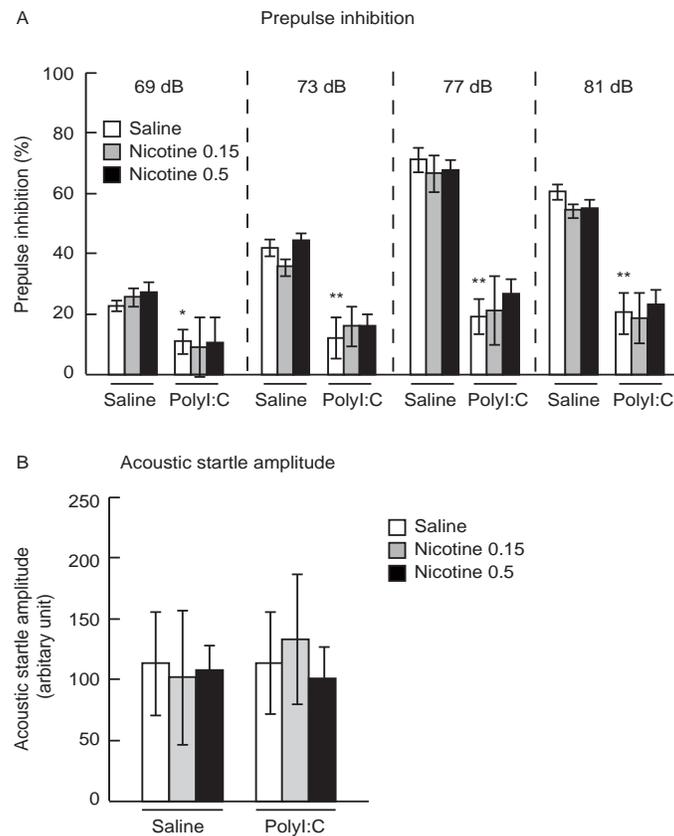


Fig. (4). Effect of nicotine on PPI deficits of startle response in polyI:C-treated mice. **(A)** PPI (%) at four different prepulse intensities (69, 73, 77, and 81 dB). **(B)** Acoustic startle amplitude as measured in trials without prepulse. Nicotine (0.15 and 0.5 mg/kg, s.c.) was administered 15 min before the behavioral test. Values indicate the mean \pm S.E. (n=8). * p <0.05 and ** p <0.01 vs. saline-treated control group (Fisher's LSD test).

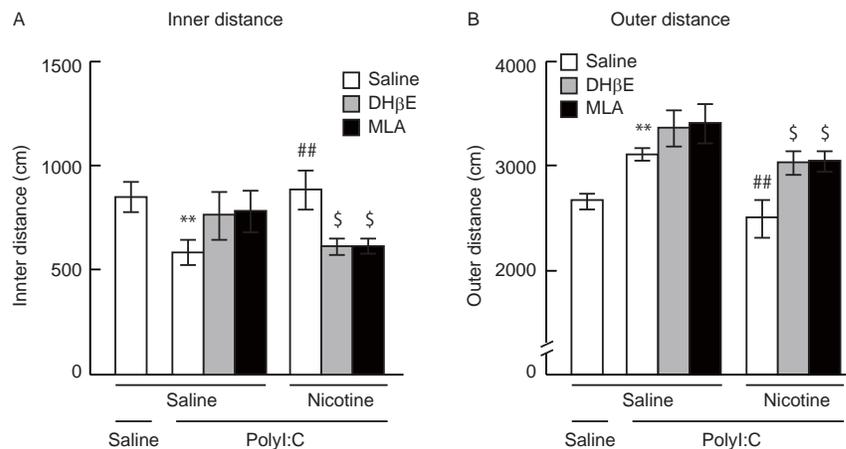


Fig. (5). Effects of nACh receptor antagonists on nicotine-induced amelioration of emotional deficits in polyI:C-treated mice. $\alpha 4\beta 2$ nACh receptor antagonist DH β E (2.0 mg/kg, s.c.) or $\alpha 7$ nACh receptor antagonist MLA (5 mg/kg, s.c.) was administered 25 min before the behavioral test. Nicotine (0.5 mg/kg, s.c.) was administered 15 min before the behavioral test. Individual mice were allowed to explore the open field freely for 5 min. **(A)** and **(B)** Distance traveled in (A) inner and (B) outer sectors. Values indicate the mean \pm S.E. (n=8-16). ** p <0.01 vs. saline-treated control group (Student's t -test). ## p <0.01 vs. saline-treated polyI:C group (Fisher's LSD test). \$ p <0.05 vs. nicotine-treated polyI:C group (Fisher's LSD test).

exploration time in either the training or retention sessions in all groups (Fig. 7A and B).

DISCUSSION

It has been reported that the first 2 weeks of postnatal life in the rat and mouse correspond to the second trimester of

pregnancy in humans [30], during which time exposure to viral or environmental insult increases the probability of subsequently developing schizophrenia in adolescence. This period is a critical time for neurogenesis in the hippocampus, and for cortical synaptogenesis [31]. According to these findings, we have developed a mouse model of viral infection during the perinatal period by repeatedly injecting

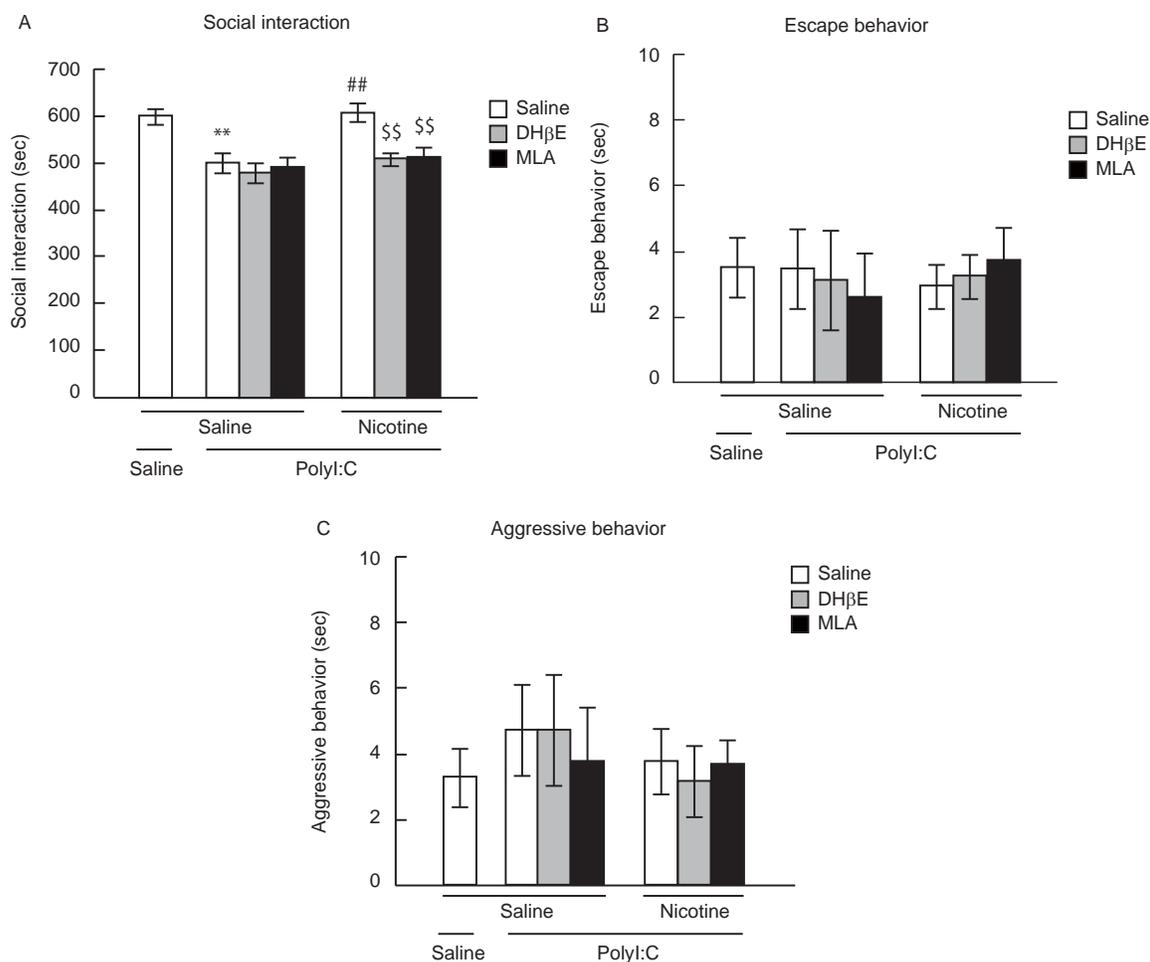


Fig. (6). Effects of nACh receptor antagonists on nicotine-induced amelioration of deficits of social behaviors in polyI:C-treated mice. (A) Social interaction, (B) escape behavior, and (C) aggressive behavior. $\alpha\beta 2$ nACh receptor antagonist DH β E (2.0 mg/kg, s.c.) or $\alpha 7$ nACh receptor antagonist MLA (5 mg/kg, s.c.) was administered 25 min before the behavioral test. Nicotine (0.5 mg/kg, s.c.) was administered 15 min before the behavioral test. Values indicate the mean \pm S.E. ($n=8-16$). ** $p<0.01$ vs. saline-treated control group (Student's t -test). ## $p<0.01$ vs. saline-treated polyI:C group (Fisher's LSD test). \$\$ $p<0.01$ vs. nicotine-treated polyI:C group (Fisher's LSD test).

polyI:C into neonatal ICR mice at postnatal days 2 to 6 [13]. Consistent with our previous study, polyI:C-treated mice showed anxiety-like behavior in the open field test, impaired social behavior in the social interaction test, impaired recognition memory in the novel object recognition test, and sensorimotor gating deficits in the PPI test after puberty. These results suggest that neonatal polyI:C treatment in ICR mice can provide an animal model exhibiting schizophrenia-like behavioral phenotypes after puberty. The abnormal behaviors, except for PPI deficits, in polyI:C-treated mice were ameliorated by the treatment with nicotine in a dose-dependent manner. Thus nicotine may have a therapeutic benefit in ameliorating clinical symptoms in schizophrenia. It is plausible that neonatal polyI:C treatment interferes in the development of cholinergic neurons, leading to abnormal behaviors in adulthood since the early postnatal period is the time for the entry of cholinergic fibers into the cortex [31].

Nicotine failed to improve the PPI deficits in polyI:C-treated mice while smoking transiently normalizes sensory gating deficits in schizophrenia [32]. Studies in rodents have

shown that $\alpha 7$ nAChR antagonists induce PPI deficits while $\alpha 7$ nAChR agonists can normalize the auditory gating deficits [33, 34]. The reason for this discrepancy remains unclear. It is unlikely that polyI:C-treated mice lack predictive validity as an animal model of schizophrenia. Because the emotional and cognitive impairments including PPI deficits in polyI:C-treated mice were ameliorated by the treatment of typical and atypical antipsychotics such as haloperidol and clozapine, respectively (data not shown). Alternatively, it is well known that dopamine receptor agonists disrupt PPI of the startle reflex [35]. Therefore, activation of dopaminergic neurons by nicotine [36] may hide the beneficial effect of nicotine on sensorimotor gating in polyI:C-treated mice.

nAChRs are pentameric, ligand-gated ion channels abundant in the central nervous system [37]. Twelve neuronal subunits have been identified, designated $\alpha 2$ to $\alpha 10$ and $\beta 2$ to $\beta 4$, which potentially assemble in multiple combinations with a broad range of pharmacological and electrophysiological properties [38]. In particular, the two most prevalent

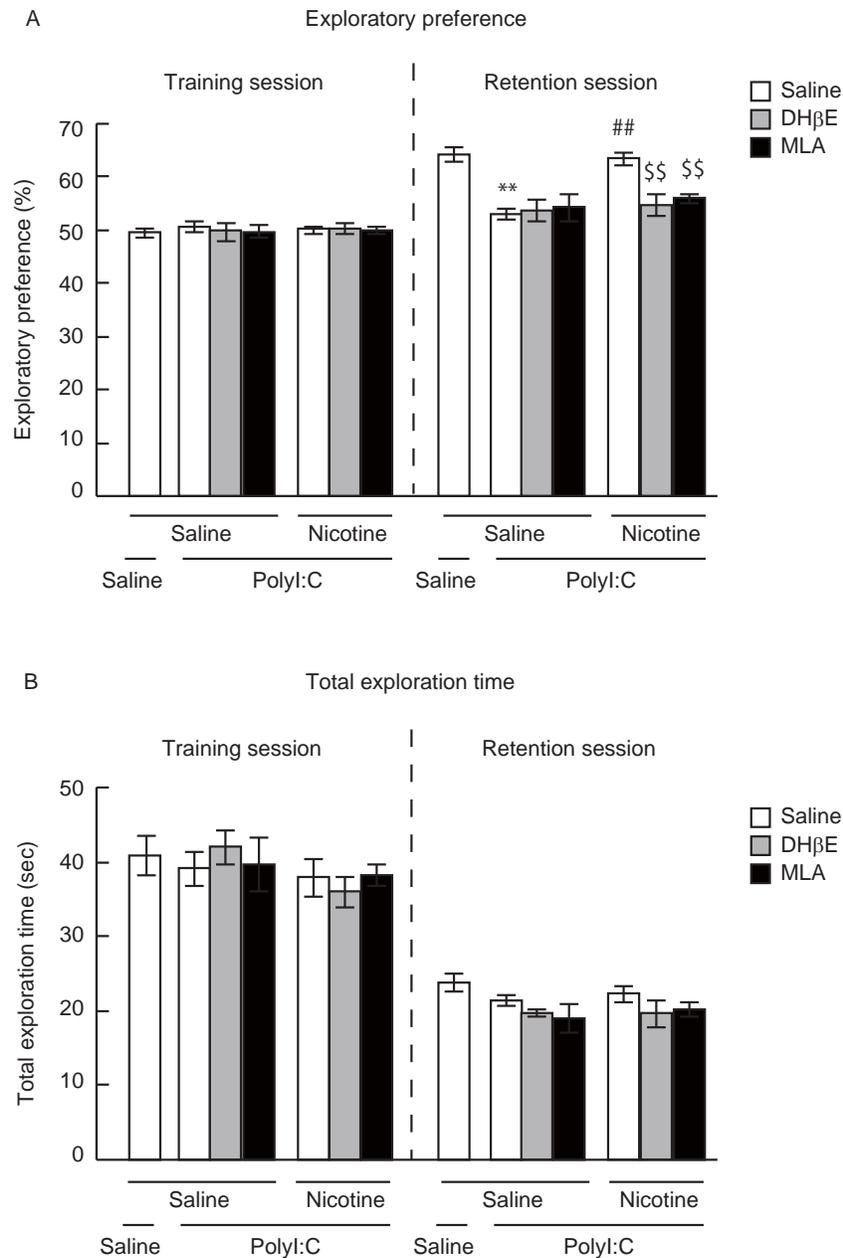


Fig. (7). Effects of nACh receptor antagonists on nicotine-induced amelioration of recognition memory impairment in polyI:C-treated mice. **(A)** Exploratory preference. **(B)** Total exploration time. $\alpha 4\beta 2$ nACh receptor antagonist DH β E (2.0 mg/kg, s.c.) or $\alpha 7$ nACh receptor antagonist MLA (5 mg/kg, s.c.) was administered 25 min before the training session. Nicotine (0.5 mg/kg, s.c.) was administered 15 min before the training session. The retention session was carried out 24 h after the training session. Values indicate the mean \pm S.E. (n=8-16). **p<0.01 vs. saline-treated control group (Student's *t*-test). ##p<0.01 vs. saline-treated polyI:C group (Fisher's LSD test). \$\$p<0.01 vs. nicotine-treated polyI:C group (Fisher's LSD test).

receptors are high-affinity $\alpha 4\beta 2$ nAChR and low-affinity $\alpha 7$ nAChR. High numbers of $\alpha 4\beta 2$ and $\alpha 7$ nAChR binding sites have been observed in several brain regions during the early developmental period [39, 40]. In the present study, we found that both antagonists for $\alpha 4\beta 2$ and $\alpha 7$ nAChRs completely blocked the effect of nicotine on anxiety, and social interaction and memory deficits in polyI:C-treated mice. These results suggest that the ameliorating effects of nicotine on abnormal behaviors in polyI:C-treated mice are mediated by the activation of $\alpha 4\beta 2$ and $\alpha 7$ nAChRs.

It has been reported that the number of $\alpha 7$ nAChRs is reduced in patients with schizophrenia [19, 41]. Functional polymorphisms have been identified in the promoter region of the $\alpha 7$ nAChR gene, which are associated with schizophrenia risk [42, 43]. In addition to $\alpha 7$ nAChR, decreased levels of $\alpha 4\beta 2$ nAChR binding have been found in the hippocampus of patients with schizophrenia [19]. $\alpha 4\beta 2$ nAChR agonists can produce a significant and long-lasting improvement of memory in aged rats and monkeys [44]. Taken together, these findings suggest that $\alpha 4\beta 2$ and $\alpha 7$

nAChRs might be molecular targets for treatment in schizophrenia.

The mechanisms by which nicotine ameliorates the schizophrenia-like behaviors *via* $\alpha 4\beta 2$ and $\alpha 7$ nAChRs remain to be determined. It is known, however, that presynaptic $\alpha 4\beta 2$ and $\alpha 7$ nAChRs stimulate neurotransmitter release, mainly dopamine, glutamate, serotonin, and noradrenaline neurotransmitters [45-49]. Interestingly, our previous study demonstrated that depolarization-evoked glutamate release in the hippocampus of polyI:C-treated mice is significantly lower than the response in saline-treated control mice [13]. Therefore, it is possible that stimulation of $\alpha 4\beta 2$ and $\alpha 7$ nAChRs may trigger glutamate release, contributing to the ameliorating effect of nicotine on emotional and cognitive dysfunction in polyI:C-treated mice. This issue should be resolved in further research.

In conclusion, single treatment with nicotine ameliorated various schizophrenia-like behavioral deficits in polyI:C-treated mice in adulthood although it had no effect on PPI deficits. The antipsychotic effects of nicotine were blocked by $\alpha 4\beta 2$ and $\alpha 7$ nAChR antagonists. These results support the hypothesis that nicotine may have some therapeutic benefits in treating clinical symptoms in schizophrenia.

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