d-Serine Ameliorates Neonatal PolyI:C Treatment–Induced Emotional and Cognitive Impairments in Adult Mice

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Abstract. Polyriboinosinic-polyribocytidilic acid (polyI:C) is a synthetic analog that elicits viral-like immune responses in mammals. We have recently found that polyI:C treatment in neonatal mice induced abnormalities of emotional, cognitive, and sensorimotor gating and dysfunction of glutamatergic neurotransmission in adulthood. In this study, we investigated the effect of the NMDA-receptor co-agonist d-serine on polyI:C-induced behavioral abnormalities in mice. Neonatal ICR mice were repeatedly injected with polyI:C for 5 days from postnatal day 2 to 6. At 10 weeks, sensorimotor gating function was analyzed in the prepulse inhibition (PPI) test. Emotional function was analyzed in open field and social interaction tests. Cognitive function was analyzed by novel object recognition tests. d-Serine dose-dependently improved polyI:C-induced impairment of emotional and cognitive behaviors whereas it had no effect on PPI deficit in adults. The ameliorating effects of d-serine were antagonized by pretreatment with an NMDA-receptor antagonist, MK-801. Although the mRNA level of d-amino acid oxidase (DAAO) was increased in the prefrontal cortex and hippocampus of neonatal polyI:C-treated mice in adulthood, no changes were observed in d-serine content and DAAO enzymatic activity. These results suggest that d-serine ameliorates emotional and cognitive impairments of the polyI:C-treated mice through potentiating NMDA receptor activity.

Keywords: cognition, emotion, d-serine, polyI:C, d-amino acid oxidase

Introduction

Maternal infection during pregnancy has been consistently associated with an increased risk of mental disorders in the offspring (1, 2). In particular, the association with schizophrenia has been found for numerous infectious agents, such as rubella, influenza, herpes simplex, toxoplasma gondii, measles, polio, and genital and reproductive infections (2). Clinical studies also suggest that severe viral infection in the central nervous system during the postnatal stage is involved in the etiology of psychiatric disorders (3 – 6). These findings have motivated the establishment of several rodent models of perinatal infection and/or immune activation, with the aim of exploring human epidemiological associations on experimental grounds (7, 8).

Numerous experimental investigations in rats and mice provide animal models of prenatal immune challenge by the viral mimic polyriboinosinic-polyriboctidilic acid (polyI:C). 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 toll-like receptor 3 (9). 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 (10 – 13): deficits in prepulse inhibition (PPI) in the acoustic startle response, deficient in exploratory behavior in both open field and novel object recognition.
tests, and deficient in social interaction. The polyI:C-induced brain dysfunctions are directly implicated in schizophrenia and other psychosis-related disorders.

We previously demonstrated that neonatal injection of polyI:C induces schizophrenia-like behavioral alterations in adulthood (14, 15). PolyI:C was subcutaneously (s.c.) administered to neonatal ICR mice as their neurevodevelopmental period matches the second trimester of human fetus having an immature blood–brain barrier and initiating glial proliferation (16, 17). Neonatal polyI:C-treated mice showed anxiety-like behavior in the open field test, impairments of object recognition memory in the novel object recognition test and social behavior in the social interaction test, and sensorimotor gating deficits in the PPI test (14). In parallel, an in vivo dialysis study revealed that depolarization-evoked glutamate release in the hippocampus was impaired in polyI:C-treated mice. These findings suggest that polyI:C treatment during the perinatal stage leads to the development of emotional and cognitive deficits in adolescence, which is accompanied by the dysfunction of glutamatergic neurotransmission in the hippocampus.

D-Serine, an endogenous co-agonist at N-methyl-D-aspartic acid (NMDA) receptors, is abundant in the central nervous system and present at lower concentration in the periphery (18). D-Serine is synthesized from L-serine by the enzyme serine racemase (SR) and is degraded by D-amino acid oxidase (DAAO). It has been reported that levels of D-serine in the serum or cerebrospinal fluid of patients with schizophrenia are lower than those of normal control subjects (19–22). Clinical trials examining the efficacy of high doses of D-serine have shown promising effects in patients with schizophrenia: D-serine reduces positive and negative cognitive deficits when administered in combination with antipsychotic medications (23–25).

In this study, we investigated the effects of D-serine and L-serine on polyI:C-induced impairment of sensorimotor gating, emotional and cognitive behaviors, in mice since D-serine has been shown to possess site- and stereo-selective actions on the NMDA-receptor glycine site to which it has a much higher affinity than the corresponding L-amino acid, L-serine (26, 27). We also measured the D-serine content, SR and DAAO mRNA levels, and DAAO activity in the brain.

Materials and Methods

Animals

Timed pregnant ICR mice were obtained from Japan SLC, Inc. (Hamamatsu) 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°C ± 1°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, D-serine, L-serine, and MK-801 were purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in saline. PolyI:C treatment was performed as previously described in our report (14). Briefly, all litters were randomly divided into saline- and polyI:C-treated groups. PolyI:C was dissolved in pyrogen-free saline. From PD 2 to 6, mice were injected s.c. and daily with either pyrogen-free saline (control group) or polyI:C at a dose of 5 mg/kg (polyI:C group). Animals were weaned at PD 21, divided by gender at PD 28, and group-housed post-weaning until use for behavioral and neurochemical analyses at 10 weeks. D-Serine (0.3 and 1.0 g/kg), L-serine (1.0 g/kg), or MK-801 (0.1 mg/kg) was intra-peritoneally (i.p.) administered to mice 30 min, 30 min, or 45 min before the behavioral tests, respectively. The dose of each drug was selected according to previous pharmacological reports (14, 28). The timing of the drug administration was referred to pharmacokinetics reports, which demonstrated the concentration of D-serine and MK-801 rapidly increase in the plasma and brain around 30 min and then gradually decrease (29, 30).

Behavioral analyses

Behavioral analyses were started at 10–12 weeks of age in the following order: PPI (Day 1), open field (Day 2), novel object recognition test (Day 3–7), and social interaction test (Day 8–10) for studying the effects of D-serine and L-serine (Figs. 1–4); open field (Day 1), novel object recognition test (Day 2–6), and social interaction test (Day 7–9) for studying the effects of MK-801 (Figs. 5–7). Two or three independent experiments were carried out in each experiment.

PPI test

The PPI test was carried out as described previously (31). After the animals were placed in the chamber (San Diego Instruments, San Diego, CA, USA), they were allowed to habituate for 10 min, during which time they
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 s and the total session lasted 17 min. The startle trial consisted of a single 120 dB white noise burst lasting 40 ms. PPI trials consisted of a prepulse (20 ms burst of white noise at 69, 73, 77, or 81 dB intensity) followed, 100 ms later, by the startle stimulus (120 dB, 40 ms 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 ms after startle stimulus onset (sampling frequency 1 kHz), rectified, amplified, and fed into a computer, which calculated the maximal response over the 100 ms. Basal startle amplitude was determined as the mean amplitude of the 10 startle trials. PPI was calculated according to the following formula: $100 \times \left[1 - \frac{(\text{PPx}/\text{P120})}{\%}\right]$, in which PPx is the mean amplitude of the 10 PPI trials (PP69, PP73, PP75, or PP80) and P120 is the basal startle amplitude.

Open field test
Mice were placed at the center of an open field (diameter, 60 cm; height, 35 cm) and allowed to explore it for 5 min, while their activity was measured automatically using the ethovision automated tracking program (Brain-science Idea Co., Ltd., Osaka) (32). 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 as well as travel distance ratio of inner vs. total travel distance in open field.

Novel object recognition test
A novel object recognition test was carried out as described previously (33). Mice were individually habituated to an open box [30 × 30 × 35 (height) 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 objects were a golf ball, wooden cylinder, and square pyramid, which were different in shape and color but similar in size. An animal was considered to be exploring the object when its head was facing the object or it was touching or sniffing the object. The time spent exploring each object was recorded by using a video camera and analyzed in a double-blind manner. 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.

Social interaction test
We used the experimental paradigm described by Tremolizzo et al. (34) 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 × 18 × 12 cm) for 2 days before the trial. We used 10- to 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 durations 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.

Quantitative analyses of DAAO and SR mRNA expression by real-time RT-PCR
DAAO and SR mRNA expression level was measured in the hippocampus of behaviorally naïve mice at the age of 10 weeks. Mice were decapitated and their brains were removed. Total RNA was isolated using the RNeasy Mini Kit (Qiagen, Hilden, Germany), as described by Ibi et al. (35). Total RNA isolated from the hippocampus was converted into cDNA using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, USA). Levels of mRNA expression were quantified using a 7300 Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA). Quantitative real-time PCR was performed in a volume of 25 μl with 500 ng of cDNA and 500 nM primers in the Power SYBR Green Master Mix (Applied Biosystems). Mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The primers used were as follows: 5′-GCAGAGGTCATCCAAACTATGG-3′ for DAAO forward, 5′-TGCACAACCCCGAGTGATT-3′ for DAAO reverse; 5′-GCTGGTCTGCTGTGAGAAGGA-3′ for SR forward, 5′-CAGTACTGAGACACATGGTTCTCT-3′ for SR reverse; 5′-TGCAAGCTCACTTCTTCTTCCCCTGTTATG-3′ for GAPDH forward, 5′-CTTACTCCTTGAGGCCCATGTAG-3′ for GAPDH reverse.
Assay of DAAO activity

DAAO activity was measured in the hippocampus and prefrontal cortex of behaviorally naïve mice at the age of 10 weeks. The brains were removed and kept frozen at −80°C until use. These organs were thawed by the addition of 7 mM pyrophosphate buffer (pH 8.3) and homogenized in a Potter-Elvehjem-type glass-Teflon homogenizer. The homogenates were centrifuged at 550 × g for 5 min. The supernatant solutions were used for the assay of DAAO activity. The enzyme activity was measured by the colorimetric method of Watanabe et al. (36) as described previously (37). Protein concentration in the supernatant solutions was determined according to the method of Lowry et al. (38) using bovine serum albumin as a standard. DAAO activity is expressed as the amount of d-alanine oxidized / min per mg protein.

Measurement of d-serine content

d-Serine content was measured in the hippocampus and prefrontal cortex of behaviorally naïve mice at the age of 10 weeks. Brain concentrations of d-serine were determined using high-performance liquid chromatography / tandem mass spectrometry. Brain homogenates were spiked with stable isotope–labeled d-serine as an internal standard followed by protein precipitation and solid phase extraction. Mass spectrometric analysis was performed on a TSQ Quantum triple quadrupole mass spectrometer (ThermoFisher Scientific, Kanagawa) using positive electrospray ionization with selected reaction monitoring. The calibration range was 5.00 – 500 nmol/g, and good accuracy and precision were obtained at all concentration levels tested.

Statistical analyses

Statistical significance was determined using Student’s t-test and one-way or two-way analysis of variance (ANOVA) with or without repeated measures followed by Fisher’s LSD post-hoc test when F ratios were significant ($P < 0.05$). Two-way ANOVA with repeated measures was used in the PPI experiment (Fig. 1A). Student’s t-test was used to analyze effect of polyI:C or l-serine (Figs. 2 – 8). One-way ANOVA was used to analyze the effect of d-serine in saline-treated or polyI:C-treated groups (Fig. 1: B and C, Figs. 2 – 4). Two-way ANOVA was used to analyze the effect of MK-801 (Figs. 5 – 7).

Results

Effects of d-serine and l-serine 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. Two-way ANOVA with repeated measures revealed the significant effects of prepulse intensity and polyI:C treatment [prepulse, $F(3,54) = 18.60$, $P < 0.01$; polyI:C, $F(1,18) = 45.51$, $P < 0.01$; prepulse × polyI:C, $F(3,54) = 2.87$, $P = 0.055$; Fig. 1A]. PolyI:C-treated mice showed a marked impairment of PPI compared with the saline-treated control group at all prepulse intensities (69, 73, 77, and 81 dB) ($P < 0.01$ by Fisher’s LSD test, Fig. 1A). Two-way ANOVA with repeated measures revealed no significant effects of d-serine were detected in polyI:C-treated groups [d-serine, $F(2,22) = 0.64$, $P = 0.54$; prepulse, $F(3,66) = 9.12$, $P < 0.01$; d-ser × prepulse, $F(6,66) = 0.87$, $P = 0.52$; Fig. 1A] and saline-treated control group [d-serine, $F(2,22) = 1.21$, $P = 0.32$; prepulse, $F(3,66) = 44.27$, $P < 0.01$; d-ser × prepulse, $F(6,66) = 0.68$, $P = 0.66$; Fig. 4A]. L-Serine also had no effect on performance of PPI in polyI:C-treated [l-serine, $F(1,15) = 0.01$, $P = 0.92$; prepulse, $F(3,45) = 3.26$, $P < 0.05$; l-ser × prepulse, $F(3,45) = 0.15$, $P = 0.93$; Fig. 1A] and saline-treated groups [l-serine, $F(1,15) = 3.08$, $P = 0.10$; prepulse, $F(3,45) = 47.52$, $P < 0.01$; l-ser × prepulse, $F(3,45) = 0.35$, $P = 0.79$; Fig. 1A]. Single treatment with d-serine (0.3 and 1.0 g/kg) and l-serine (1.0 g/kg) had no effects on acoustic startle amplitude (Fig. 1B) and baseline activity at background stimulus (65 dB) in the polyI:C-treated group or saline-treated control groups (Fig. 1C).

Effects of d-serine and l-serine on emotional deficits in polyI:C-treated mice in open field test

To investigate the effects of d-serine on emotional deficits in polyI:C-treated mice in adulthood, the 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. Vehicle-treated control mice showed that the time spent in the inner sector (43.4 ± 4.3 s) was significantly less than that in the outer sector (256.6 ± 4.3 s) ($P < 0.01$ by Student’s t-test, Fig. 2), indicating a natural aversion to illuminated open areas in our experimental condition. The time spent in the inner sector was significantly decreased while the time spent in the outer sector was significantly increased in polyI:C-treated mice compared with those of saline-treated control mice ($P < 0.01$ by Student’s t-test, Fig. 2: A and B). One-way ANOVA analysis revealed a significant effect of d-serine on the time spent in inner sectors [$F(2,22) = 9.02$, $P < 0.01$] and outer sectors [$F(2,24) = 9.86$, $P < 0.01$] in the polyI:C-treated group. Post-hoc analysis revealed that d-serine dose-dependently and significantly (1.0 g/kg) increased the time spent in the inner sector ($P < 0.01$ by Fisher’s
LSD test, Fig. 2B). Likewise, \( \alpha \)-serine (1.0 g/kg) treatment ameliorated the altered ratio of inner vs. total distance traveled in open-field in polyI:C-treated mice \([F(2,24) = 5.91, P < 0.01; \text{Fig. 2C}]\), whereas it had no effect on total distance traveled \([F(2,24) = 0.14, P = 0.87; \text{Fig. 2D}]\). \( \alpha \)-Serine itself had no effect on performance in the saline-treated control group \([\text{time spent in inner sector, } F(2,22) = 1.21, P = 0.78; \text{time spent in outer sector, } F(2,24) = 9.86, P = 9.86; \text{travel distance ratio of the inner sectors, } F(2,22) = 0.67, P = 0.52; \text{total distance traveled, } F(2,22) = 0.29, P = 0.75; \text{Fig. 2}]\), although the group treated with 1.0 g/kg dose of \( \alpha \)-serine appears to have a slight reduction of time spent in inner sectors and travel distance ratio. Furthermore, \( \beta \)-serine (1.0 mg/kg, i.p.) treatment had no effect on the performance in the saline-treated control \([\text{time spent in inner sector, } P = 0.65 \text{ by Student’s } t\text{-test}; \text{time spent in the outer sector, } P = 0.66 \text{ by Student’s } t\text{-test}; \text{travel distance ratio, } P = 0.94 \text{ by Student’s } t\text{-test}; \text{total distance traveled, } P = 0.84 \text{ by Student’s } t\text{-test}]\) and polyI:C-treated mice (time spent in inner sector, \( P = 0.08 \text{ by Student’s } t\text{-test}; \text{time spent in outer sector, } P = 0.07 \text{ by Student’s } t\text{-test}; \text{travel distance ratio, } P = 0.25 \text{ by Student’s } t\text{-test}]. \)

**Fig. 1.** Effects of \( \alpha \)-serine and \( \beta \)-serine on PPI deficits of the 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. \( \alpha \)-Serine (0.3 and 1.0 mg/kg, i.p.) or \( \beta \)-serine (1.0 mg/kg, i.p.) was administered 30 min before the behavioral test. C) Baseline activity at background stimulus (65 dB). Values indicate the mean ± S.E.M. \((n = 10 \text{ for the saline-treated control and polyI:C, } n = 8 \text{ for } \alpha\text{-serine } 1.0\text{-treated control and polyI:C, } n = 7 \text{ for other groups}). **P < 0.01 vs. corresponding saline-treated control group (Student’s \( t\text{-test}).)
Effects of D-serine and L-serine on deficits of object recognition memory in polyI:C-treated mice

To examine the effect of D-serine treatment on neonatal polyI:C treatment-induced memory impairment in adults, the novel object recognition test was carried out at the age of 10 – 12 weeks. During the training session, there was no biased exploratory index in either group (\(P = 0.35\) by Student’s \(t\)-test, Fig. 3A), and both polyI:C-treated and saline-treated control mice spent equal amounts of time exploring either one of two objects (\(P = 0.58\) by Student’s \(t\)-test, Fig. 3B), suggesting no differences in motivation and curiosity about novel objects and in motor function between polyI:C-treated and saline-treated control mice. The retention session was carried out 24 h after the training session. The level of exploratory index to the novel object was significantly decreased in polyI:C-treated mice compared with that in saline-treated control mice (\(P < 0.01\) by Student’s \(t\)-test, Fig. 3C). Total exploration time in the retention session did not differ between two groups (\(P = 0.30\) by Student’s \(t\)-test, Fig. 3D), suggesting that polyI:C-treated mice have impaired recognition memory in adulthood. In polyI:C-treated mice, D-serine had no effect on the level of exploratory index in the training session when D-serine was administered 30 min before the training session (\(F(2,22) = 0.49\), **\(P < 0.01\) vs. saline-treated control group (Student’s \(t\)-test). #\(P < 0.05\), ##\(P < 0.01\) vs. saline-treated polyI:C group (Fisher’s LSD test).
One-way ANOVA analysis revealed significant effect of D-serine on the level of exploratory index in the retention session in polyI:C-treated mice \(F(2,22) = 14.20, P < 0.01\); Fig. 3C). A single treatment with D-serine (1.0 g/kg) significantly improved cognitive impairment in polyI:C-treated mice \(P < 0.01\) by Fisher’s LSD test, Fig. 3C). Instead of D-serine, L-serine had no effect on the level of exploratory index in the training session \(P = 0.74\) by Student’s \(t\)-test) and retention session \(P = 0.68\) by Student’s \(t\)-test) in polyI:C-treated mice (Fig. 3A). The total exploration time in polyI:C-treated mice was not affected by D-serine and L-serine in either the training \(F(2,22) = 0.09, P = 0.91\) by one-way ANOVA analysis; L-serine, \(P = 0.55\) by Student’s \(t\)-test; Fig. 3B) or retention session \(F(2,22) = 1.04, P = 0.37\) by one-way ANOVA analysis; L-serine, \(P = 0.39\) by Student’s \(t\)-test; Fig. 3D). In saline-treated control mice, D-serine and L-serine had no effect on the level of exploratory index \(F(2,22) = 1.07, P = 0.36\) by one-way ANOVA analysis; D-serine in the retention session, \(F(2,22) = 0.09, P = 0.91\) by one-way ANOVA analysis; L-serine in the training session, \(P = 0.99\) by Student’s \(t\)-test; L-serine in the retention session, \(P = 0.16\) by Student’s \(t\)-test], or total exploration time \(F(2,22) = 2.91, P = 0.08\) by one-way ANOVA analysis; D-serine in the retention session, \(F(2,22) = 1.77, P = 0.38\) by one-way ANOVA analysis; L-serine in the training session, \(P = 0.99\) by Student’s \(t\)-test; L-serine in the retention session, \(P = 0.16\) by Student’s \(t\)-test] throughout the experiment, although the 1.0 g/kg dose of D-serine
appears to show a slight reduction of total exploration time in the training session (Fig. 3).

**Effects of d-serine and l-serine 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 \( \text{polyI:C, } F(1,18) = 17.82, P < 0.01; \text{ trial, } F(3,54) = 59.24, P < 0.01; \text{ polyI:C × trial, } F(3,54) = 0.04, P = 0.99 \) by repeated measured two-way ANOVA; data not shown. Therefore, total time for social interaction was evaluated in the following analysis. One-way ANOVA revealed that a significant effect of D-serine on social interaction \( \text{F(2,22) = 6.81, } P < 0.01; \text{ Fig. 4A} \) was observed in the polyI:C-treated group. A single treatment with d-serine (1.0 g/kg) significantly increased total time for social interaction in polyI:C-treated mice \( \text{F(2,22) = 2.19, } P = 0.10 \) by one-way ANOVA; Fig. 4A). d-Serine had no significant effect on escape \( \text{saline-treated control, } F(1,34) = 0.52, P = 0.60; \text{ polyI:C-treated control, } F(2,22) = 2.64, P = 0.10 \) by one-way ANOVA; Fig. 4B) or aggressive behavior \( \text{saline-treated control, } F(2,22) = 0.22, P = 0.81; \text{ polyI:C-treated control, } F(2,22) = 0.10, P = 0.90 \) by one-way ANOVA; Fig. 4C) in polyI:C-treated and saline-treated control mice.

Treatment with l-serine (1.0 g/kg) failed to improve deficits of social interaction in polyI:C-treated mice \( \text{F(1,34) = 0.10, } P = 0.72; \text{ polyI:C-treated group, } F(2,22) = 0.97 \) by Student’s \( t \)-test in polyI:C-treated and saline-treated mice.

**Effect of MK-801 on ameliorative effect of d-serine against emotional and cognitive deficits in polyI:C-treated mice**

To clarify the involvement of NMDA receptors in the ameliorative effect of d-serine on the emotional and cognitive deficits in polyI:C-treated mice, the mice were pretreated with a non-competitive NMDA-receptor antagonist, MK-801, prior to d-serine treatment. In the open field test, since two-way ANOVA indicated significant interactive effects of d-serine and MK-801 in time spent in inner sectors \( \text{F(1,34) = 5.79, } P < 0.05; \text{ Fig. 5A} \) and outer sectors \( \text{F(1,34) = 10.69, } P < 0.01; \text{ Fig. 5B} \), multiple comparisons were performed to determine

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**Fig. 4.** Effects of d-serine and l-serine on performance in the social interaction test in polyI:C-treated mice. A) Social interaction, B) escape behavior, and C) aggressive behavior. d-Serine (0.3 and 1.0 mg/kg, i.p.) or l-serine (1.0 mg/kg, i.p.) was administered 30 min before the behavioral test. Values indicate the mean ± S.E.M. (n = 10 for the saline-treated control and polyI:C, n = 8 for the n-serine 1.0–treated control and polyI:C, n = 7 for other groups). **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).
which group is statistically significant. The ameliorating effect of D-serine on anxiety-like behavioral changes in polyI:C-treated mice was completely blocked by pretreatment with MK-801 ($P < 0.05$ by Fisher’s LSD test, Fig. 5: A and B). Treatment with MK-801 alone did not affect the change in time spent in the inner ($P = 0.21$ by Fisher’s LSD test, Fig. 5A) or outer sector for salinetreated polyI:C mice ($P = 0.16$ by Fisher’s LSD test, Fig. 5B). Similar results were observed in the altered travel distance ratio of inner vs. total distance traveled in the open-field [D-serine × MK-801, $F(1,34) = 5.44, P < 0.05$ by two-way ANOVA; Fig. 5C]. Pretreatment with MK-801 blocked the ameliorating effect of D-serine on travel distance ratio of inner sector ($P < 0.05$ by Fisher’s LSD test, Fig. 5C), although it slightly increased total distance traveled in polyI:C-treated mice [$F(1,34) = 5.99, P < 0.05$ by two-way ANOVA, $P < 0.05$ by Fisher’s LSD test; Fig. 5D].

In the novel object recognition test, MK-801 significantly and completely blocked the ameliorating effect of D-serine on the impairment of object recognition memory in polyI:C-treated mice [D-serine, $F(1, 28) = 11.708, P < 0.01$; MK-801, $F(1, 28) = 4.920, P < 0.05$; MK-801 × D-serine, $F(1, 28) = 1.983, P = 0.170$ by two-way ANOVA; Fig. 6C]. Treatment with MK-801 did not affect the exploratory preference in saline-treated mice ($P = 0.57$ by Fisher’s LSD test) or the total exploration time in either the training or the retention session in all groups (Fig. 6: A, B, and D).

In the social interaction test, pretreatment with MK-801 significantly attenuated the ameliorating effect of D-serine in polyI:C-treated mice, although the same treat-
ment failed to affect saline-treated polyI:C mice [D-serine, F(1,28) = 13.49, P < 0.01; MK-801, F(1,28) = 10.89, P < 0.01; D-serine × MK-801, F(1,28) = 0.03, P = 0.87 by two-way ANOVA; Fig. 7A]. Furthermore, MK-801 had no effect on escape [D-serine, F(1,28) = 0.02, P = 0.90; MK-801, F(1,28) = 2.71, P = 0.11; D-serine × MK-801, F(1,28) = 0.08, P = 0.78 by two-way ANOVA; Fig. 7B] or aggressive behaviors [D-serine, F(1,28) = 0.07, P = 0.80; MK-801, F(1,28) = 2.44, P = 0.13; D-serine × MK-801, F(1,28) = 0.01, P = 0.92 by two-way ANOVA; Fig. 7C] in either saline-treated or D-serine–treated polyI:C mice.

**Fig. 6.** Effects of MK-801 on D-serine–induced amelioration of memory impairment in polyI:C-treated mice. A and C) Exploratory preference in training session (A) and retention session (C). B and D) Total exploration time in training session (B) and retention session (D). NMDA-receptor antagonist MK-801 (0.1 mg/kg, i.p.) was administered 45 min before the training session. D-Serine (1.0 mg/kg, i.p.) was administered 30 min before the training session. The retention session was carried out 24 h after the training session. Values indicate the mean ± S.E.M. (n = 8 for all groups). **P < 0.01 vs. saline-treated control group (Student’s t-test). #P < 0.05 vs. saline-treated polyI:C group (Fisher’s LSD test). $P < 0.05 vs. D-serine–treated polyI:C group (Fisher’s LSD test).**

D-Serine metabolism in the brains of polyI:C-treated mice

To investigate possible alterations of D-serine metabolism in polyI:C-treated mice, we examined the levels of SR and DAAO mRNA in the prefrontal cortex and hippocampus. No marked differences in the expression level of SR mRNA were observed between polyI:C-treated and saline-treated control groups (P = 0.61 by Student’s t-test, Fig. 8A; P = 0.47 by Student’s t-test, Fig. 8B). The mRNA level of DAAO was significantly increased in the prefrontal cortex and hippocampus of polyI:C-treated mice compared with that in saline-treated control mice (P < 0.05 by Student’s t-test, Fig. 8: C and D). DAAO activities and D-serine contents in the brains examined,
went, did not differ between polyI:C-treated and saline-treated control groups (Table 1, Table 2).

**Discussion**

Recent evidence indicates that the pathophysiology of schizophrenia involves widespread perturbation in several closely interacting neurotransmitter systems, such as dopamine, glutamate, and γ-aminobutyric acid, in the cortical and subcortical regions (39). NMDA-receptor hypofunction in particular has been proposed to contribute to the pathophysiology of schizophrenia. The NMDA-receptor hypofunction hypothesis of schizophrenia originates from the discovery that administration of NMDA-receptor antagonists, such as phencyclidine and ketamine, to schizophrenic patients exacerbated their core psychotic and cognitive symptoms (40 – 42) and induced a similar psychotic state in human volunteers (43 – 45). Now, several other lines of evidence suggest that NMDA receptors are involved (46).

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 (16, 17), 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 (47). According to these findings, we have developed a mouse model of viral infection during the perinatal period by repeatedly injecting polyI:C into neonatal ICR mice at PD 2 – 6 (14). 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 d-serine, but not L-serine, in a dose-dependent manner. In saline-treated control mice, since D-serine (1.0 g/kg) slightly, but not significantly, reduced time spent in inner sectors in the open field test and social interaction time in the social interaction test, the sedative-like effect of D-serine might be confounded. In contrast, D-serine significantly increased time spent in inner sectors and social interaction time in polyI:C-treated mice. Thus, D-serine may have a therapeutic benefit in ameliorating clinical symptoms in schizophrenia. It is plausible that neonatal polyI:C treatment interferes in the development of glutamatergic neurons, leading to abnormal behaviors in adulthood.

The ameliorating effect of D-serine on social interaction deficit was partial in polyI:C-treated mice, whereas the treatment completely improved anxiety-like behavior.

![Fig. 7. Effects of MK-801 on d-serine–induced amelioration of deficits of social behaviors in polyI:C-treated mice. A) Social interaction, B) escape behavior, and C) aggressive behavior. The NMDA-receptor antagonist MK-801 (0.1 mg/kg, i.p.) was administered 45 min before the behavioral test. D-Serine (1.0 mg/kg, i.p.) was administered 30 min before the behavioral test. Values indicate the mean ± S.E.M. (n = 8 for all groups). **P < 0.01 vs. saline-treated control group (Student’s t-test). *P < 0.05 vs. saline-treated polyI:C group (Fisher’s LSD test). $P < 0.05 vs. D-serine–treated polyI:C group (Fisher’s LSD test).]
in the open field test and impaired recognition memory in the novel object recognition test. Furthermore, D-serine failed to improve the PPI deficits in polyI:C-treated mice although both haloperidol and clozapine ameliorated these deficits (Nagai et al., unpublished observation). Similarly, the PPI disruptive effect of MK-801 can be reversed by clozapine, but not by D-serine (48). We have no precise explanation for the differential effect of D-serine on behavioral abnormalities in polyI:C-treated mice. One possible explanation is the insufficient concentration in the brain. Hashimoto et al. (28) have recently reported that co-administration of a potent DAAO inhibitor, 5-chloro-benzo[d]isoxazol-3-ol, significantly enhanced the efficacy of D-serine in attenuating MK-801-

![Fig. 8. Expression levels of SR and DAAO mRNA in the prefrontal cortex and hippocampus of polyI:C-treated mice. A and B) SR mRNA expression in the prefrontal cortex (A) and hippocampus (B). C and D) DAAO mRNA expression in the prefrontal cortex (C) and hippocampus (D). Mice were injected with either pyrogen-free saline or polyI:C (5 mg/kg, s.c.) from PD 2 to 6 and sacrificed when they were 10-week-old. Values indicate the mean ± S.E.M. (n = 6). *P < 0.05 vs. saline-treated control group (Student’s t-test).]

**Table 1.** Content of D-serine in the brain of polyI:C-treated mice

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>PolyI:C</th>
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<tbody>
<tr>
<td>Prefrontal cortex</td>
<td>209 ± 16.6</td>
<td>254 ± 10.6</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>262 ± 17.9</td>
<td>250 ± 17.3</td>
</tr>
</tbody>
</table>

Mean ± S.E.M. (n = 6).

**Table 2.** DAAO activity in the brain of polyI:C-treated mice

<table>
<thead>
<tr>
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<th>Saline</th>
<th>PolyI:C</th>
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<tbody>
<tr>
<td>Prefrontal cortex</td>
<td>5.49 ± 0.72</td>
<td>6.85 ± 1.15</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>4.58 ± 0.89</td>
<td>5.75 ± 0.16</td>
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</table>

V<sub>max</sub> (nmol/min per mg protein)

<table>
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<tr>
<th></th>
<th>Saline</th>
<th>PolyI:C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefrontal cortex</td>
<td>11.28 ± 1.22</td>
<td>14.58 ± 1.79</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>9.62 ± 1.84</td>
<td>13.68 ± 4.10</td>
</tr>
</tbody>
</table>

Mean ± S.E.M. (n = 5).
induced PPI deficits. Therefore, co-treatment with D-serine and DAAO inhibitor may be required to ameliorate PPI deficits in polyI:C-treated mice. Another possibility is the involvement of several different neuronal systems in the behavioral deficits of the polyI:C-treated animals. Vuillermot et al. (49) has demonstrated prenatal immune challenge by polyI:C induces dopamine-related structural changes including tyrosine hydroxylase, dopamine receptors, and dopamine transporter expression in the mesolimbic pathway and behavioral abnormalities in an age-dependent manner. Prenatally, immune activation leads to an increase in the levels of dopamine and its major metabolites in the lateral globus pallidus and prefrontal cortex, whereas it decreases serotonin and its metabolite in the hippocampus, nucleus accumbens, and lateral globus pallidus (50).

In addition to activation on the glycine site of NMDA receptors, D-serine serves as an endogenous ligand for δ2 glutamate receptor to regulate long-term depression at synapses between parallel fibers and Purkinje cells in the immature cerebellum (51). In the present study, we found that the NMDA-receptor antagonist MK-801 blocked the effect of D-serine on anxiety and social interaction and memory deficits in polyI:C-treated mice. These results suggest that the ameliorating effects of D-serine on abnormal behaviors in polyI:C-treated mice are mediated by the activation of NMDA receptors.

In the adult human and rodent brain, SR is expressed in forebrain areas, with high levels in the cortex and hippocampus (52), while DAAO is most abundant in the cerebellum and brainstem (53, 54). Several genetic linkage studies have shown an association of schizophrenia with single nucleotide polymorphisms in SR and DAAO (55 – 59). SR knockout mice, in which the first exon is deleted, show impaired spatial and recognition memory (60, 61). SR mutant mice that have complete loss of SR protein and activity due to a point mutation in the SR gene have demonstrated impairment in NMDA receptor–dependent spatial memory, deficits in social interaction, and sensorimotor gating deficit (62). In contrast, DAAO mutant mice that lack DAAO activity owing to a spontaneous missense mutation display enhanced reversal memory (63). Genetic inactivation of DAAO also improves social approach and spatial memory retention and induces reversal of abnormally persistent latent inhibition and a partial normalization of startle responses in NMDA-receptor mutant mice that results in a reduction of the affinity of the glycine site (64). Accordingly, we examined whether changes in the expression level of SR and DAAO contributed to behavioral abnormalities in the neonatal polyI:C model. We observed no differences in SR mRNA levels in the prefrontal cortex and hippocampus of polyI:C-treated mice compared to those in control mice. Moreover, there were no differences in DAAO activities and D-serine contents in the brains examined between the two groups, although the mRNA level of DAAO was significantly increased in the prefrontal cortex and hippocampus of polyI:C-treated mice. The reason for the discrepancy between DAAO mRNA and its activity remains unclear. DAAO mRNA may be unstable due to developmental compensatory changes in polyI:C-treated mice. Future study is needed to conclude the contribution of the endogenous D-serine pathway to behavioral abnormalities induced by neonatal polyI:C treatment.

In conclusion, the present study demonstrated that D-serine ameliorates emotional and cognitive impairments of polyI:C-treated mice through activation of NMDA receptors. Pharmacological interventions related to the D-serine pathway may have therapeutic potential for the treatment of neurodevelopmental psychiatric disorders such as schizophrenia.

Acknowledgments

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