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REGULAR RESEARCH ARTICLE

Prenatal Poly I:C Challenge Affects Behaviors and Neurotransmission via Elevated Neuroinflammation Responses in Female Juvenile Rats

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Abstract

Background: Exposure to polyriboinosinic-polyribocytidylic acid (Poly I:C) in pregnant rats has been reported to cause schizophrenia-like behaviors and abnormal neurotransmissions in adult, particularly male, offspring. However, what is less well understood are the effects of maternal Poly I:C exposure on adolescent behaviors and neurotransmission in female juvenile rats.
Methods: Female adolescent Poly I:C offspring were constructed by treating with 5 mg/kg Poly I:C on timed pregnant rats (gestation day 15). A battery of behavioral tests was conducted during postnatal day 35–60. Neurotransmitter receptors and inflammation markers in brain regions were evaluated by RT-qPCR on postnatal day 60.
Results: Open field, elevated plus maze, and forced swimming tests revealed that prenatal Poly I:C exposure led to elevated anxiety-like and depression-like behaviors in female adolescent offspring. Deficits in pre-pulse inhibition and social interaction were also observed. However, the Poly I:C rats had better performance than the controls in the novel object recognition memory test, which demonstrated a behavioral phenotype with improved cognitive function. Prenatal Poly I:C exposure caused brain region–specific elevation of the P2X7 receptor- and NF-κB-NLRP3-IL-1β inflammatory signaling in female juvenile rats. Prenatal Poly I:C exposure decreased expression of $GABA_A$ receptor subunits *Gabrb3* in the prefrontal cortex and *Gabrb1* and dopamine D2 receptor in the hippocampus, but increased NMDA receptor subunit *Grin2a* in the prefrontal cortex, 5-HT2A in the hippocampus, and *Gabrb3* and D2 receptor in the nucleus accumben.
Conclusions: Prenatal Poly I:C challenge causes behavioral deficits and brain-specific neurotransmission changes via elevated neuroinflammation responses in female adolescent offspring rats.

Keywords: Adolescent, behavior, female offspring, inflammation marker, maternal immune activation, neurotransmitter receptor

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Significance Statement

Maternal immune activation induced by bacterial or viral infection is associated with psychiatric disorders in the offspring, including autism and schizophrenia. Exposure to polyriboinosinic-polyribocytidylic acid (Poly I:C) in pregnant rats has been reported to cause schizophrenia-like behaviors and abnormal neurotransmissions in adult, particularly male, offspring. However, what is less well understood are the effects of maternal Poly I:C exposure on adolescent behaviors and neurotransmission in female juvenile rats. This study found that prenatal Poly I:C challenge causes behavioral deficits in female adolescent rats, while Poly I:C rats had better performance in the novel object recognition memory test. This study presented a behavioral phenotype with improved cognitive function in the Poly I:C model. Other important findings included that prenatal Poly I:C exposure had caused brain region-specific elevation of the P2X7 receptor– and NF-κB–NLRP3–IL-1β inflammatory signaling and region-specific neurotransmission changes in female juvenile rats.

INTRODUCTION

Maternal immune activation (MIA), an immune activation by bacterial or viral infection during pregnancy acting as a neurodevelopmental disease's primer, is sufficient to impact lifelong neuropathological and behavioral changes in the offspring via altered neuroimmune modulations (Solek et al., 2018). Double-stranded RNA synthetic analogue, polyinosinic-polycytidylic acid (Poly I:C) treatment, mimicking infection of pregnant rodent females or postnatal offspring, has been considered to be a well-validated MIA method modeling neurodevelopmental mental disorders, such as autism spectrum disorders and schizophrenia, in rodents (Murray et al., 2019). A number of rodent studies on maternal Poly I:C exposure showed that the full spectrum of behavioral abnormalities usually appears after the offspring have grown to late adolescence or early adulthood (Meyer, 2014; Brown and Meyer 2018). However, current research has largely ignored the disease progression at the adolescent stage. In addition, sex differences have been observed in behaviors of rodent MIA models for autism and schizophrenia (Kokras and Dalla 2014; Gogos et al., 2020). However, the majority of preclinical studies have been conducted in male and adult offspring only, largely to avoid possible influence by estrogens (Prendergast et al., 2014), while limited research in females showed conflicting results (Lins et al., 2019; Osborne et al., 2019). Therefore, this project investigated the behavioral effects of prenatal Poly I:C exposure in female adolescent rats.

In line with the long-lasting alterations of neuroinflammation markers in MIA offspring, previous studies, largely in male adult rats/mice, demonstrated that MIA caused deficits in dopaminergic, serotonergic, glutamatergic, and γ-Aminobutyric acid (GABA) neurotransmission (Bergdolt and Dunaevsky 2019). In addition, dopamine D2, 5-HT1A/2A, NMDA, and $GABA_A$ receptors are involved in the neurodevelopment and pathophysiology of various mental disorders in adolescents (Andersen 2003; Belujon and Grace 2017; Naaijen et al., 2017; De Santis et al., 2018; Fetit et al., 2021). The genes encoding the subunits of NMDA and $GABA_A$ receptors have been identified as candidate genes for neuropsychiatric disorders, including autism and schizophrenia (Purkayastha et al., 2015; Yang et al., 2017). However, it is not clear how maternal inflammation caused neurotransmission dysfunction in the brain. One potential link is the ATP-sensitive homomeric P2X7 receptor (P2X7R), since it acts as a key regulator in the inflammasome complex in inflammatory diseases (Khalafalla et al., 2017). Nucleotide-binding domain-like receptors (NLRP3), another key inflammasome component in the innate immune system, leads to the cleavage and release of pro-inflammatory cytokines such as pro-IL-1β (Rathinam et al., 2012). P2X7R activated NLRP3 and subsequently catalyzed the maturation of cytokines IL-1β (Karmakar et al., 2016). Thus, P2X7R-NLRP3-IL-1β may play a critical role in MIA-induced brain dysfunction. Therefore, this study investigated the effects of prenatal Poly I:C exposure on the expression of P2X7R, NLRP3, IL-1β, and various neurotransmitter receptors in the brain of female adolescent offspring.

MATERIALS AND METHODS

Animals and Treatment

Two batches of 14 pregnant Sprague-Dawley rats (supplementary Table 1) were obtained at gestation day (GD) 8 from the Animal Resource Centre (Perth, Australia). On GD15 (mid-late gestation), they were divided randomly into 2 groups: (1) one-half of pregnant rats received an i.p. injection with 5 mg/kg Poly I:C (Invivo Gen, Toulouse, France) dissolved in 0.2 mL 1% phosphate buffer saline (PBS); and (2) the other one-half were injected with an equivalent volume of PBS. Female pups were weaned on PD21 and then housed in Techniplast GR1800 double-decker rat ventilated cages (Tecniplast Australia, Lane Cove, NSW, Australia) with a divider under environmentally controlled conditions (22°C, light/dark cycle: 7:00 AM–7:00 PM/7:00 PM–7:00 AM). Each cage housed 2 rats from the same treatment group, and the divider separated the cage into 2 chambers of equal size with its own enrichment devices, including a plastic tunnel, a wood stick, and nesting materials with corncob bedding. All animals were allowed ad libitum access to water and standard laboratory chow diet. The divider with perforated holes allows 2 rats to see, hear, and smell each other. All of female offspring (34 Poly I:C and 36 control) pups from batch 1 were used for the behavioral tests. In batch 2 (supplementary Table 1), 1 female pup from each dam (of 6 Poly I:C and 6 saline dams) was randomly taken for quantitative real-time polymerase chain reaction (qRT-PCR) analysis. All experimental procedures were approved by the Animal Ethics Committee, University of Wollongong, Australia (AE17/12).

Behavioral Tests

A series of 6 behavioral tests was conducted during PD35-60 (supplementary Figure 1) (McIlwain et al., 2001; Blokland et al., 2012). All behavioral experiments were performed between 9:30 AM and 5:00 PM and recorded using video cameras for further analysis.

Open Field Test (PD35-37)—The open field test, conducted to examine locomotor activity and exploratory behavior, followed the procedures reported previously (Lian et al., 2015). A single rat was placed in the center of a black rectangular arena (60 cm × 60 cm) with 40-cm-high walls. The light intensity was 20–30 lx (average 25 lx) across the entire arena. Rat behavior

in the field was recorded for 30 minutes. Video images were analyzed using EthoVision video-tracking software (Noldus Information Technology, Wageningen, Netherlands), in which the arena was divided into a center (30-cm × 30-cm square in the inner space) and periphery zones (outside the inner space) to measure the moving distance and time spent in different zones. Rearing times were also calculated.

Social Interaction (PD38-39)—As previously reported (De Santis et al., 2016), the social interaction test was performed to examine social and anxiolytic behaviors in a black circular arena (90-cm diameter × 30-cm-high walls) made of black acrylic, with light intensity at average 25 lx across the entire arena. Rats had 10 minutes' habituation in the arena per day before the test. Two similar sized but unfamiliar rats from the same treatment group were placed on opposing sides of the arena and allowed to move around freely. Behavior was recorded for 7 minutes. All rats were tested once only. EthoVision software was used to analyze (1) time spent within a given proximity (set at 20 cm – average body length) of one another, (2) mean distance between each other, and (3) duration of the relative movement between the 2 rats (total time of the 2 rats approach each other). Data gathered from each social interaction test involving 2 rats were analyzed as a single data point.

Novel Object Recognition Memory Test (PD42-46)—The novel object recognition memory test ,which was conducted for assessing cognitive function, contained 3 phases (Lueptow, 2017): (1) habituation phase in which a rat was placed in the arena (60 cm × 60 cm) that had been used for the open field test previously to habituate for 10 minutes each time for 3 days; (2) training phase in which 2 similar sized objects, but different in shape and color, were placed at opposite corners of the testing arena. Each rat was allowed to explore freely in the arena for 10 minutes. The rat was considered to be exploring the object when its head was touching the object or the rat was standing on the object. The time that the rat spent exploring each object was recorded; and (3) test phase: on the next day, one of the familiar objects during training was replaced by a novel object. The rat was allowed to explore freely for 5 minutes, and the time spent exploring each object was recorded. The used objects were counter-balanced throughout the experiment in terms of their physical complexity and emotional neutrality.

Elevated Plus Maze Test (PD48/49)—The elevated maze is a black wooden structure elevated 60 cm above the floor used to test anxiogenic/anxiolytic behavior (De Santis et al., 2016). It consisted of 2 open arms (50 cm × 7 cm) and 2 closed arms (50 cm × 7 cm × 30 cm) with an open roof arranged around a central platform (7 cm × 7 cm). Like-arms opposed each other across the central platform. The light intensity was set at 100 lx at the open arms and central platform. A single rat was placed on the central platform of the elevated maze facing an open arm and was recorded for 7 minutes. The number of open and closed arm entries (whole body and 4 paws entry), arm changes, as well as the duration of time spent in the open and closed arms, central platform and "open part" (open arms plus central platform) were scored manually. The rat was considered to be in the central platform zone if its head and front paws were in the central platform, with its body positioned in the closed arm.

Pre-pulse Inhibition (PPI) of the Acoustic Startle Response (ASR) (PD51-56)—PPI of ASR was assessed to evaluate sensorimotor gating in the startle chamber (SR-LAB, San Diego, CA), which contained a plexiglass cylinder (20 cm long × 8.9 cm diameter) mounted with a piezoelectric accelerometer underneath, which detected the vibrations caused by the movements of the animals. Before the formal test, all rats were placed in the plexiglass cylinder in the startle chambers to habituate for 10 minutes daily for 3 days. During the test period, they first underwent a 5-minute acclimation phase under constant background noise (70 dB white noise) to assess background level of activity. Then, 100 trials were evaluated for each rat, including 5 trials of maximal startle pulses (120 dB, 20 ms), 30 trials of startle stimulus intensity from 70 dB to 120 dB (10 dB between each stimuli, 5 trials for each stimulus) to assess ASR, 60 trials with a pre-pulse to assess PPI, and ended with the replication of 5 initial startling pulses (120 dB) allowing for the assessment of within-test habituation of the ASR. For the PPI trials, 80 dB was used as pre-pulse intensity, 110 dB as startle pulse, and 6 different pulse intervals (8, 16, 32, 64, 128, and 256 ms; 10 trials each) of pre-pulse to assess maximal PPI. The startle response was measured by the average amplitude of each trial type. PPI was calculated using the formula [(amplitude of trial without pre-pulse – amplitude of trial with pre-pulse)/amplitude of trial without pre-pulse] ×100 (Kesby et al., 2012).

Forced Swimming Test (PD57-60)—The forced swimming test was modified from previous designs looking at depressive-like behaviors (De Santis et al., 2016). A blue plastic cylinder (30 cm × 50 cm) was filled with water at 29 ± 1°C to a predetermined level and adjusted the depth to 1.25 of the rat length. Rats were tested over 2 days at a light intensity of 100 lx. On the first day, a rat was placed in the water and removed 10 minutes later. On the second day, the rat was placed in the cylinder with fresh water for 5 minutes. Before returning to its own cage, the rat was dried with towels and then warmed under a heat lamp for 20 minutes. The time spent floating (immobile, no movement of the paws except for that required to keep its head above water), climbing (vertical movements of the forepaws, usually up against the cylinder), and swimming (movement throughout the cylinder, usually in a horizontal direction) were evaluated manually.

Quantitative Real-Time PCR (qRT-PCR)

Rats were killed by isoflurane anesthesia and decapitation on PD60. The whole brain was immediately frozen in liquid nitrogen and then stored at –80°C. Since pathological changes in the nucleus accumbens (NAc), caudate putamen (CPu), hippocampus (Hip), and prefrontal cortex (PFC) were reported in MIA animal models and various neuropsychiatric disorders (Bakhshi and Chance 2015; Belujon and Grace 2017; Bergdolt and Dunaevsky 2019; Fetit et al., 2021), these brain regions were dissected using micro-punches in a cryostat based on the rat brain atlas (Paxinos and Watson 2007). Total RNA was isolated using the PureLink RNA Mini Kit (#12183025; Invitrogen, Carlsbad, CA), and quantified by NanoDrop2000 (ThermoFisher, Waltham, MA). cDNA was converted using the High Capacity cDNA Reverse transcription kit (#4368814; ThermoFisher, Waltham, MA). qRT-PCR was performed in duplicate on a Quant Studi qRT-PCR system (ThermoFisher, Waltham, MA) using SYBR Green PCR Master Mix (Life Technologies, Sydney, NSW, Australia) for *Rela* encoding the subunit of NF-kappaB (forward primer: gggatggttctatgaggctgaac, reverse primer: cttgctccaggtctcgcttcttc), and TaqMan Gene Expression Assays (Life Technologies, Sydney, NSW, Australia) for *P2rx7* (Rn00570451_m1), *Nlrp3*(Rn04244620_m1), *Il-1b* (Rn00580432_m1), *Grin1* (Rn01436034_m1), *Grin2a* (Rn00561341_m1), *Grin2b* (Rn00680474_m1), *Gabrb1* (Rn00564146_m1), *Gabrb2* (Rn00564149_m1), *Gabrb3* (Rn00567029_m1), *Htr1a* (Rn00561409_s1), *Htr2a* (Rn00568473_m1), *Drd2* (Rn00561126_m1), β-*actin* (Hs01060665_g1) and *Gapdh* (Rn01775763_g1). Target gene relative expression was normalized by the housekeeping genes β-*actin* and *Gapdh*, then calculated according to the 2-ΔΔCT method.

Statistical Analysis

Data from all experiments were analyzed on SPSS25.0 (IBM, Armonk, New York, USA). A Kolmogorov-Smirnov test was applied to examine their distribution before further analysis. For data with normal distribution, a Student *t* test was used to compare the difference between Poly I:C and saline-treated groups. A nonparametric Mann–Whitney U test was performed for data without normal distribution. Statistical significance was accepted when $P<.05$. The results were presented as the mean ± SEM.

RESULTS

Effects of Maternal Poly I:C Exposure on Adolescent Behaviors

Open Field Test—As presented in Table 1, there were no significant differences between Poly I:C and saline-treated offspring in total travel distance, travel velocity, and total duration of moving in the whole arena. However, compared with the saline-treated animals, Poly I:C offspring showed significantly less center duration ($t=1.721$, $df=65$, $P=.045$). Consistently, Poly I:C rats spent significantly longer in the periphery ($t=1.865$, $df=64$, $P=.033$), while Poly I:C had no significant difference in travel distance in the periphery zone ($P>.05$). In addition, Poly I:C offspring showed significantly lower rearing frequency than that of saline-treated rats ($t=1.862$, $df=65$, $P=.034$).

Elevated Plus Maze Test—Maternal Poly I:C exposure significantly increased closed arm duration ($t=1.765$, $df=66$, $P=.041$; Figure 1A) compared with the control group. However, Poly I:C rats significantly decreased the time spent in the center platform ($t=2.701$, $df=66$, $P=.004$) and the open part ($t=2.456$, $df=65$, $P=.008$) compared with the control (Figure 1A). Interestingly, Poly I:C treatment also resulted in a significant increase in closed arm entries ($t=2.031$, $df=65$, $P=.023$), but not in open arm entries ($t=0.683$, $df=66$, $P=.248$; Figure 1B).

Forced Swimming Test—Although there was no significant difference in climbing duration between Poly I:C and saline-treated offspring ($t=0.988$, $df=66$, $P=.163$; Figure 2A), Poly I:C rats showed a significantly longer floating time ($t=2.510$, $df=66$, $P=.007$; Figure 2B) but a shorter swimming duration than the saline group ($t=2.410$, $df=66$, $P=.009$; Figure 2C).

Social Interaction Test—The mean of distance between 2 rats was significantly larger in Poly I:C treatment offspring than in controls ($t=2.065$, $df=31$, $P=.024$; Figure 3A). Consistently, an analysis of the relative movement between the 2 rats showed that the duration for moving away was significantly longer in Poly I:C rats than that of the controls ($t=1.834$, $df=31$, $P=.038$; Figure 3B). On the other hand, Poly I:C rats spent significantly less time in proximity than the controls ($t=1.923$, $df=30$, $P=.032$; Figure 3C).

Novel Object Recognition Test—In the training session, there was no difference in exploration time on either Object 1 or Object 2 between Poly I:C-treated and saline-treated juvenile rats (Figure 4A). In the testing session, Poly I:C rats showed significantly longer exploration time on the novel object than the controls ($t=2.851$, $df=65$, $P=.003$); however, there was no significant difference on the familiar object ($t=0.736$, $df=65$, $P=.232$; Figure 4B). The discrimination index [(time spent exploring the novel object – time spent exploring the familiar object)/(time spent exploring the novel object + time spent exploring the familiar object] in the Poly I:C group was significantly higher than that of the saline group ($t=2.171$, $df=66$, $P=.017$; Figure 4C).

Pre-Pulse Inhibition Test (PPI)—As shown by the "S" curve in Figure 5A, the startle amplitude in both saline- and Poly I:C-treated rats was not significantly different at 80 and 90 dB from 70 dB (all $P>.05$), while the startle response was sharply and significantly increased at 110 dB (saline: $t=8.337$, $df=66$, $P<.001$; Poly I:C: $t=8.927$, $df=62$, $P<.001$). Interestingly, Poly I:C rats had a relatively lower startle amplitude at 110 dB than those of the control ($t=1.909$, $df=66$, $P=.030$), while this difference disappeared at 120 dB ($t=0.297$, $df=66$, $P=.383$). The PPI tests revealed that the PPI index in Poly I:C-treated rats was significantly decreased at the 256-ms stimulus interval compared with saline-treated rats ($t=1.670$, $df=65$, $P=.0499$), while no significant differences in other pre-pulse intervals were observed (Figure 5B). These data indicated a PPI deficit was exhibited in juvenile female rats with maternal Poly I:C exposure. The habituation test revealed that the intensities of startle amplitude in response to 120 dB in the final 5 tests were slightly lower than those in the first 5 tests, but not significantly, in both Poly I:C- and saline-treated groups, and there was also no significant difference between the 2 groups (Figure 5C).

Effects of Maternal Poly I:C Exposure on the Expression of Neurotransmitter Receptors

GABAA Receptors—The expression of *Gabrb1* mRNA was significantly decreased by maternal Poly I:C exposure in the hippocampus ($t=2.372$, $df=9$, $P=.021$). Moreover, a significant decrease of *Gabrb3* mRNA expression was observed in the PFC as well as the CPu (PFC: $t=2.139$, $df=10$, $P=.029$; CPu: $t=2.542$ $df=10$, $P=.015$; Figure 6B,D). On the other hand, *Gabrb3* mRNA expression was significantly upregulated in the NAc region ($t=2.11$, $df=9$, $P=.032$; Figure 6C). No significant difference was observed in the rest of the $GABA_A$ receptor subtypes among the 4 brain regions (Figure 6A–D).

Table 1. Behaviors in the Open Field Test

Groups	Saline (n = 35)	Poly I:C (n = 34)
Total travel distance (cm)	7291.79 ± 332.37	6936.84 ± 364.50
Average velocity (cm/s)	4.06 ± 0.19	3.94 ± 0.20
Total duration of moving (%)	47.11 ± 2.09	47.73 ± 1.98
Travel distance in the periphery (cm)	6637.68 ± 291.36	6403.01 ± 327.43
Periphery duration (s)	1729.91 ± 7.02	1746.27 ± 4.63*
Travel distance in the centre (cm)	652.94 ± 56.22	533.83 ± 51.69[a]
Centre duration (s)	68.48 ± 6.93	53.52 ± 4.63*
Rearing frequency	82.39 ± 7.71	63.42 ± 6.34*

[a]$P=.065$, saline vs Poly I:C groups. Data are presented as mean ± SEM. *$P<.05$.

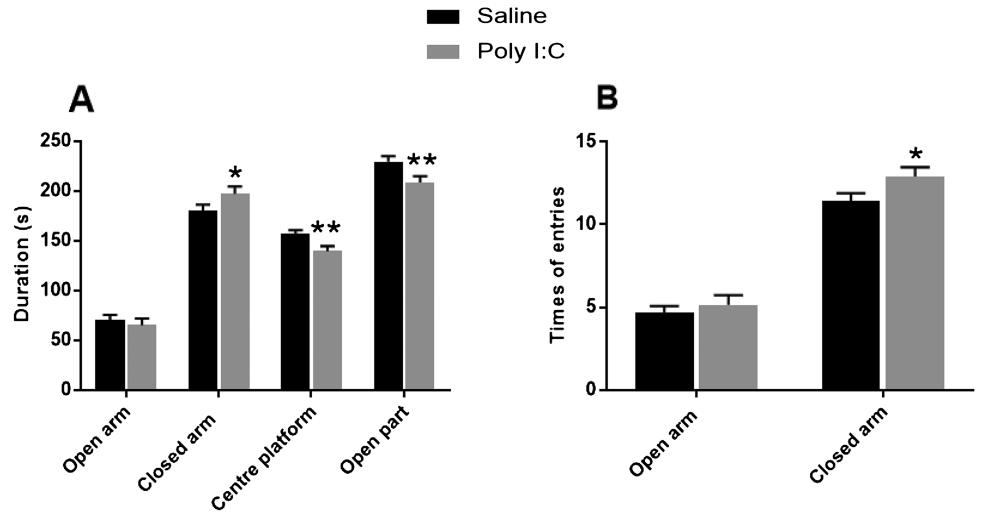

Figure 1. Behaviors in the elevated plus maze test. (A) Duration spent in closed arms, centre platform and open part. (B) Times of open arms and closed arms entries. Data were presented as mean ± SEM (saline n = 35, Poly I:C n = 34). *P < .05, **P < .01, Poly I:C vs saline.

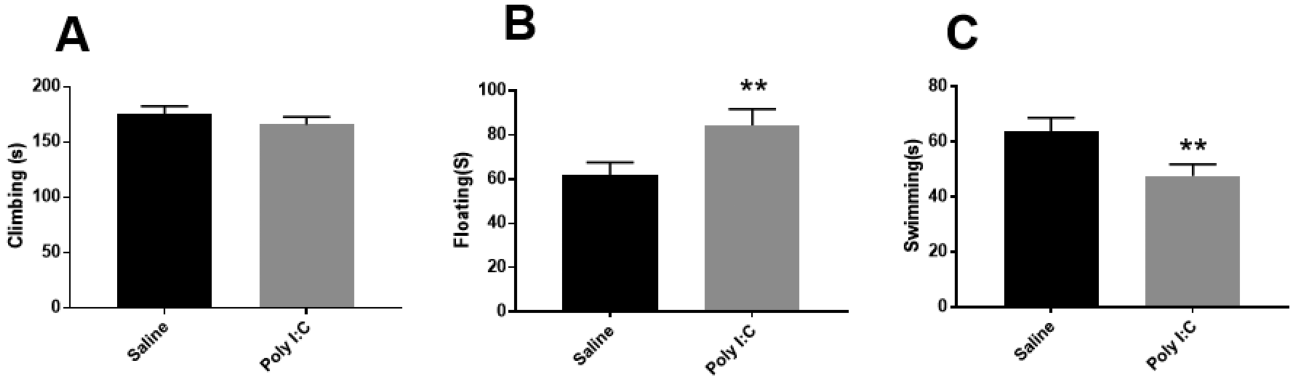

Figure 2. Behaviors in the forced swimming test. (A) Duration for climbing. (B) Duration for floating. (C) Duration for swimming. Data were presented as mean ± SEM (saline n = 35, Poly I:C n = 34). **P < .01, vs saline.

NMDA Receptors—The offspring from the mother with maternal Poly I:C exposure had a significant decrease in mRNA expression of the NMDA receptor *Grin1* subunit in the hippocampus ($U_{6,6} = 7$, $P = .044$; Figure 6A) but a significantly increased mRNA expression of the *Grin2a* subunit in the PFC ($t = 1.841$, df = 10, $P = .048$; Figure 6B) compared with the control. However, there were no significant differences in mRNA expression of the *Grin2b* subunit in these brain regions (Figure 6A,B). In addition, the expression of *Grin1*, *Grin2a*, *and Grin2b* in the NAc and CPu was not affected by maternal Poly I:C exposure (Figure 6C,D).

Serotonin 5-HT1A, 5-HT2A, and Dopamine D2 Receptors—*Htr2a* mRNA expression in the PFC of Poly I:C-treated rats was significantly downregulated compared with the control ($t = 1.902$, df = 10, $P = .043$; Figure 6B), while no significant difference in *Htr2a* mRNA expression was observed in the other 3 brain regions (all $P > .05$; Figure 6A,C,D). No significant changes in *Htr1a* mRNA expression were observed in the 4 regions examined (all $P > .05$; Figure 6A–D). The *Drd2* mRNA level in the hippocampus of female juvenile rats with Poly I:C treatment was significantly lower than in the saline-treated rats ($t = 2.293$, df = 10, $P = .022$; Figure 6A), while it was significantly increased in the NAc of the Poly I:C rats ($t = 2.439$, df = 10, $P = .019$; Figure 6C). However, no significant difference in *Drd2* mRNA expression in the PFC and CPu was observed (all $P > .05$, Figure 6B,D).

Effects of Maternal Poly I:C Exposure on the P2X7R and NF-κB-NLRP3-IL-1β Inflammatory Pathway

The mRNA expression of *Il1b* was significantly increased in the hippocampus, PFC, and NAc of juvenile female rats with maternal Poly I:C exposure ($U_{6,5} = 5$, $P = .041$ in hippocampus; $t = 3.415$, df = 10, $P = .003$ in PFC; $t = 3.378$, df = 10, $P = .004$ in NAc; Figure 7A–C). The mRNA expression of the *Rela* encoding NF-κB subunit was significantly increased in all of the brain regions examined ($U_{6,6} = 3$, $P = .008$ in hippocampus; $t = 1.903$, df = 10, $P = .043$ in PFC; $t = 2.818$, df = 10, $P = .010$ in NAc; $t = 2.021$, df = 10, $P = .035$ in CPu; Figure 7A–D). The expression of *Nlrp3* mRNA was significantly increased in the PFC and NAc of the Poly I:C group ($t = 1.949$, df = 10, $P = .040$ in PFC; $t = 2.082$, df = 10, $P = .036$ in NAc; Figure 7B,C). In the NAc, significantly increased mRNA expression of *P2rx7* was also observed ($t = 1.937$, df = 10, $P = .044$; Figure 7C). However, maternal Poly I:C exposure did not have any significant effects on the mRNA expression of these markers in the CPu (Figure 7D).

DISCUSSION

The present study revealed that a single dose i.p. injection of 5.0 mg/kg Poly I:C at GD15 caused both anxiety- and

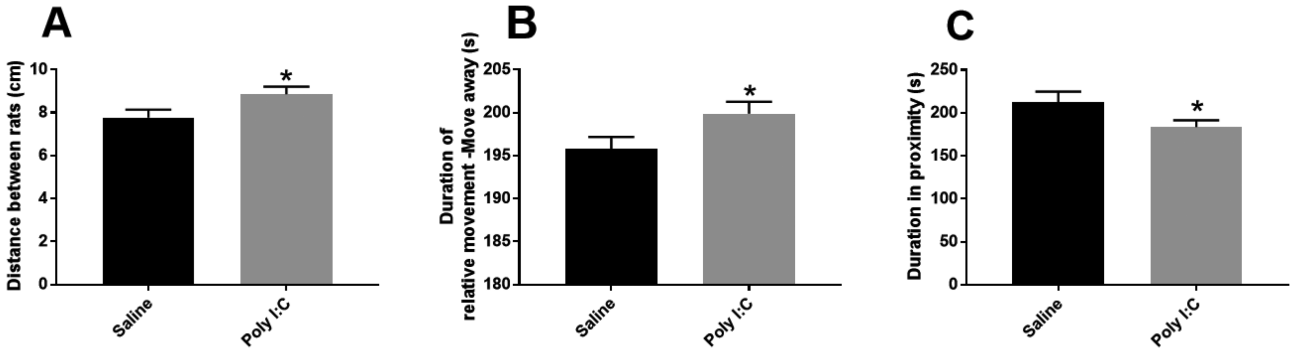

Figure 3. Behaviors in the social interaction test. (A) Mean of distance between 2 rats. (B) Duration of relative movement (total time of the 2 rats approach each other). (C) Duration in proximity. Data were presented as mean ± SEM. The sample size was 17 pairs per group. *P < .05 vs saline.

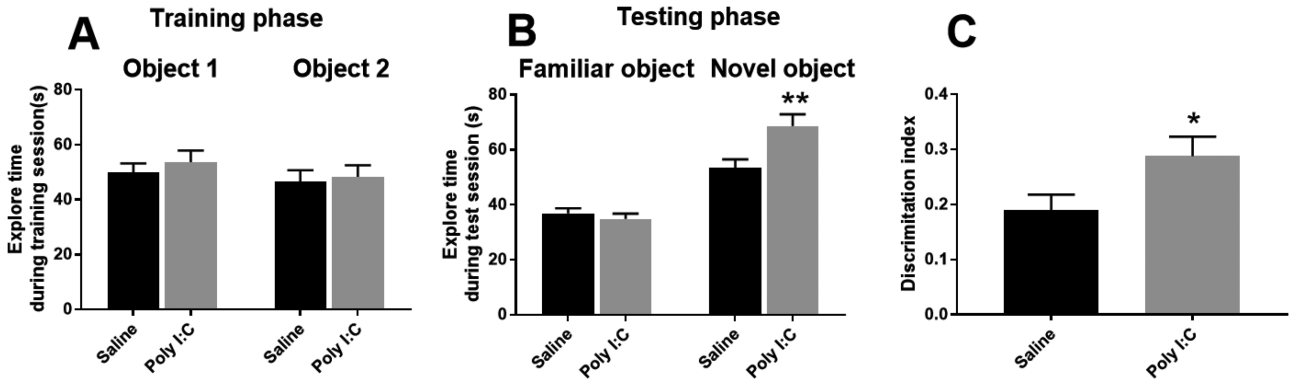

Figure 4. Behaviors in the novel object recognition test. (A) Exploring time during training session. (B) Exploring time during testing session. (C) Discrimination index. Data were presented as mean ± SEM (saline n = 35, Poly I:C n = 34). *P < .05, ** P < .01 vs saline.

depressive-like behaviors, deficits in social behavior and sensorimotor gating, and an unexpected improvement in discrimination ability in adolescent female rats. The behavioral and neuronal effects of prenatal Poly I:C exposure on offspring appear to be dependent on the dosage used and timing of treatment (Murray et al., 2019; Haddad et al., 2020). Poly I:C treatment at early gestation (such as GD9/10) causes dopamine-related positive symptom-like behavioral changes, while its exposure at mid-late gestation (such as GD15/17/19) leads to cognitive deficits and negative symptom-like behavioral changes in adult rat offspring (Meehan et al., 2017; Gogos et al., 2020). Since Poly I:C exposure at GD15 may intersect these 2 windows of sensitivity (Meyer et al., 2006; Gogos et al., 2020), a single 5.0 mg/kg i.p. injection of Poly I:C at GD15 was used in this study. Previously, a majority of studies in the rat MIA model have used single 4-5 mg/kg Poly I:C exposure via i.v. injection at GD14.5-15 (Haddad et al., 2020). On the other hand, in most of the studies in the mouse MIA model, Poly I:C was delivered via i.p. injection (Haddad et al., 2020). To date, there was no evidence to show any differences in offspring phenotypes through the 2 administration routes (i.v. vs i.p.) of Poly I:C. Our results in this study provided evidence that prenatal Poly I:C exposure via i.p. injection is effective to establish a rat MIA model in offspring phenotypes with behavioral and neurotransmission deficits as well as abnormal neuroinflammation responses.

Previous animal studies in rodent Poly I:C models reported that the anxiety- and depressive-like behaviors are sex dependent and more frequently appeared in males (Majidi-Zolbanin et al., 2015). For example, in adult male, but not female, C57BL/6 mice, offspring from mothers with i.p. injections of 20 mg/kg Poly I:C at GD12 presented elevated anxiety- and depressive-related behaviors (Majidi-Zolbanin et al., 2015; Arenas et al., 2017). A recent study in rats reported that 10 mg/kg Poly I:C treatment at GD9 resulted in progressively worsening anxiety-like behaviors in male adolescent and adult offspring (Hao et al., 2019). Previous studies indicated that 5-HTergic, dopaminergic, and GABAergic dysfunction in the hippocampus, PFC, and NAc are related to anxiety- and depression-like behaviors (Lever et al., 2006; Belujon and Grace, 2017; Fogaça and Duman, 2019; Villas-Boas et al., 2021). In this study, we observed the downregulated expression of 5-HT2AR and GABAA β3 in the PFC, D2R, and GABAA β1 in the hippocampus and an upregulation of D2R mRNA in the NAc. Taken together, our results first revealed that Poly I:C prenatal challenge in mid-gestation caused anxiety- and depression-like behaviors in adolescent female offspring, and this alteration may be correlated with brain-region specific modulations of GABAA, 5-HT2A, and dopamine D2 receptors.

Impaired sensorimotor gating assessed by PPI test has been repeatedly reported in both male and female adult offspring of rats with prenatal Poly I:C exposure (Carreno et al., 2020; Gogos et al., 2020); however, the results are not consistent in adolescent offspring. Recently, it has been reported that early gestation exposure to Poly I:C at GD9 did not cause PPI deficits in male adolescent offspring (Ding et al., 2019; Hao et al., 2019), while Poly I:C exposure at GD15 led to PPI deficits in male adolescent offspring (Wolff and Bilkey 2008). This study extended these findings to female adolescent offspring with prenatal Poly I:C exposure at GD15, finding that PPI deficits emerged in a 256-ms interstimulus interval. The $GABA_AR$ α3 knockout mice exhibited

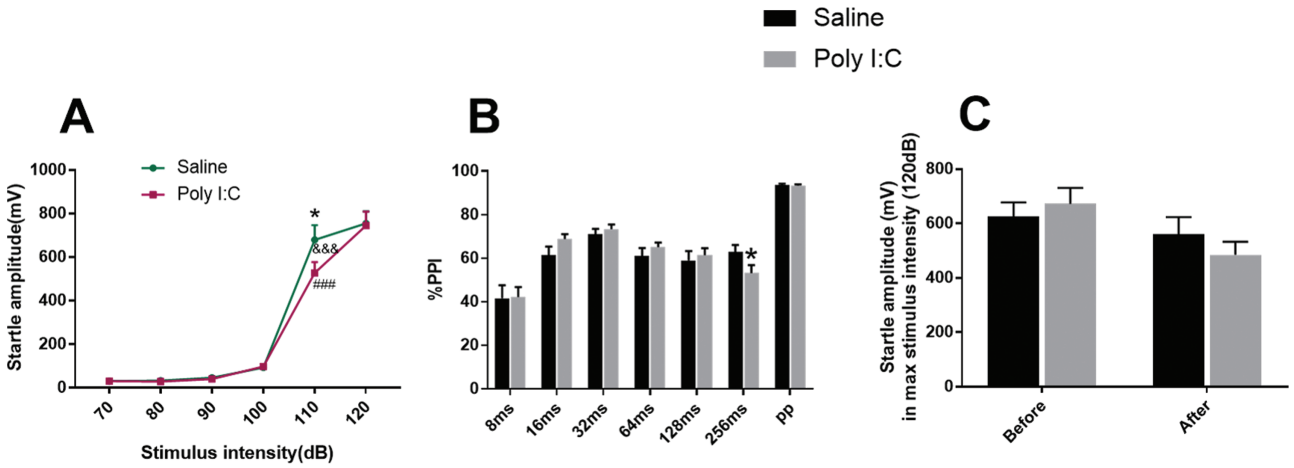

Figure 5. Effects of prenatal Poly I:C exposure on pre-pulse inhibition in female adolescent offspring. (A) Startle amplitude in different stimulus intensity (dB). (B) PPI index in different time of pre-pulse intervals. Value of 80 dB was used as pre-pulse intensity, and 110 dB as startle pulse. "pp" means PPI value without pre-pulse and acts as negative control. (C) Changes of startle amplitude in the max stimulus intensity (120 dB) after PPI trials. Data were presented as mean ± SEM (saline n = 35, Poly I:C n = 34). *P < .05, Poly I:C vs Saline; ###P < .001, Poly I:C $_{110db\ vs}$ Poly I:C $_{100db}$; &&&P < .001, saline $_{110db\ vs}$ saline $_{100db}$.

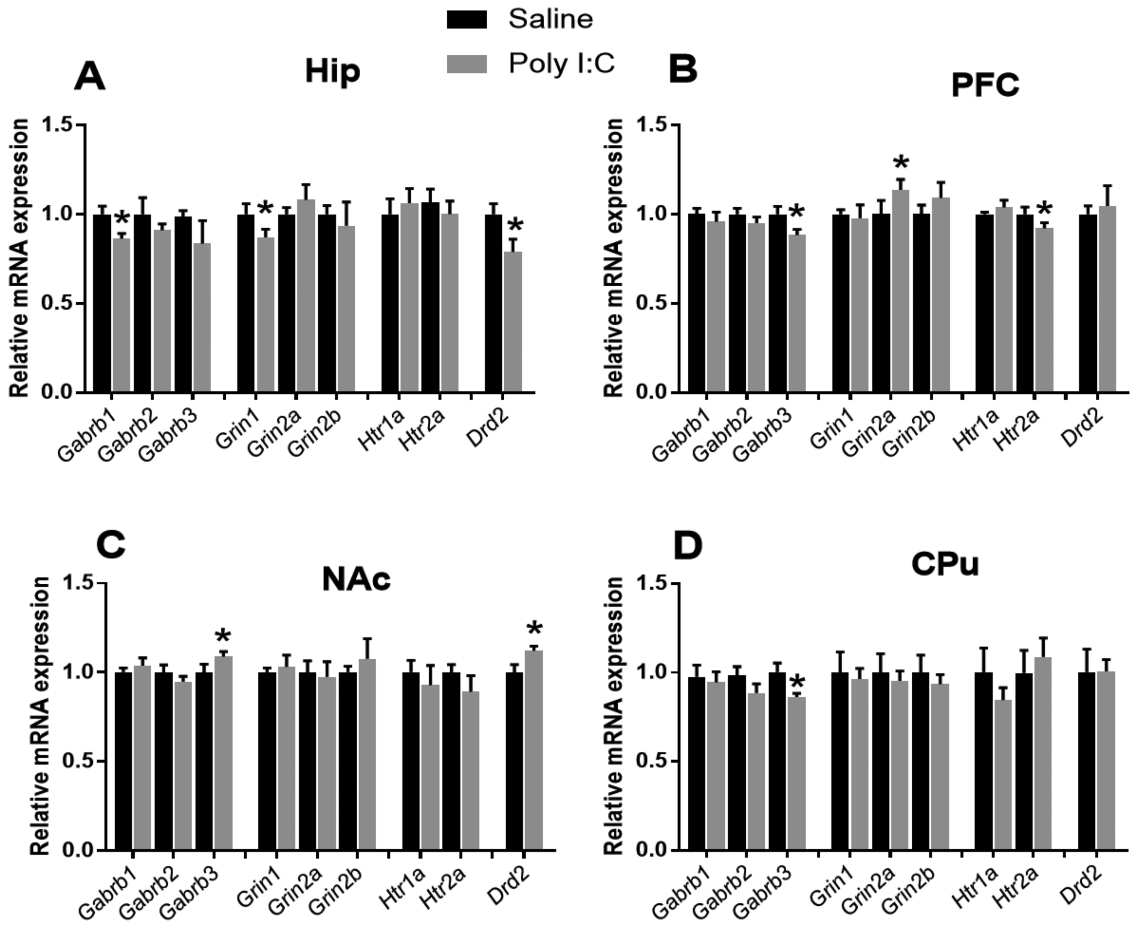

Figure 6. mRNA expression of $GABA_A$ subunits, NMDA receptor subunits, 5-HT1A/2A, and D2 receptors in various brain regions. (A) Hippocampus (Hip). (B) Pre-frontal cortex (PFC). (C) Nucleus accumbens (NAc). (D) Caudate putamen (CPu). Data were presented as mean ± SEM (n = 6/group). *P < .05 vs saline.

a deficit in sensorimotor information processing that could be reversed by haloperidol (Yee et al., 2005). It is interesting that we found decreased mRNA expression of $GABA_AR$ α3 in the PFC and CPu but increased *Gabrb3* expression in the NAc. Consistent with the exhibition of sensorimotor impairment in preweaning NMDA NR1 knockdown mice (Moy et al., 2012), the expression

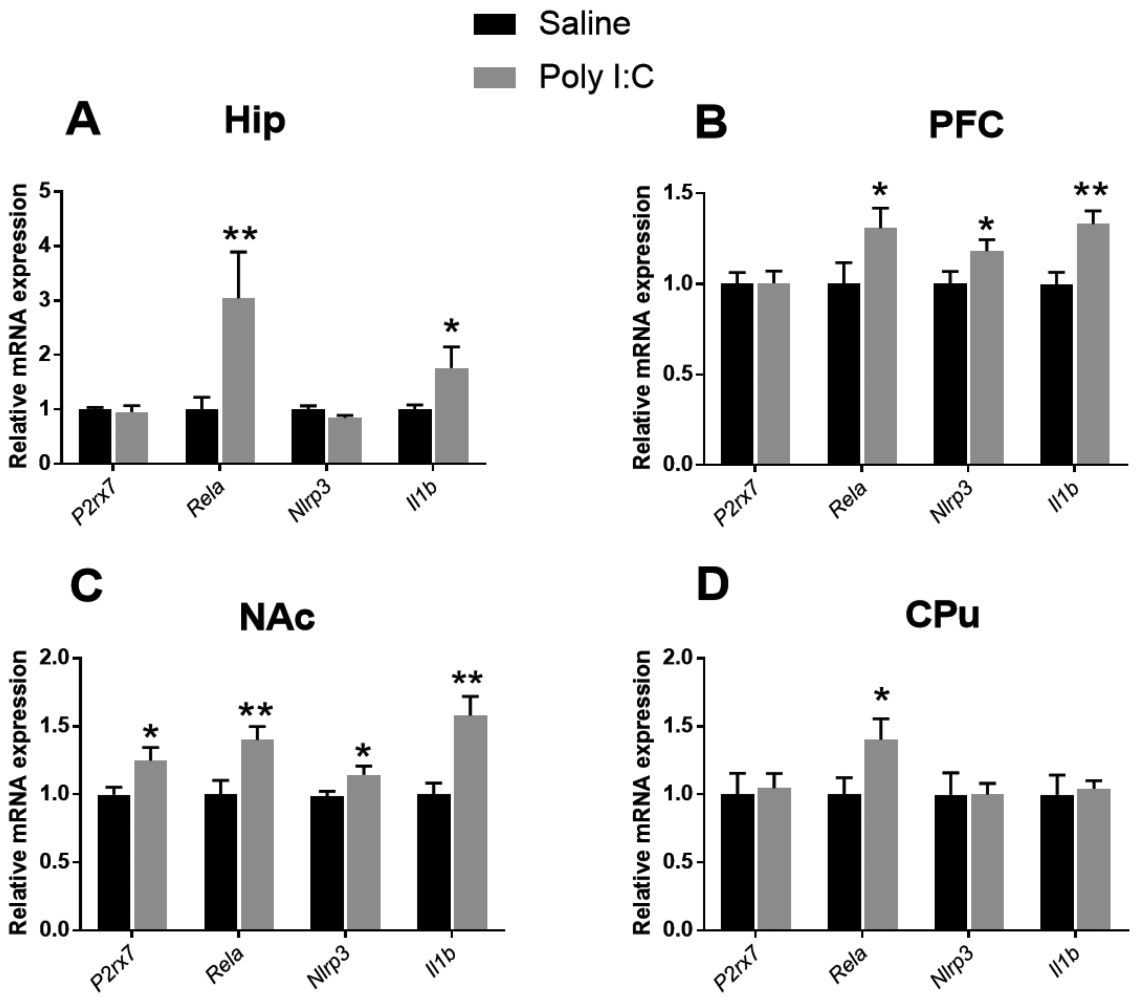

Figure 7. Relative mRNA expression of the P2X7-Rela-Nlrp3-IL-1β inflammatory pathway in 4 brain regions. (A) Hippocampus. (B) Pre-frontal cortex (PFC). (C) Nucleus accumbens (NAc). (D) Caudate putamen (CPu). Data were presented as mean ± SEM (n = 6/group). *$P < .05$, **$P < .01$; vs saline. IL-1β, interleukin-1 beta; NLRP3, nucleotide-binding domain-like receptor 3; P2X7R, P2X7 receptor.

of *Grin1*, encoding NR1, was significantly downregulated in the hippocampus in this study.

Consistent with previous reports (Amodeo et al., 2019; Osborne et al., 2019), the female adolescent Poly I:C rats showed deficits in the social interaction test. It has been reported that blockage of $GABA_A$ receptors with bilateral infusions of bicuculline methiodide into the PFC decreased the amount of time and the number of social interactions in the social interaction test (Paine et al., 2017). Furthermore, a selective reduction in NMDA NR1 subunits (*Grin1*) on parvalbumin-containing GABA neurons was linked to a decline in social approach, while FG-7142 (a $GABA_A$ receptor inverse agonist) reduced sociability, which was reversed by flumazenil (a $GABA_A$ receptor antagonist) (Hanks et al., 2013). In this study, prenatal Poly I:C exposure significantly decreased expression of *Gabrb3* in the PFC as well as *Gabrb1* and *Grin1* in the hippocampus.

This study found that the female adolescent Poly I:C rats had a better performance than controls in the novel object recognition memory test, which provided evidence that MIA may lead to an improvement in cognitive function. However, previous studies in the MIA models have reported cognitive deficits, particularly in adult offspring, which was often proposed as a phenotype of schizophrenia models (Reisinger et al., 2015; Haddad et al., 2020). Cognitive impairment is a major source of disability in neuropsychiatric diseases (Gallagher et al., 2017). However, some autistic phenotype patients often exhibited superior perception, peaks of ability, and savant skills generally related to memory (Mottron et al., 2013; Hughes et al., 2018). To date, there are limited genetically modified mouse models available for studying autistic savants and underlying mechanisms (Bader et al., 2011; Morimura et al., 2017). This study demonstrated a behavioral phenotype with improved cognitive function in a MIA rat model. One argument is that the improved novel object recognition could be due to higher "obsessions with objects" (one of the symptoms of autism) (Ne'eman et al., 2020). Since obsessions are an internal experience that can only be measured via self-report, behavioral assessment in rodents is limited to compulsive-like behaviors (Zike et al., 2017). The novel object recognition test is a highly validated test for recognition memory in rodents (Leger et al., 2013), while there is no evidence that it could be used to measure compulsive-like behaviors (Zike et al., 2017; Chen et al., 2021). If the MIA rats did not have a better recognition between the familiar and novel objects, "obsessions with objects" may lead to rats randomly approaching objects but not recognizing between novel and familiar objects; in fact, rodent models for obsessive–compulsive disorder showed recognition deficits (Zike et al., 2017; Chen et al., 2021). Supporting this study, a recent study in an autism

model found that i.p. injections of Poly I:C (5 mg/kg) at GD13-15 increased spatial working memory performance in young adult mice (at PD70) as well as reduced fetal mRNA levels of *Gad1* and adult hippocampal mRNA levels of *Pvalb* (Nakamura et al., 2021). Dynamic expression of NMDA receptors, especially the *Grin2* subunit in the PFC, was crucial in cognitive functions (Baez et al., 2018), while the D2 receptor is required for visual discrimination and reversal learning (Morita et al., 2016). Consistently, a significantly higher expression of *Grin2* in the PFC and D2 mRNA in the NAc was observed in this study.

There is evidence that MIA-elicited neuroinflammation responses, including increased IL-1β expression, contribute to the pathophysiological changes in 5-HTergic, GABAergic, and glutamatergic neurotransmissions in the PFC and hippocampus (Comer et al., 2020; Ravaccia and Ghafourian, 2020). Consistent with our findings of abnormal expression of 5-HT2A, $GABA_A$, and NMDA receptor subunits in the PFC and hippocampus, there is also an elevated IL-1β expression in these brain regions. This study further extended these findings that an increased expression of both IL-1β and D2 receptors was observed in the NAc of the female juvenile Poly I:C rats, suggesting elevated neuroinflammation via IL-1β contributed to pathophysiological changes in dopamine neurotransmission.

Poly I:C could be specially recognized by the transmembrane protein toll-like receptor 3 (TLR3) and transit into intracellular signals for modulating *IL-1β* mRNA expression and pro-IL-1β synthesis (Akira and Takeda 2004). In line with elevated expression of *IL-1β*, we observed the upregulation of *Rela* mRNA expression, which encoded the subunit of NF-κB. This suggests that prenatal Poly I:C activation of TLR3 elevated long-lasting NF-κB transcription leading to an increased expression of *IL-1β* (Figure 8). The activation of NF-κB after ligation of the TLR3 by Poly I:C also promoted NLRP3 production (Rajan et al., 2010). Activation of the NLRP3 inflammasome promotes cleavage of pro-IL-1β. We found that increased expression of *Nlrp3* mRNA in the PFC and NAc of female juvenile Poly I:C offspring implies that the NLRP3 inflammasome activation pathway may contribute to IL-1β elevation.

Recently, it has been reported that the P2X7R drives maternal Poly I:C induced autism-like behavior in adult male mice (Horvath et al., 2019). This study extended this result, finding that prenatal Poly I:C exposure induced not only autism-like behavior but also elevated P2X7R expression in female juvenile offspring rats. Accumulated evidence shows that activation of P2X7R receptors enhances the recruitment of the NLRP3 inflammasome-caspase-1 complex, then stimulates the IL-1β maturation and release (Giuliani et al., 2017). Therefore, elevated P2X7R expression and NF-κB (Rela) in female juvenile Poly I:C rats may lead to an increase in IL-1β expression, then maturation and release via the recruitment of the NLRP3 inflammasome-caspase-1 complex, which could be potential mechanisms for MIA-induced schizophrenia-like and autism-like behaviors (Figure 8). One limitation is that, due to the time and space limitations for conducting a series of behavioral tests in a short

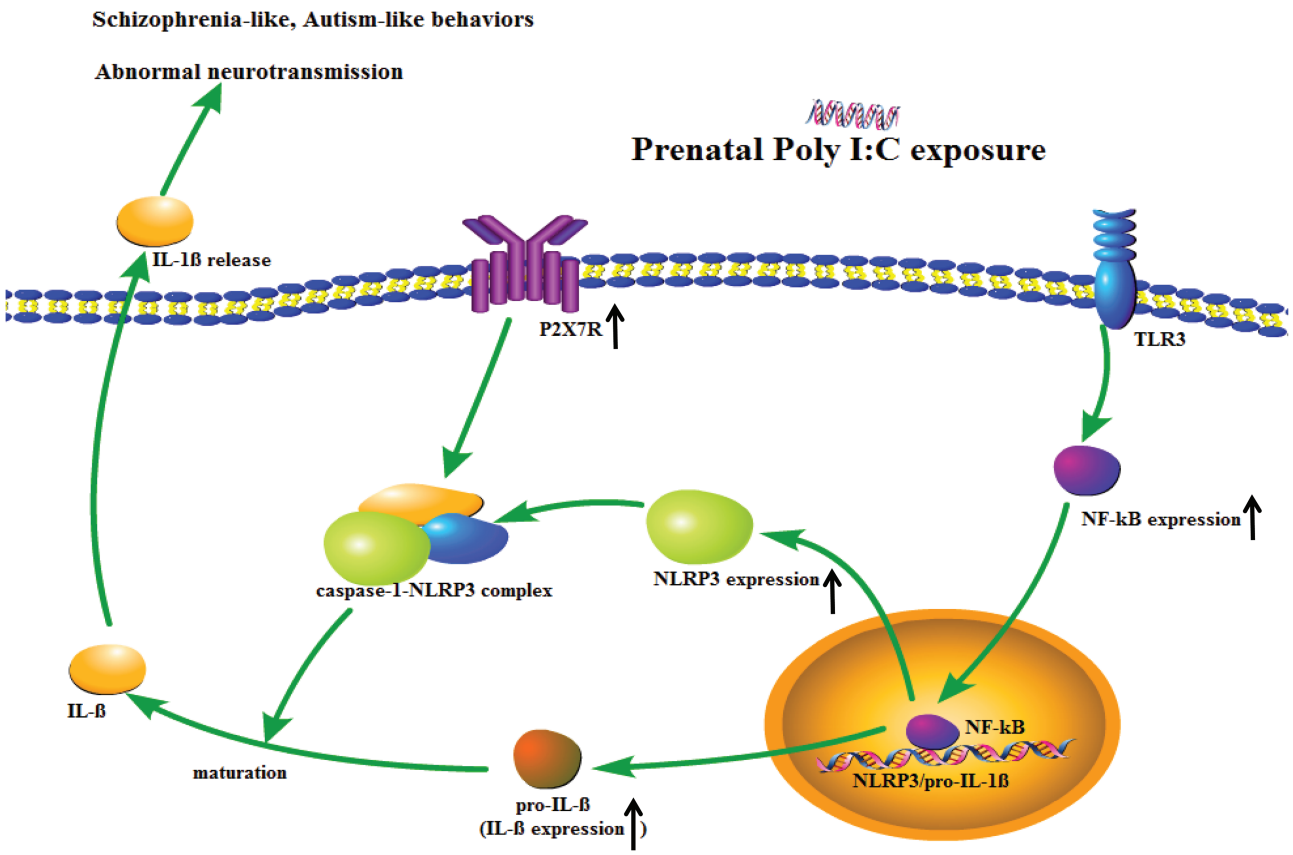

Figure 8. The possible mechanism of prenatal Poly I:C exposure induced long-lasting alterations of neuroinflammation responses, and brain functions in adolescent offspring. Prenatal Poly I:C exposure may activate TLR3, which initiates the intracellular signaling cascade that caused increased expression of NF-κB transcription factors, *IL-1β*, and *NLRP3* in the brain of Poly I:C offspring. The upregulated purinoceptor P2X7 receptor (P2X7R) enhances the recruitment of the NLRP3 inflammasome-caspase-1 complex, then promotes maturation and release of IL-1β, which could be a potential mechanism underlying MIA-induced schizophrenia-like and autism-like behaviors in juvenile Poly I:C rats.

period (PD35–60), only female juvenile rats have been examined in this study. Therefore further studies are necessary to investigate the effects of prenatal Poly I:C exposure in male juvenile rats using the similar housing and treatment conditions.

CONCLUSION

This study fills the gap in investigations into MIA-exposed female juvenile offspring and provides solid evidence that prenatal Poly I:C exposure has significant impacts on behaviors of female adolescent offspring. One of the novel findings in this study is that an improvement in discrimination ability was observed in female adolescent offspring rats with prenatal Poly I:C exposure, which demonstrated a behavioral phenotype with improved cognitive function in a MIA rat model. Since only 1 cognitive test was examined, further investigations are necessary using other cognitive behavioral tests to verify this finding. It is also important to examine possible sex differences in behaviors of adolescent Poly I:C offspring. Other important findings of this study include that prenatal Poly I:C exposure could cause brain region–specific elevation of the P2X7R and NF-κB–NLRP3–IL-1β inflammatory signaling as well as region-specific neurotransmission changes in female juvenile rats (Figure 8). Further research should be extended to explore the full spectrum of changes in neuroinflammation responses behind neurotransmitter alterations and schizophrenia-like and autistic-like behaviors caused by prenatal Poly I:C exposure.

Supplementary Materials

Supplementary data are available at *International Journal of Neuropsychopharmacology (IJNPPY)* online.

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Interest Statement

None of the authors has a conflict of interest to disclose.

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