Neurobiology of Learning and Memory 141 (2017) 150–156

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Maternal immune activation during pregnancy in rats impairs working memory capacity of the offspring

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ARTICLE INFO

Article history:
Received 30 January 2017
Revised 28 March 2017
Accepted 16 April 2017
Available online 19 April 2017

Keywords:
PolyI:C
Odor span task
Variable delay
Schizophrenia
Medial prefrontal cortex
Nonmatching-to-sample

ABSTRACT

Maternal immune activation during pregnancy is an environmental risk factor for psychiatric illnesses such as schizophrenia in the offspring. Patients with schizophrenia display an array of cognitive symptoms, including impaired working memory capacity. Rodent models have been developed to understand the relationship between maternal immune activation and the cognitive symptoms of schizophrenia. The present experiment was designed to test whether maternal immune activation with the viral mimetic polyinosinic:polycytidylic acid (polyI:C) during pregnancy affects working memory capacity of the offspring. Pregnant Long Evans rats were treated with either saline or polyI:C (4 mg/kg; i.v.) on gestational day 15. Male offspring of the litters (2–3 months of age) were subsequently trained on a nonmatching-to-sample task with odors. After a criterion was met, the rats were tested on the odor span task, which requires rats to remember an increasing span of different odors to receive food reward. Rats were tested using delays of approximately 40 s during the acquisition of the task. Importantly, polyI:C- and saline-treated offspring did not differ in performance of the nonmatching-to-sample task suggesting that both groups could perform a relatively simple working memory task. In contrast, polyI:C-treated offspring had reduced span capacity in the middle and late phases of odor span task acquisition. After task acquisition, the rats were tested using the 40 s delay and a 10 min delay. Both groups showed a delay-dependent decrease in span, although the polyI:C-treated offspring had significantly lower spans regardless of delay. Our results support the validity of the maternal immune activation model for studying the cognitive symptoms of neurodevelopmental disorders such as schizophrenia.

1. Introduction

Maternal immune activation (MIA) during pregnancy may contribute to the development of schizophrenia, autism spectrum disorder, and bipolar disorder in the offspring (Brown, 2012; Brown, 2015; Brown & Derkits, 2010; Brown & Patterson, 2011; Fineberg & Ellman, 2013; Khandaker, Zimbron, Lewis, & Jones, 2013; Pearce, 2001). However, whether MIA contributes to the cognitive symptoms of these disorders remains poorly understood. In one study, Brown et al. (2009) showed that patients with schizophrenia whose mothers had confirmed viral infections during pregnancy performed worse on set-shifting as assessed by the Wisconsin Card Sorting Task than patients whose mothers did not have an infection. These findings raise the possibility that MIA may be particularly detrimental to higher order cognitive processes. One such process is working memory. Working memory impairments are consistently observed in schizophrenia patients in the domains of goal maintenance, interference control, and capacity (Barch, Moore, Nee, Manoach, & Luck, 2012; Barch & Smith, 2008). Interestingly, Gold et al. (2010) found that memory capacity (number of items stored) is impaired in schizophrenia patients while precision of stored representations and maintenance over different delays are intact. Thus, we sought to assess the effects of MIA on working memory capacity in a rodent model.

Rodent models of MIA during pregnancy recapitulate a variety of behavioral changes relevant to schizophrenia in the offspring (Meyer, Feldon, & Fatemi, 2009; Piontkewitz, Arad, & Weiner, 2012; Meyer, 2014). Following MIA in mice, working memory has been assessed using the alternating y maze (Krstic et al., 2012; Ribeiro et al., 2013) and matching-to-sample tasks using the cheeseboard maze (Bitanihirwe, Weber, Feldon, & Meyer, 2010; Richetto, Calabrese, Meyer, & Riva, 2013; Richetto, Calabrese, Riva, & Meyer, 2014) and Morris water maze (Meyer, Feldon, Schedlowski, & Yee, 2005; Meyer, Knuesel, Nyffeler, & Feldon, 2010; Meyer, Nyffeler, Yee, Knuesel, & Feldon, 2008). In general, working memory impairments in the offspring from MIA

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http://dx.doi.org/10.1016/j.nlm.2017.04.005
1074-7427/

litters have been observed, particularly with longer delays and in older animals. In rats, no effects on a matching-to-sample working memory task in the water maze were observed in offspring following MIA during pregnancy (Vorhees et al., 2015). Importantly, the tasks used in these previous studies require rodents to remember a limited amount of information without manipulating capacity. Thus, we tested the effects of MIA on working memory capacity using the odor span task (OST) in rats (Dudchenko, Talpos, Young, & Baxter, 2013).

The OST (Fig. 3A), developed by Dudchenko, Wood, and Eichenbaum (2000), is an incremental nonmatching-to-sample task in which rats or mice receive a food reward by choosing to dig in a bowl of sand with a novel scent (Davies, Greba, & Howland, 2013; Davies, Molder, Greba, & Howland, 2013; Dudchenko et al., 2000; Rushforth, Allison, Wonnacott, & Shoaib, 2010; Rushforth, Steckler, & Shoaib, 2011; Young et al., 2007) or by flipping scented lids (April, Bruce, & Galizio, 2013; Galizio, Deal, Hawkey, & April, 2013; MacQueen, Bullard, & Galizio, 2011). If the subject chooses the novel bowl, additional bowls are added one at a time with the previous bowl(s) repositioned on the platform until the subject chooses a previously rewarded bowl (recorded as an error). The number of bowls correctly selected minus 1 is the span of the rat. Average spans of approximately 7–9 odors are reported when rats are stopped after their first error (Dudchenko et al., 2000 but see April et al., 2013; Davies, Greba et al., 2013; Davies, Molder et al., 2013). Odor span capacity is decreased following reversible inactivation of the medial prefrontal cortex (mPFC) (Davies, Molder et al., 2013) and dorsomedial striatum (Howland, Davies, Greba, Selk, & Syed, 2014), but not permanent lesions of dorsal hippocampus (Dudchenko et al., 2000) in rats. Studies in rats have shown that span capacity is impaired following various treatments, such as acute stress (Davies, Molder et al., 2013), 192 IgG-saporin-induced cholinergic lesions of the basal forebrain (Turchi & Sarter, 2000), N-methyl-D-aspartate (NMDA) receptor antagonists (Davies, Greba et al., 2013; Galizio et al., 2013; MacQueen et al., 2011; Rushforth et al., 2011), and the γ-aminobutyric acid (GABA) A receptor modulator chlordiazepoxide (April et al., 2013). Odor span capacity is also increased in rats by systemically administered nicotinic (Rushforth et al., 2010). Given the neural substrates mediating the OST, we expected that the offspring of rats treated with the viral mimetic polyinosinic:polycytidylic acid (polyI:C) during pregnancy would be impaired on the task.

2. Materials and methods

2.1. Subjects

Timed pregnant Long–Evans rats [gestational day (GD) 7; Charles River Laboratories, Quebec, Canada] were individually housed in clear plastic cages in a temperature-controlled (21 °C) colony room on a 12/12-h light/dark cycle (lights on at 0700 h). Food (Purina Rat Chow) and water were available ad libitum. Male offspring of 3 separate squads of dams were used in the current experiments. The experiments were conducted during the light phase and offspring were handled 3 times before experiments commenced. Offspring had water available ad libitum and were food restricted to maintain 85% of their free feeding weight during behavioral experiments. Experimenters were blind to the treatment of the dams and pups during the course of all experiments. All experiments were performed in accordance with the Canadian Council on Animal Care and were approved by the University of Saskatchewan Animal Research Ethics Board.

2.2. Gestational and neonatal treatment

Treatment methods closely followed those reported previously (Ballendine et al., 2015; Howland, Cazakoff, & Zhang, 2012; Sangha, Greba, Robinson, Ballendine, & Howland, 2014; Zhang, Cazakoff, Thai, & Howland, 2012). On GD 15, dams were individually transported to a room where weight and rectal temperature (Homeothermic Blanket System, Harvard Instruments, MA, USA) were measured. Dams were then anesthetized with isoflurane (5% induction and 2.5% maintenance) and injected intravenously with a single dose of either saline (n = 8) or polyI:C (4.0 mg/kg, high molecular weight; InVivoGen, San Diego, CA, USA; n = 8) via the tail vein. This procedure took an average of 10 min/animal, and care was taken to ensure the saline treated dams were anesthetized for the same duration as the polyI:C-treated dams. Weight and temperature were measured again at 8, 24, and 48 h after the injection. Dams were otherwise left undisturbed until the day after parturition. The day of parturition was designated postnatal day (PND) 0. On PND 1, litters were weighed and culled to a maximum of 10 pups per litter (six males and four females where possible). Other than routine husbandry (including recording litter weights on PND 8, 14, and 21), litters were left undisturbed until weaning on PND 21. Weaned male pups from the same litter were housed in same-sex cages of 2–4 animals. Care was taken to ensure that one offspring per litter was included in each group to reduce the influence of litter effects. Training was initiated when the offspring were young adults (2–3 months of age).

2.3. Apparatus

Training and testing followed previously established protocols (Davies, Greba et al., 2013; Davies, Molder et al., 2013). A 91.5 cm^2 platform covered with black corrugated plastic with a 2.5 cm tall border around the outer edge was used. The platform was secured to a metal frame with casters and stood 95 cm above the floor. The platform was surrounded by a beige curtain to block visual cues in the room. Velcro was used to secure white porcelain bowls (4.5 cm high, 9 cm in diameter) to the platform and prevent the rats from spilling the sand. Pieces of Velcro were equally spaced along the edge of the platform (one piece in each corner and five additional pieces on each side). The bowls for a given trial were randomly positioned on the pieces of Velcro.

2.4. Odors

Premium Play Sand (Quikrete Cement and Concrete Products, Atlanta, GA) was sifted to remove rocks and then odors were mixed into the sand. Sand (100 g) was scented by mixing it with 0.5 g of a single dried spice. The odor and sand mixtures were stored in separate Ziploc bags when unused and new batches of sand and odors were freshly mixed every 7 days. Twenty-four different spices were used in the experiments: allspice, anise seed, basil, caraway, celery seed, cinnamon, cloves (0.1 g), cocoa, coffee, cumin, dill, fennel seed, garlic, ginger, lemon and herb, marjoram, mustard powder, nutmeg, onion powder, orange, oregano, paprika, sage, and thyme. Spices were purchased from a local grocery store. The sequence of the odors used each day were selected randomly and rats were regularly exposed to all odors. Sand filled bowls were placed on the platform as needed for each trial.

2.5. Training on the odor span task

Dig Training/Shaping. First, rats were trained to dig for a food reward (Kellogg's Froot Loops) in a bowl filled with 100 g of unscented sand. Rats were placed opposite to a bowl on the platform for three separate phases. In the first phase, the food reward

was placed on top of the sand, in the second phase the food reward was partly buried, and in the third phase the food reward was completely buried. Rats were trained until they would consistently dig for the food reward regardless of the bowl's position on the platform (range: 4–9 days to complete). *Odor nonmatching-to-sample.* Once the rats reliably dug in unscented bowls, they moved onto the nonmatching-to-sample task (Fig. 2A). In the sample phase of a trial, the rat was presented with a bowl of sand with odor randomly located on the platform. After the rat dug and consumed the food reward, it was removed from the platform and placed behind a curtain to obscure its view of the platform for a delay (40 s). The researcher then placed the bowl at a random position on the opposite end of the platform and added a second bowl with a different odor on the platform. In the choice phase of the trial, the rat was placed on the platform opposite to both bowls and then allowed access to both bowls. A food reward was only in the bowl with the novel odor for that trial. A choice was scored if a rat dug or placed its paws or nose on the sand and an error was scored if the rats chose a previously rewarded bowl. The rats completed 6 trials each day until they chose the novel odor on at least five of the six trials for three days. *Odor span task.* After attaining criterion on the nonmatching-to-sample task, rats were introduced to the OST (Fig. 3A). Trials were run as described for the nonmatching-to-sample task except that bowls with novel odors (for that trial) were added to the platform and previous bowls remained until the rat made an error (i.e., dug in any of the bowls except the novel one) which resulted in ending the trial. Previously presented bowls were randomly repositioned before each novel bowl was added to the platform. Thus, rats could not use spatial cues to guide their response. The span for a given trial was measured as the number of odor bowls correctly chosen minus one. Each rat performed 1–3 spans per day (rats with high spans performed 1 span while rats with medium spans performed 2 spans, and rats with low spans performed 3 spans) with a break between spans occurring while other rats were trained. Averaging the spans reduced variance in our sample, as reported with other memory tests (Winters & Reid, 2010). The mean of all spans for a given day is reported in the figures where indicated. After rats completed 12 days of acquisition on the OST, they were given 5–7 days off. Afterwards, the OST was trained with a shorter (40 s) delay for 2 days followed by 2 days of a longer (10 min) delay. During the longer delay trials, rats were placed in their home cage and taken out of the testing room during each delay while other rats were tested.

2.6. Probe sessions

To determine if the rats were using the odor to solve the task, two types of probe sessions were conducted. The first probe session tested whether the scent of the Froot Loop guided behavior. In this session, the rats were presented with bowls as described in the OST without any rewards present. When the rat made a correct choice, the researcher dropped a Froot Loop on top of the sand in the correct bowl. The second probe tested if the rats were marking the bowls of sand when examining them. During a session, all the bowls and sand were replaced with new bowls and new sand that contained the same odors between each trial. If rats marked bowls and sand, their performance would be reduced on these trials. The rats' performance was 100% during both of these probe sessions (data not shown).

2.7. Data analysis

Odor spans were manually recorded during testing and entered into Microsoft Excel (2010) and Statistical Package for the Social Sciences (SPSS version 19) for analysis. All descriptive values are reported as means ± standard error of the mean. Analyses were performed using t-tests, Kaplan-Meier survival analysis, repeated measures ANOVA, and Newman-Keuls post hoc tests where appropriate. Statistical tests were considered significant if p values were <0.05.

3. Results

3.1. Effects of polyI:C treatment on dams and offspring

Weight (Fig. 1A) and rectal temperature (Fig. 1B) were taken from the dams at 0, 8, 24, and 48 h after treatment with saline or polyI:C. There was no significant difference in the weight of dams before treatment (saline 286.25 ± 8.37 g; polyI:C 305.38 ± 12.45 g; $t(14) = -1.28$ $p = 0.22$). Results of a repeated measures ANOVA on the percent weight change from baseline revealed significant main effects of Treatment ($F(1,14) = 6.08$, $p = 0.03$) and Time ($F(3,42) = 58.54$, $p < 0.001$), as well as a significant Time by Treatment interaction ($F(3,42) = 5.21$, $p = 0.01$). Post hoc analysis indicated that dams treated with polyI:C lost significantly more weight than saline-treated rats 24 h after treatment. Rectal temperature changes were found during the monitoring period with a main effect of Time ($F(3,42) = 4.99$, $p = 0.01$); however, neither the main effect of Treatment ($F(1,14) = 3.13$, $p = 0.10$) nor the interaction of Time by Treatment ($F(3,42) = 0.66$, $p = 0.58$) reached significance.

PolyI:C-treated dams had more pups than saline-treated dams (Table 1; $t(14) = -2.30$, $p = 0.04$). A repeated measures ANOVA revealed that pups gained weight in both treatment groups at a similar rate from PND1 to PND21 (Table 1; main effect of Time with every comparison significantly different; $F(3,42) = 2495.34$, $p < 0.001$; post hoc $p < 0.05$). There was not a significant main effect of Treatment ($F(1,14) = 1.41$, $p = 0.26$), nor a Time by Treatment interaction ($F(3,42) = 2.66$, $p = 0.10$) for pup weight.

3.2. Training

Offspring from saline- and polyI:C-treated dams underwent a series of shaping tasks before acquisition of the OST (see Methods). After dig training, rats were trained in the nonmatching-to-sample task until they selected the novel odor in at least 5/6 trials for three sessions (saline-treated rats 6.00 ± 0.27 days, range = 5–7 days; polyI:C-treated offspring 6.63 ± 0.65 days, range = 4–9 days; Fig. 2). The survival distribution of the days trained on the nonmatching-to-sample task was not significantly different between groups ($\chi^2(1) = 1.49$, $p = 0.22$), which shows acquisition of the nonmatching-to-sample task did not differ between polyI:C- and saline-treated groups.

3.3. Acquisition of the OST

The 12 acquisition days of the OST were divided into early, middle, and late phases consisting of 4 days, similar to previous analyses of attention and visual discrimination tasks (Bussey, Muir, Everitt, & Robbins, 1996; Bussey, Muir, Everitt, & Robbins, 1997; Muir, Bussey, Everitt, & Robbins, 1996). When all spans in a day (1–3 spans per day) were examined, there was no difference in performance in the early phase between saline- and polyI:C-treated offspring (Time by Treatment interaction; $F(3,42) = 0.77$, $p = 0.49$, Fig. 3B), nor was there a significant main effect of Treatment ($F(1,14) = 0.01$, $p = 0.98$). Offspring showed a significant increase in span on day 4 relative to day 1 (main effect of Time; $F(3,42) = 11.34$, $p < 0.001$; post hoc $p < 0.05$). In the middle phase of training, polyI:C-treated offspring had lower spans compared to saline-treated offspring on day 8 of training (Time by Treatment interaction; $F(3,42) = 7.92$, $p = 0.01$; post hoc $p < 0.05$; Fig. 3C). Offspring had a significant increase in span on day 8 relative to day's 5 and

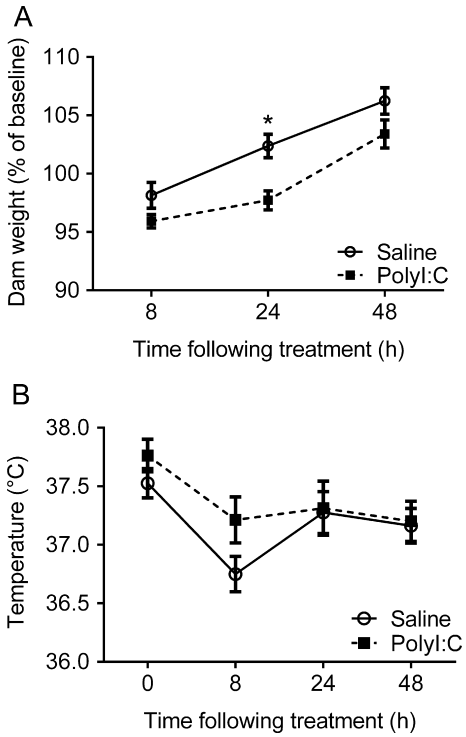

Fig. 1. Weight and temperature were taken at 0, 8, 24, and 48 h after polyI:C or saline injection on gestational day 15. (A) Weight of dams as a percentage of their baseline weight. PolyI:C-treated dams gained significantly less weight relative to saline-treated dams 24 h after injection. (B) Rectal temperature of the dams did not differ significantly between the groups at any time point. A significant main effect of Time was observed.

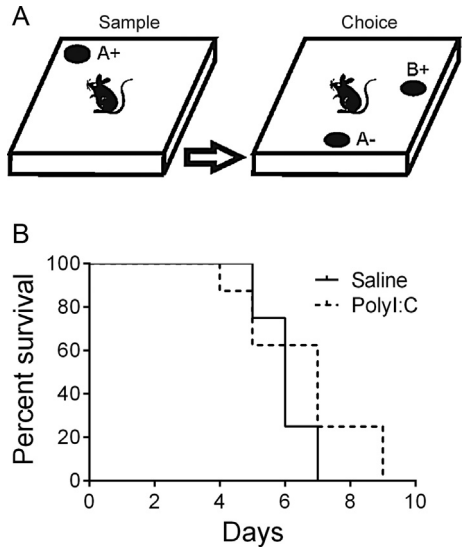

Fig. 2. Performance of the nonmatching-to-sample task in saline or polyI:C offspring. (A) Illustration of the nonmatching-to-sample task. See text for details. Odors are indicated with letters. The sample phase involved the presentation of a scented bowl (black circle) that was rewarded (+), and the choice phase involved the presentation of a novel scented bowl containing a reward (B+) with the sample bowl relocated on the platform without a reward (A−). A correct response was scored if the rat dug into the bowl containing the novel odor, whereas an incorrect response was scored if the rat dug into the sample bowl. (B) Kaplan-Meier survival analysis of the number of days rats were trained on the nonmatching-to-sample task. Rats completed six non-matching-to-sample trials each day until they chose the novel odor on at least five of the six trials for three days, which ended nonmatching-to-sample training for that particular rat. No significant differences were observed between the groups.

6 (main effect of Time; $F(3,42) = 8.23$, $p = 0.01$; post hoc $p < 0.05$). The main effect of Treatment was not significant ($F(1,14) = 1.19$, $p = 0.29$). In the last phase of training, polyI:C-treated offspring had lower spans on days 9 and 12 of training (Time by Treatment interaction; $F(3,42) = 2.87$, $p = 0.048$; post hoc $p < 0.05$; Fig. 3D). Offspring had increased span on day's 11 and 12 relative to day 9 (main effect of Time; $F(3,42) = 7.28$, $p < 0.001$; post hoc $p < 0.05$). The main effect of Treatment was not significant ($F(1,14) = 2.38$, $p = 0.15$).

We also analyzed the data using the first span of each training day to examine if there was a difference between the groups' first spans. No differences were found between the polyI:C- and saline-treated offspring in the early phase (main effect of Treatment: ($F(1,14) = 0.01$, $p = 0.94$; Time by Treatment interaction: $F(3,42) = 1.20$, $p = 0.32$; Fig. 4A). Offspring had increased span on day 4 relative to day's 1 and 2 (main effect of Time; $F(3,42) = 12.76$, $p < 0.001$; post hoc $p < 0.05$). In the middle phase of training, polyI:C-treated offspring had a lower first span than saline-treated offspring on day 8 of training (Time by Treatment interaction; $F(3,42) = 5.80$, $p = 0.01$; post hoc $p < 0.05$; Fig. 4B), and offspring showed an increase in span on day 8 relative to day's 5 and 6 (main effect of Time; $F(3,42) = 5.24$, $p = 0.01$; post hoc $p < 0.05$). The main effect of Treatment was not significant ($F(1,14) = 3.01$, $p = 0.10$). In the last phase of training, there was no difference between polyI:C- and saline-treated offspring (Time by Treatment interaction; $F(3,42) = 1.33$, $p = 0.28$; Fig. 4C). The main effects of Time ($F(3,42) = 2.43$, $p = 0.08$) and Treatment ($F(1,14) = 3.93$, $p = 0.07$) were not significant.

3.4. Delay-dependence of OST performance

We tested rats for 2 days of short delays (40 s) and then 2 days of 10 min delays. When all spans were analyzed, there was a main effect of Delay ($F(1,10) = 73.05$, $p < 0.001$; Fig. 5) and Treatment ($F(1,10) = 6.36$, $p = 0.03$). PolyI:C-treated offspring had significantly lower spans than saline-treated offspring and sessions with a 10 min delay resulted in significantly lower spans that sessions with a shorter delay.

4. Discussion

The present experiment revealed that polyI:C-treated-offspring have reduced span capacity during acquisition and performance of the OST. Importantly, the rate of acquisition of an odor-based nonmatching-to-sample task did not differ between the treatment groups. These results suggest that working memory capacity may be specifically altered in the offspring following MIA during pregnancy in rats.

4.1. Short-term effects of polyI:C treatment on pregnant dams

To assess the acute effects of polyI:C treatment on the dams, weight and rectal temperature were measured (Fig. 1). Consistent with previous findings from our laboratory, polyI:C treatment reduced maternal weight in Long Evans rats (Ballendine et al., 2015; Howland et al., 2012; Paylor et al., 2016; Sangha et al., 2014; Zhang et al., 2012). Others have reported that maternal weight is not consistently reduced following polyI:C administration in Sprague Dawley rats (Bronson, Ahlbrand, Horn, Kern, & Richtand, 2011; Wolff & Bilkey, 2010) and that weight loss was observed in Wistar rats for roughly a day, although the magnitude of loss was not quantified (Zuckerman, Rehavi, Nachman, & Weiner, 2003; Zuckerman & Weiner, 2005). Maternal temperature changes in response to polyI:C administration are inconsistent in our laboratory (Ballendine et al., 2015; Howland et al., 2012;

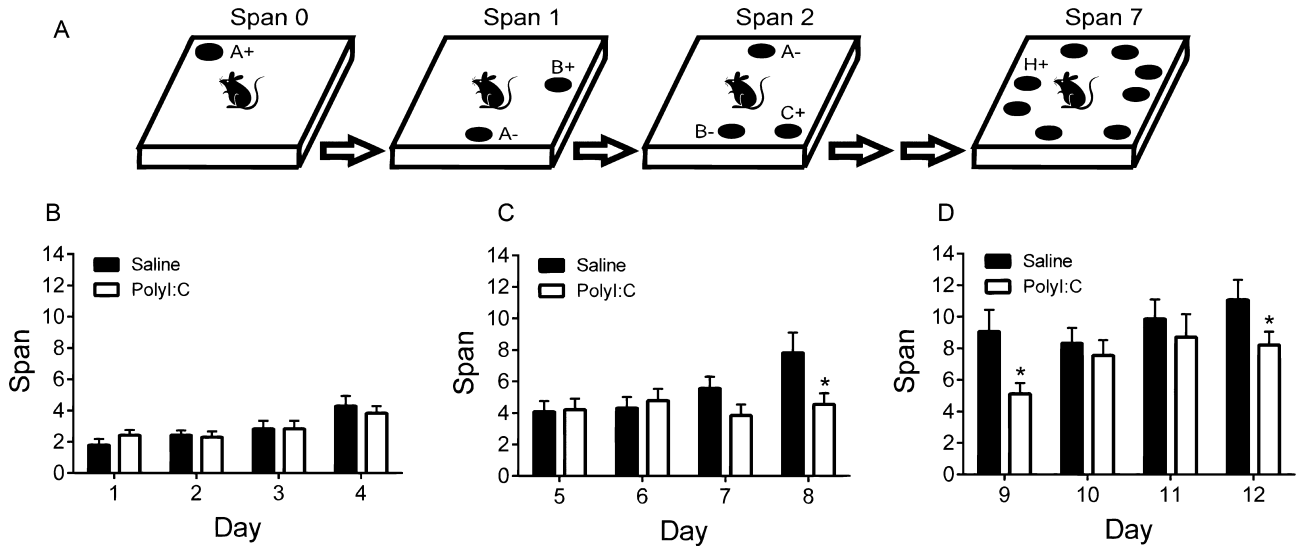

Fig. 3. Effects of polyI:C treatment on acquisition of the odor span task (OST). (A) Illustration of the OST. See text for details. Odors are indicated with letters. On subsequent trials for a given span, the bowl (black circle) that contained the novel odor was rewarded (+) while previously encountered bowl(s) were not (−). Bowls were added one at a time to the platform until an error occurs. Span was calculated as the number of bowls on the platform for the last error free trial minus 1. Note that all bowls were moved around the platform before each new trial. (B) The first 4 days of OST acquisition of all spans (1–3 spans per day) revealed no difference between poly:I:C- and saline-treated offspring. (C) The middle of OST acquisition of all spans (1–3 spans per day; days 5–8) show that polyI:C-treated offspring have reduced span on day 8 relative to saline-treated offspring. (D) The last phase of OST acquisition of all spans (1–3 spans per day; days 9–12) reveal that polyI:C-treated offspring have reduced span on days 9 and 12 relative to saline-treated offspring.

Table 1
Number and weight of the pups born to saline- and polyI:C-treated dams. Means are listed with standard error of the mean indicated with ±. PND, postnatal day.

Pups per litter		Average weight per pup (g)			
		PND1	PND8	PND14	PND21
Saline	11.00 ± 0.89[a]	8.58 ± 0.23	18.94 ± 0.35	35.93 ± 0.65	60.76 ± 1.16
PolyI:C	13.38 ± 0.53	8.82 ± 0.46	18.28 ± 1.20	34.58 ± 0.89	57.60 ± 1.51

[a] Indicates a main effect of maternal Treatment.

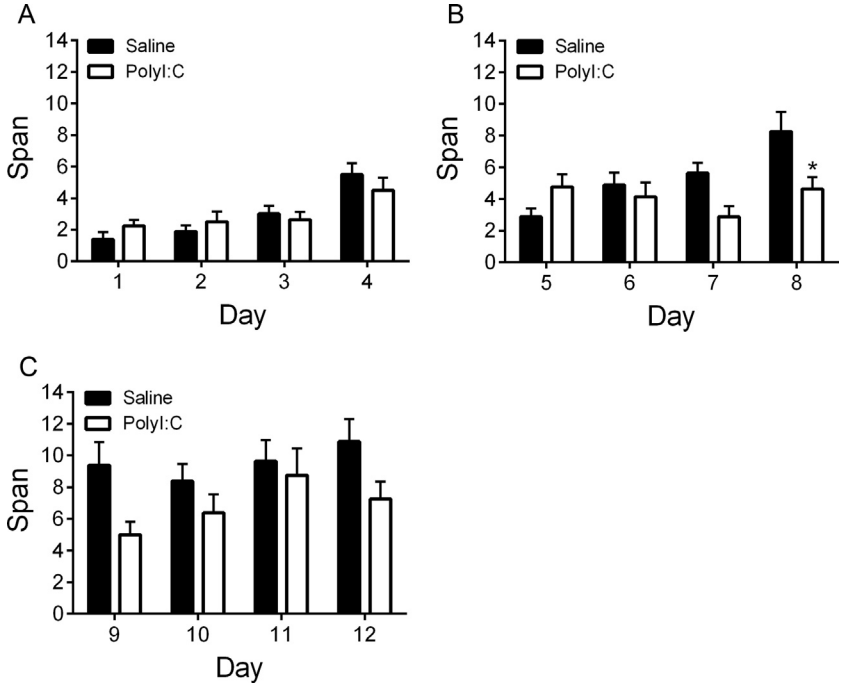

Fig. 4. Effects of polyI:C treatment on acquisition of the odor span task (OST) for the first span of each acquisition day. (A) The early phase (days 1–4) of OST acquisition revealed no difference between polyI:C- and saline-treated offspring for their first span of each day. (B) During the middle of OST acquisition (days 5–8), polyI:C-treated offspring had reduced span on day 8 relative to saline-treated offspring. (C) PolyI:C offspring had lower first spans during days 9, 10, and 12 of the late phase of OST acquisition, although these differences did not reach the threshold for statistical significance ($p = 0.07$).

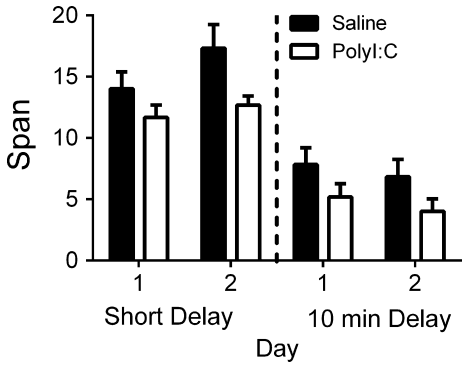

Fig. 5. Performance of the OST depended on delay and maternal treatment. Rats were tested for 2 days of shorter delays (40 s) followed by 2 days of 10 min delays with all spans (1–3 per day) assessed. Main effects of Treatment (polyI:C-treated offspring have lower spans relative to saline-treated offspring) and Delay (spans were lower on days with 10 min delays compared to shorter delays) were observed.

Sangha et al., 2014; Zhang et al., 2012). Surprisingly, polyI:C treated dams had significantly larger litters than saline treated dams (Table 1), as other studies show no difference (Howland et al., 2012; Sangha et al., 2014; Wolff & Bilkey, 2008; Wolff & Bilkey, 2010; Zhang et al., 2012; Zuckerman & Weiner, 2005; Zuckerman et al., 2003) or smaller litters (Ballendine et al., 2015) following polyI:C treatment. Pup growth was not significantly affected by treatment (Table 1), consistent with other studies (Ballendine et al., 2015; Howland et al., 2012; Sangha et al., 2014; Zhang et al., 2012).

4.2. PolyI:C offspring are impaired on the OST

Some previous studies have shown impaired working memory in the offspring of polyI:C-treated mice (Bitanihirwe et al., 2010; Meyer et al., 2005; Meyer et al., 2008; Meyer et al., 2010; Richetto et al., 2013; Richetto et al., 2014); however, others have failed to see effects in young adult mice tested at short delays (Krstic et al., 2012; Meyer et al., 2005) and rats (Vorhees et al., 2015). Notably, the present results show no difference between polyI:C and saline offspring during the initial nonmatching-to-sample phase of the OST, which has a working memory component. Thus, our lack of effect with odor stimuli is consistent with the findings of Vorhees et al. (2015) using a spatial matching-to-sample task. When the polyI:C-offspring were presented with the more challenging OST, deficits in task acquisition and performance emerged, consistent with impaired working memory capacity. While our results suggest the impairment observed in the OST is the result of reduced working memory capacity, impairments in other cognitive functions such as attention may have contributed. In addition, it is possible that maternal polyI:C treatment impaired olfactory function of the offspring. For example, cytokines, such as TNF, are elevated in the offspring of polyI:C dams (Garay, Hsiao, Patterson, & McAllister, 2013; Han, Nanxin, Meng, Shao, & Wang, 2011) and elevated cytokines may be involved in with impaired olfaction in mouse models (Lane, Turner, May, & Reed, 2010) and patients with hyposmia (Henkin, Schmidt, & Velicu, 2013). However, the similar performance between groups on the nonmatching-to-sample task helps to rule out differences in attention or other nonspecific factors such as motivation and odor discrimination in the offspring. Previous research has also shown delay dependent impairments in matching-to-position spatial working memory tasks for polyI:C offspring (Bitanihirwe et al., 2010; Meyer et al., 2005; Meyer et al., 2008; Meyer et al., 2010). Thus, it may be that if longer delays were used in the odor-based nonmatching-to-sample task, impairments would have been observed in the polyI:C offspring.

To the best of our knowledge, this is the first time that the effects of delay between subsequent presentations of odors have been examined for the OST. We found a delay-dependent decrease in span for both saline and polyI:C-treated rats, and polyI:C-treated rats performed worse at both delays. Dudchenko et al. (2000) examined delay-dependent effects using a nonmatching-to-sample study that included a capacity component. In their design, rats were exposed to 12 familiar 'sample' odors. After a variable delay, they were presented with one of the previously sampled odors and a novel odor. Performance decreased from greater than 80% correct with a 15 min delay to chance performance when a 3 h delay was used (Dudchenko et al., 2000). Taken together, these manipulations suggest that the memory component of the OST is sensitive to delays between the presentations of stimuli.

The OST depends on a distributed circuitry including the mPFC and dorsomedial striatum (Davies, Greba et al., 2013; Davies, Molder et al., 2013; Howland et al., 2014) but notably does not involve the hippocampus (Dudchenko et al., 2000). NMDA receptors in these areas are also critically involved in span as overexpression of GluN2B-containing NMDA receptors in the forebrain increased span on the OST (Cui et al., 2011) while span is reduced after administration of NMDA receptor antagonists (Davies, Greba et al., 2013; Galizio et al., 2013; MacQueen et al., 2011; Rushforth et al., 2011). Maternal polyI:C treatment has an array of neuropathological effects on frontal-striatal circuits (Meyer et al., 2009; Piontkewtiz et al., 2012; Meyer, 2014) which may explain the deficits in OST acquisition and performance we have observed. Interestingly, sub-chronic administration of clozapine in early adulthood improves working memory in polyI:C offspring when longer delays are used (Meyer et al., 2010). Thus, future research to test the effects of atypical antipsychotic drug treatment on the acquisition and performance of the OST may offer options for treatment of working memory impairments in individuals with schizophrenia (Barch & Smith, 2008; Dudchenko et al., 2013).

Acknowledgements

BGM and JJM collected the data. DAD analysed the data, constructed the figures, and wrote the manuscript. JGH designed the experiment, analysed the data, and wrote the manuscript. The authors wish to thank Quentin Greba and Stephanie A. Ballendine for assistance treating the dams used in the present experiment. These experiments were supported by a Canadian Institutes for Health Research Open Operating Grant to JGH (#125984). DAD held a postgraduate scholarship from the Natural Sciences and Engineering Research Council of Canada. JJM and BGM received salary support from the College of Medicine, University of Saskatchewan.

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