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Sex differences in the effect of maternal immune activation on cognitive and psychosis-like behaviour in Long Evans rats

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Abstract

Maternal immune activation during pregnancy is associated with increased risk of development of schizophrenia in later life. There are sex differences in schizophrenia, particularly in terms of age of onset, course of illness and severity of symptoms. However, there is limited and inconsistent literature on sex differences in the effects of maternal immune activation on behaviour with relevance to schizophrenia. The aim of this study was therefore to investigate sex differences in the effects of maternal immune activation by treating Long Evans rats with poly(I:C) on gestational day 15. We compared adult male and female offspring on spatial working memory in the touchscreen trial-unique nonmatching-to-location task, pairwise discrimination and reversal learning, as well as on prepulse inhibition and psychotropic drug-induced locomotor hyperactivity. Male, but not female poly(I:C) offspring displayed a deficit in spatial working memory, particularly at the longer delay. Neither pairwise discrimination nor reversal learning showed an effect of poly(I:C), but female controls outperformed male controls in the reversal learning task. Significant reduction of prepulse inhibition and enhancement of acute methamphetamine-induced locomotor hyperactivity was found similarly in male and female poly(I:C) offspring. These results show that maternal immune activation induces a range of behavioural effects in the offspring, with sex-specificity in the effects of maternal immune activation on some aspects of cognition, but not psychosis-like behaviour.

1. Introduction

Evidence suggests schizophrenia is neurodevelopmental in nature, involving one or more 'hits' during early brain development. One such insult may be infection in utero (Brown, 2006; Brown & Derkits, 2010) with previous research showing that infection during pregnancy is associated with increased risk of development of schizophrenia in later life (Brown *et al.*, 2004). The preclinical maternal immune activation (MIA) model, particularly the poly(I:C) model, has emerged as a valuable tool to explore the relationship between prenatal infection and schizophrenia (Meyer & Feldon, 2012). Poly(I:C) is a synthetic analogue of double-stranded RNA that mimics the acute phase of the response to viral infection. Administration of poly(I:C) to a pregnant dam causes an increase in levels of pro-inflammatory cytokines including interleukin-1 β , interleukin-6 and tumour necrosis factor- α (Cunningham, Campion, Teeling, Felton, & Perry, 2007) by activating the transmembrane protein toll-like receptor 3 (Akira & Takeda, 2004). Following gestational treatment with poly(I:C), offspring exhibit cognitive and behavioural aberrations reminiscent of schizophrenia symptomatology, including impaired sensorimotor gating, reduced social interaction, decreased cognitive performance, and altered locomotor hyperactivity and response to psychotomimetic drugs (Wolff & Bilkey, 2010; Howland *et al.*, 2012; Luchicchi *et al.*, 2016; van den Buuse, 2010). Neurochemical changes seen in schizophrenia patients, including dysregulation of striatal dopamine (Abi-Dargham *et al.*, 1998; Howes *et al.*, 2011), are also observed in poly(I:C) offspring (Piontkewitz *et al.*, 2011a; Luchicchi *et al.*, 2016). Current literature suggests mid-late gestation exposure of intravenous poly(I:C) in rats is most effective in producing prepulse inhibition (PPI) impairments and aberrations in locomotor hyperactivity following psychotomimetic drugs in the offspring (Piontkewitz *et al.*, 2011a; Howland *et al.*, 2012; Meehan *et al.*, 2016).

There are prominent sex differences in schizophrenia, particularly an earlier onset of approximately 3-5 years in males compared to females. Further, females also have a second peak of incidence at 40-45 years of age, a period suggested to correspond with low sex hormone levels due to menopause (Häfner *et al.*, 1993; Häfner, 2003). Course of illness, severity of symptoms, and response to antipsychotic medication differ between sexes, with females having a better outcome compared to males (as reviewed in: Canuso & Pandina,

2007; Abel *et al.*, 2010; Markham, 2012). Presentation of the illness also differs, with men affected by more negative symptoms and women suffering more affective symptoms (Canuso & Pandina, 2007). Sex hormones are postulated to be a prominent factor in the dissimilar presentation of illness between men and women (Gogos *et al.*, 2015). Symptom severity in women with schizophrenia and risk of relapse increase during the postpartum period, postmenopause, and during the luteal phase of the menstrual cycle, all times of reduced hormone levels (Kendell *et al.*, 1987; Brockington *et al.*, 1988; Seeman, 1997).

Sex differences have received limited attention in studies on the effect of MIA, with most studies testing male rats only, and the data appear inconsistent. For example, some previous studies using both male and female subjects found no significant sex differences in spatial working memory (Bitanhirwe *et al.*, 2010; Richetto *et al.*, 2013; MacDowell *et al.*, 2017) but other studies reported impairments in male but not female poly(I:C) offspring in PPI (Meehan *et al.*, 2016) and strategy set-shifting (Zhang *et al.*, 2012). The mechanism behind the discrepant sex differences in the effects of MIA remain unclear, although it is feasible that endogenous estrogen may be protecting female rodents from certain cognitive-behavioural impairments but not others. The effect of estrogen on cognition has been shown to differ depending on the cognitive domain tested (Gogos, 2013; Sbisà *et al.*, 2017b). Moreover, methodological differences between laboratories, including the timing of poly(I:C) treatment or batch of poly(I:C) used (Mueller *et al.*, 2019) and factors such as rat/mouse strain, could play a role and make direct comparison between studies difficult.

In the current study, we therefore sought to further characterise sex differences in the effects of poly(I:C) treatment on a range of behaviours with relevance to schizophrenia, in offspring born to dams injected intravenously on gestational day 15 (GD15). In two cohorts of Long Evans rats held in the same animal housing facility, from dams treated on the same gestational day, we determined: (1) the effects on three different cognitive domains: spatial working memory in the trial-unique nonmatching-to-location task (TUNL), pairwise discrimination learning, and reversal learning; and (2) the effects on two psychosis-like behaviours: PPI and psychotomimetic drug-induced locomotor hyperactivity. The results confirm that sex differences in the effects of gestational poly(I:C) depend on the behavioural

domain investigated, with cognitive deficits found predominantly in males but psychosis-like behaviour affected in both sexes similarly.

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2. Material and methods

2.1 Animals

22 female and 7 male outbred Long Evans rats were obtained from a breeding colony at the Florey Institute of Neuroscience and Mental Health, Melbourne, Australia, or the Australian Resource Centre, Murdoch, Australia. These rats were used to generate the offspring that were later tested for behavioural changes. The Long Evans rat strain was chosen as its superior visual acuity compared to albino strains makes it an ideal choice for cognitive testing in the touchscreen platform which strongly depends on visual recognition of stimuli (Bryce & Howland, 2015; Kumar *et al.*, 2015). All rat breeding, treatments and behavioural testing were carried out in the Behavioural Neuroscience suites at the La Trobe Animal Research and Teaching Facility, La Trobe University, Melbourne, Australia. All surgical procedures and experiments were approved by the La Trobe University Animal Ethics Committee and performed at the Central Animal House at La Trobe University Animal Research and Teaching Facility (Bundoora, VIC, Australia). Experiments were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes set out by the National Health and Medical Research Council of Australia.

All rats were housed in groups of 2-4 in individually-ventilated cages (Tecniplast, Italy) at a constant temperature of 20-22 °C and a 12/12h light cycle. The animals used for touchscreen cognitive testing (N=43) were moved at weaning (3 weeks of age) to housing in a reverse light cycle room (white lights on 8pm, off 8am). These animals' dark period was mimicked by using lighting covered with Light Gard Spectral Control Window Film (Solar Graphics, Clearwater, FL, USA) to restrict illumination to red light. These rats were food restricted to 90-95% of their free-feeding weight beginning 3 days before pre-training. Food restriction included reduction by 1-2 g of food per day (beginning at 10 g) until goal body weight was reached, which took approximately 5 days and overlapped with the habituation phase of TUNL pre-training. These rats were maintained on food restriction for the entire experiment, however, daily food allowance increased over time, allowing for continued growth. For the animals tested for PPI and locomotor hyperactivity (N=42), lights were on at 7am and off at 7pm and these rats received standard rat chow and tap water *ad libitum*.

2.2 Maternal Immune Activation (MIA)

Females were approximately 4 months of age at the time of successful mating which was verified by the presence of a vaginal plug the next morning. This was designated as GD1 and the dams were subsequently housed with fellow female breeders (4 per cage) until GD15. Dams were then randomly allocated to control or poly(I:C) treatment conditions. On GD15, the rats were briefly anaesthetised using an isoflurane/oxygen gas mixture and injected with saline or 4 mg/kg of poly(I:C) (salt weight; polyinosinic–polycytidylic acid potassium salt, P9582 Sigma Chemical Company, MO, USA) into the tail vein in a volume of 1 ml/kg. Poly(I:C) was dissolved in saline and prepared fresh the morning of each injection. Following injection on GD15, dams were individually housed. Prior to injection on GD15 and 24 hours later on GD16, body weight and rectal temperature were taken and then dams were left undisturbed for the remaining duration of pregnancy. All dams delivered pups normally, litters were weaned at postnatal day 21, and littermates were separated into the two cohorts (Supplementary table 1). To generate poly(I:C) offspring, 8 litters were born to form cohort 1 for touchscreen testing and 12 litters to form cohort 2 for PPI/locomotor testing. To generate control offspring, 7 litters were born to form cohort 1 and 10 litters to form cohort 2. Cohort 1 groups included no more than three offspring per sex from one litter and cohort 2 had ≤ 2 offspring/sex/litter. See Supplementary Table 1.

2.3 Touchscreen tasks

Automated touchscreen operant systems (Campden Instruments, Leics, UK) were used to test learning and spatial working memory (Bussey *et al.*, 2012; Nithianantharajah & Grant, 2013; Sbisà *et al.*, 2017a). Briefly, automated trapezoidal-shaped operant boxes were equipped with a LED house light, tone, and click generator, a touch-sensitive LCD screen, a pellet dispenser delivering 45 mg dustless precision sugar reward pellets (Bio-Serv, NJ, USA), and an infrared detection system to record screen touches and magazine entries. The system was controlled by ABET software (Campden Instruments). Performance was measured as the percentage of correctly completed trials (%correct; correct trials/total completed trials $\times$ 100). Rats underwent behavioural testing between 9am and 2pm of their reverse light cycle each day.

2.4 Trial-Unique Nonmatching-to-Location (TUNL) task

The TUNL task is a prefrontal cortex and hippocampus-dependent test of spatial working memory and pattern separation (Talpos *et al.*, 2010). Touchscreen pre-training, TUNL training, and testing of rats followed the protocol as outlined previously (Oomen *et al.*, 2013) with some modifications (Sbisa *et al.*, 2017a). Rats were trained to associate stimuli on one of 15 response windows with a sugar pellet reward and to touch this stimulus in order to obtain this reward. Incorrect responses were not rewarded and resulted in 'punishment' (5 sec timeout and illumination of the house light). Testing commenced immediately following training, and occurred over a 4-week period. To better reveal possible differences between the groups, TUNL testing sessions included an additional separation and a more extended delay between the sample and the non-matching stimulus, 6 sec. Thus, rats were tested on one of four separation types (S0-3) per week (for 5 consecutive days with a 2 day break in-between types) and each trial had a random 1 or 6 sec delay between the sample and non-matching stimuli. Separation refers to the number of squares between the sample and non-matching stimulus. The inter-trial interval (ITI) was 5 sec and rats were to complete up to 80 trials or 60 min per testing day.

2.5 Pairwise discrimination and reversal learning task

Immediately following testing on the TUNL task, rats were trained on the pairwise discrimination and reversal learning task, which involves perceptual learning and nonhippocampal associative stimuli-reward learning, and assesses cognitive flexibility (Romberg *et al.*, 2011). During this task a 1 x 2 screen mask was used (10 x 6 cm, spaced 1 cm apart). Due to previous experience in using the touchscreen apparatus, rats underwent only one training stage to familiarise with the new stimuli. The task involved viewing one image on the screen displayed in 1 of 2 response windows. Touching the blank square instead of the image resulted in a 5 sec timeout and illumination of the house light; nose-poke to the stimuli resulted in a reward. Rats were to complete 100 trials in 60 min (5 sec ITI) and achieve at least 80% correct. Following training and a 1-day break, rats were tested for 5 consecutive days on pairwise discrimination, which involved simultaneous presentation of two different stimuli: correct and incorrect. Location of stimuli was random; selecting the correct image elicited a tone in conjunction with a reward, and selecting the incorrect image resulted in a 5 sec timeout and illumination of the house light. Following a

1-day break, rats were tested for another five consecutive days in reversal learning, during which the correct stimulus became incorrect.

2.6 Prepulse Inhibition (PPI)

PPI of the acoustic startle response was measured with eight automated startle chambers (SR-Lab; San Diego Instruments, CA, USA), as described previously (Gogos *et al.*, 2010). Rats were placed individually into a transparent acrylic cylinder in a sound-attenuating cabinet. The PPI session comprised 80 trials presented with variable intervals (8-27 sec), including 32 startle pulse-alone trials (4 blocks of eight 115 dB trials) and 40 prepulse-pulse trials. Prepulse-pulse trials consisted of a prepulse of an intensity of 2, 4, 8, 12, or 16 dB above the 70 dB background (eight per intensity), followed 100 msec later by the startle pulse. Startle data were measured using all four blocks of pulse-alone trials; the middle 16 pulse-alone trials were used to calculate %PPI. The %PPI was calculated as $[(\text{pulse-alone trials startle response amplitude} - \text{prepulse-pulse trials startle amplitude}) / (\text{pulse-alone trials startle amplitude})] \times 100\%$. One outlier (male control) was excluded due to low baseline startle response.

2.7 Locomotor activity

Locomotor activity was measured using eight automated photocell chambers (ENV-520, MED Associates, VT, USA), as published previously (Gogos *et al.*, 2012). Each locomotor activity session was 120 min and distance moved was recorded in consecutive 5 min time blocks. Rats were placed in the locomotor chamber for 30 min, after which they were injected with saline or methamphetamine (1 mg/kg, (+)methamphetamine HCl, National Measurement Institute, NSW, Australia) and locomotor activity was recorded for a further 90 minutes. Methamphetamine was dissolved in 0.9% sterile saline and injected subcutaneously at a volume of 1 ml/kg. The 5 min interval during which rats were injected was excluded from data analysis. The average locomotor activity of the 30-min period before injection was calculated as a measure of novelty-induced locomotor hyperactivity and habituation. Two outliers (female control) were excluded due to high baseline activity.

2.8 Data analysis

All data were expressed as mean $\pm$ standard error of the mean (SEM) and analysed using the statistical software package SPSS Statistics 23 (IBM, IL, USA). Body weight and temperature were assessed with one-way analysis of variance (ANOVA) with treatment (control or poly(I:C)) as the between-subject factor. We were unable to collect temperature data from two female dams (control treatment). ANOVA was used to test between-subject factors, including poly(I:C) treatment and sex, and repeated-measures analyses were used where appropriate to test within-subject factors including spatial separation, prepulse intensity and time (day, week or block). Bonferroni post-hoc correction for multiple comparisons was applied when overall ANOVA were significant. Effect size was estimated using Partial eta squared (η^2), where 0.01 is small, 0.06 is medium and 0.138 is large (Richardson 2011). Results were considered significant if $P < 0.05$.

3. Results

3.1 Body weight and temperature

One-way ANOVA showed no significant difference in dam body weight between control and poly(I:C) treatment groups at the beginning of pregnancy (GD1; control = 192 ± 5.0 g, poly(I:C) = 194 ± 5.4 g). Further, analysing weight gain from GD1 to G15 showed no difference between treatment groups (control = 27.7 ± 2.4 g, poly(I:C) = 30.4 ± 2.3 g), however the change in weight from GD15 to GD16 was significantly different between conditions (control = 2.3 ± 0.6 g, poly(I:C) = -1.5 ± 0.9 g; day x treatment interaction: $F_{1,20} = 11.16$, $P = 0.003$). Temperature change from immediately prior to treatment to 24 hours post-injection also significantly differed between conditions (day x treatment interaction: $F_{1,18} = 5.12$, $P = 0.04$); poly(I:C) dams on average had decreased rectal temperature (control = $36.3 \pm 0.4^\circ\text{C}$ vs. $36.7 \pm 0.2^\circ\text{C}$, poly(I:C) = $36.6 \pm 0.2^\circ\text{C}$ vs. $36.2 \pm 0.1^\circ\text{C}$).

3.2 TUNL training

Over the six-week TUNL training period (Supplementary figure 1), all rats gradually increased their level of correct responding (main effect of week: $F_{5,195} = 33.61$, $P < 0.001$, partial $\eta^2 = 0.46$) and performed better with greater separation between the sample and the non-matching stimulus (main effect of separation: $F_{2,78} = 74.11$, $P < 0.001$, partial $\eta^2 = 0.66$). There was also a main effect of sex ($F_{1,39} = 6.59$, $P = 0.01$, partial $\eta^2 = 0.14$) reflecting an overall better performance in males, and a main effect of poly(I:C) treatment ($F_{1,39} = 4.80$, $P = 0.03$, partial $\eta^2 = 0.11$) reflecting overall lower scores in poly(I:C) treated offspring. There were no significant interactions. These data suggest that poly(I:C) offspring show impaired learning of the TUNL task although the group differences are subtle (Supplementary figure 1). Indeed, during the last week of TUNL training, there was no more effect of poly(I:C) treatment although a sex difference persisted (Table 1).

3.3 TUNL testing

As expected, analysis of the percentage correct responding in the TUNL task revealed highly significant main effects of separation ($F_{3,117} = 181.22$, $P < 0.001$, partial $\eta^2 = 0.82$) and of delay ($F_{1,39} = 179.82$, $P < 0.001$, partial $\eta^2 = 0.82$) as well as a separation x delay interaction

($F_{3,117} = 23.32$, $P < 0.001$, partial $\eta^2 = 0.37$), reflecting the reduced number of correct responses with increased difficulty of the task due to reduced separation of the sample and the non-matching stimulus or due to increased delay (Figure 1). There was also a main effect of poly(I:C) treatment ($F_{1,39} = 4.16$, $P = 0.05$, partial $\eta^2 = 0.10$) reflecting impaired performance in poly(I:C) offspring. There was a delay x sex interaction ($F_{1,39} = 4.16$, $P = 0.05$, partial $\eta^2 = 0.10$), therefore further analysis was done with data separated by delay and sex (Figure 1).

Analysis of percentage correct responding at the shorter 1 sec delay in males revealed a separation x poly(I:C) treatment interaction ($F_{3,63} = 3.28$, $P = 0.03$, partial $\eta^2 = 0.14$) as well as the expected main effect of separation ($F_{3,63} = 129.30$, $P < 0.001$, partial $\eta^2 = 0.86$). Further analysis of each separation showed significantly lower correct responding of poly(I:C)-treated males at separation 1 ($P = 0.03$) but not any of the other separations (Figure 1). Analysis of percentage correct responding at the longer 6 second delay in males again revealed the main effect of separation ($F_{3,63} = 31.53$, $P < 0.001$, partial $\eta^2 = 0.60$) and also a main effect of poly(I:C) treatment ($F_{1,21} = 4.84$, $P = 0.04$, partial $\eta^2 = 0.19$). Inspection of the data (Figure 1) suggests this result reflects generally less correct responding in the poly(I:C) group as compared to controls, irrespective of the separation level. Analysis of percentage correct responding in females showed main effects of separation at both the 1 and 6 sec delay ($F_{3,54} = 81.76$ and 21.51 , respectively, both $P < 0.001$, partial $\eta^2 = 0.82$ and 0.54), however there were no effects of poly(I:C) treatment (Figure 1).

3.4 Pairwise discrimination and reversal learning

Over the five days of pairwise discrimination learning, the percentage correct responses significantly increased (main effect of day: $F_{4,156} = 142.87$, $P < 0.001$, partial $\eta^2 = 0.79$). However, there were no main effects or interactions with sex or poly(I:C) treatment, suggesting all groups learned this task equally (Figure 2A, Table 1).

On the first day of reversal learning, as expected, correct responding dropped to low levels but the animals rapidly learned the new rule as indicated by the increasing percentage of correct responses over the five-day period (main effect of day: $F_{4,156} = 108.71$, $P < 0.001$,

partial $\eta^2 = 0.74$). While there were no interactions with sex or poly(I:C) treatment, there was a main effect of sex ($F_{1,39} = 6.39$, $P = 0.02$, partial $\eta^2 = 0.14$) which reflected less correct responding in males as compared to females, particularly in control males (Figure 2B, Table 1). Indeed, further analysis revealed significantly lower reversal learning in male controls vs. female controls (main effect of sex: $F_{1,18} = 6.58$, $P = 0.02$, partial $\eta^2 = 0.27$; main effect of day: $F_{4,72} = 41.33$, $P < 0.001$, partial $\eta^2 = 0.70$; no interaction) but no sex difference in poly(I:C) offspring (only a main effect of day: $F_{4,84} = 71.75$, $P < 0.001$, partial $\eta^2 = 0.77$).

3.5 Prepulse inhibition (PPI) of acoustic startle

As expected, percentage PPI increased as a function of prepulse intensity ($F_{4,152} = 126.39$, $P < 0.001$, partial $\eta^2 = 0.77$). There was a main effect of poly(I:C) treatment ($F_{1,38} = 29.55$, $P < 0.001$, partial $\eta^2 = 0.44$), reflecting lower PPI in poly(I:C) offspring (Figure 3), irrespective of the sex of the animals. Indeed, analysis of either male or female data revealed significantly lower PPI in poly(I:C) offspring in both sexes (males: $F_{1,21} = 17.03$, $P < 0.001$, partial $\eta^2 = 0.45$; females: $F_{1,17} = 16.10$, $P = 0.001$, partial $\eta^2 = 0.49$; Figures 3A and 3B, respectively).

Startle amplitudes habituated over the course of the PPI session (main effect of startle block, $F_{3,114} = 7.53$, $P < 0.001$, partial $\eta^2 = 0.17$, Supplementary Figure 2) and were lower in female rats than in male rats (main effect of sex: $F_{1,38} = 9.76$, $P = 0.003$, partial $\eta^2 = 0.20$, Figure 3C). Females also tended to habituate less to repeated startle stimuli (block x sex interaction: $F_{3,114} = 2.78$, $P = 0.04$, partial $\eta^2 = 0.07$), possibly because of their already lower startle amplitudes (Supplementary Figure 2). Average startle amplitudes (Figure 3C) or startle habituation (Supplementary Figure 2) were not affected by poly(I:C) treatment.

3.6 Locomotor hyperactivity

Spontaneous, novelty-induced locomotor hyperactivity habituated rapidly after the animals were placed in the locomotor photocell chambers (main effect of time: $F_{5,185} = 71.26$, $P < 0.001$, partial $\eta^2 = 0.66$). There was a time x sex x poly(I:C) interaction ($F_{5,185} = 4.24$, $P = 0.001$, partial $\eta^2 = 0.10$), reflecting that spontaneous activity was slightly higher in female rats than in male rats, particularly at the first 5-min time block (sex x poly(I:C) interaction at time block 1 only, $F_{1,37} = 4.57$, $P = 0.039$, partial $\eta^2 = 0.11$). There was no effect of poly(I:C)

treatment on overall levels of locomotor activity or habituation during this phase (Supplementary Figure 3).

Acute treatment with methamphetamine (1 mg/kg) induced marked locomotor hyperactivity ($F_{1,37} = 137.68$, $P < 0.001$, partial $\eta^2 = 0.79$). This effect was greater in female rats compared to male rats (methamphetamine x sex interaction: $F_{1,37} = 4.50$, $P = 0.04$, partial $\eta^2 = 0.11$; main effect of sex: $F_{1,37} = 4.67$, $P = 0.04$, partial $\eta^2 = 0.11$; Figure 4) and in poly(I:C) offspring compared to controls (methamphetamine x poly(I:C) interaction: $F_{1,37} = 5.07$, $P = 0.03$, partial $\eta^2 = 0.12$; main effect of poly(I:C) treatment: $F_{1,37} = 4.62$, $P = 0.04$, partial $\eta^2 = 0.11$). However, the lack of a sex x poly(I:C) interaction or sex x poly(I:C) x methamphetamine interaction suggested that methamphetamine-induced locomotor hyperactivity was similarly enhanced in male and female poly(I:C) offspring compared to controls (Figure 4).

4. Discussion

This study aimed to compare the effects of MIA on cognition and psychosis-like behaviour in male and female Long Evans rat offspring. We injected pregnant dams with poly(I:C) on GD15 and assessed the effects of this treatment in the offspring in adulthood on spatial working memory in the TUNL task, pairwise discrimination and reversal learning, as well as on PPI and locomotor hyperactivity. Key findings include that poly(I:C) offspring showed a deficit in spatial working memory but not pairwise discrimination or reversal learning. Further, when TUNL data were separated by delay and sex, there appeared a difference where male poly(I:C) offspring had lower performance compared to male controls, particularly at the longer 6 second delay, but this was not observed in females. Poly(I:C) treatment caused significant reduction of PPI and enhancement of acute methamphetamine-induced locomotor hyperactivity; these changes in psychosis-like behaviour were independent of the sex of the animals. This study also found sex differences which were independent of poly(I:C) treatment, such as lower correct responding in females than in males in the TUNL task but higher percentages in reversal learning. Startle amplitudes were generally lower in female compared to male rats but spontaneous locomotor activity and methamphetamine-induced locomotor hyperactivity were higher in females than in males. Our findings further characterise the poly(I:C) model of schizophrenia, in the Long Evans rat strain, and highlight that effects of MIA are sex-specific on select cognitive domains, but not psychosis-like behaviour.

While poly(I:C) offspring can successfully model symptoms as seen in schizophrenia patients, some studies show contrasting results (Zuckerman & Weiner, 2003; Meyer *et al.*, 2006; Piontkewitz *et al.*, 2009). Some of these discrepancies could be due to the timing and method of poly(I:C) administration. For example, it appears that early gestation poly(I:C) exposure (e.g. GD10) leads to dopamine-related positive symptom-like behavioural changes in offspring, while mid-late gestation exposure (e.g. GD15, GD19) produces glutamatergic dysfunction and leads to behavioural changes reminiscent of negative and cognitive symptoms (Meehan *et al.*, 2016); hence the most common exposure at GD15 aims to intersect these two windows of sensitivity (Meyer *et al.*, 2006).

4.1 Male poly(I:C) offspring exhibit impaired spatial working memory but not pairwise discrimination or reversal learning

Male, but not female poly(I:C) offspring showed an impairment in this task compared to controls, and a tendency for impairment during the training period. While several studies have shown deficits in poly(I:C) offspring in spatial working memory in tasks including the Morris watermaze, cheeseboard maze and alternation T-maze (Meyer *et al.*, 2008; Bitanirwe *et al.*, 2010; Richetto *et al.*, 2013; Meehan *et al.*, 2016), these applied treatment during late gestation (mouse: GD17, rat: GD19). Thus the findings of the current study, using mid-late gestation (GD15) treatment, extends the time frame for which spatial working memory deficits can be observed in poly(I:C) offspring. Of note, a previous study in poly(I:C) mice found significant impairments in spatial working memory only following a long (30 sec) delay (Meyer *et al.*, 2008), similar to our results where a deficit was particularly evident when using a greater delay. Of the mid-late gestation exposure studies, whether they tested male only poly(I:C) offspring or both sexes, impairment was found in males only (Howland *et al.*, 2012; Zhang *et al.*, 2012). In contrast, studies using late gestation administration showed no significant sex differences in poly(I:C)-induced impairments (Bitanirwe *et al.*, 2010; Richetto *et al.*, 2013; MacDowell *et al.*, 2017), suggesting that spatial working memory impairments in female poly(I:C) offspring may only be evident after MIA during late gestation. While we selected to use the Long Evans rat strain in this study due to their enhanced visual acuity, they are not as commonly used as other strains in MIA models. One study used a similar protocol to the current study where Long Evans dams were administered poly(I:C) intravenously on GD15 (Murray *et al.*, 2017). They assessed male offspring in an olfactory working memory task (nonmatching-to-sample) and found that poly(I:C) offspring showed poorer performance (Murray *et al.*, 2017), similar to the findings of the current study.

In line with Zuckerman and Weiner (Zuckerman & Weiner, 2005) who used Wistar rats, we did not observe an effect of poly(I:C) treatment in Long Evans rats on discrimination learning. However in contrast, poly(I:C) offspring in the current study did not demonstrate enhanced reversal learning. Although not reaching statistical significance, male poly(I:C) offspring had increased reversal learning (34.8%) compared to control males (29.5%), while female performance did not differ. The literature on reversal learning and MIA is highly

variable even though all studies administered poly(I:C) on GD15; some studies have shown enhancements in poly(I:C) offspring (Zuckerman & Weiner, 2005; Zhang *et al.*, 2012), while others showed deficits (Han *et al.*, 2011; Savanthrapadian *et al.*, 2013) or no effect of poly(I:C) (Ballendine *et al.*, 2015). The reason for these discrepancies is unclear but may be due to the different techniques used to measure reversal learning.

While unknown, it is possible that the sex difference in the effect of poly(I:C) treatment on spatial working memory may be due to greater in utero susceptibility to the effects of poly(I:C) in male offspring, the protective effect of estrogen during postnatal development in females, or a combination of both factors. Considering the enhancing effect estrogen can have on rodent cognition (Luine *et al.*, 2003; Sbisa *et al.*, 2017b) and estrogen's effects on spine density in the hippocampus (Maclusky *et al.*, 2005), it is possible endogenous estrogen may be preventing impairment. Indeed, we have previously shown that ovariectomy, i.e. removing the main endogenous source of circulating estrogen in female rats, results in significant impairment of correct responding in the TUNL task (Sbisa *et al.*, 2017a). Previous TUNL studies have shown the spatial component of the task (separation) is hippocampus-dependent (McAllister *et al.*, 2013), while the working memory component (delay) is reliant on both intact prefrontal cortex and hippocampus (Talpos *et al.*, 2010; McAllister *et al.*, 2013). Literature suggests MIA interferes with foetal brain development, inducing long-lasting neurochemical and structural changes within these brain areas. For example, offspring of dams treated on GD15 exhibit disrupted hippocampal expansion during adolescence, and smaller hippocampal and prefrontal-cortical volume in adulthood, compared to control offspring (Piontkewitz *et al.*, 2011b). Poly(I:C) treatment resulted in moderate to severe cell loss in the hippocampus and entorhinal cortex of adult rat offspring, which is consistent with morphological abnormalities in schizophrenia (Falkai & Bogerts, 1986; Zaidel *et al.*, 1997; Zuckerman *et al.*, 2003). Collectively, evidence suggests gestational poly(I:C) exposure is capable of inducing morphological changes in brain areas important for intact performance in the TUNL task, namely the hippocampus, while the lack of impairment in pairwise discrimination and reversal learning may be due to the nonhippocampal nature of these cognitive domains (Graybeal *et al.*, 2011). Further studies investigating the neurobiological mechanisms underlying the cognitive deficits (or lack thereof) that are observed in poly(I:C) offspring are needed.

4.2 No sex differences in the effect of MIA on PPI and locomotor hyperactivity

The current results showed both male and female poly(I:C) offspring exhibited a deficit in baseline PPI without significant change in startle responses. This is consistent with previous literature administering poly(I:C) intravenously on GD15 (Wolff & Bilkey, 2008; Dickerson *et al.*, 2010; Wolff & Bilkey, 2010; Yee *et al.*, 2011; Howland *et al.*, 2012; Klein *et al.*, 2013; Ballendine *et al.*, 2015; Bikovsky *et al.*, 2016; Luchicchi *et al.*, 2016). Baseline exploratory locomotor activity, locomotor habituation and locomotor activity following saline injection were not significantly altered in either male or female poly(I:C) offspring. In contrast, poly(I:C) offspring showed increased methamphetamine-induced locomotor hyperactivity, consistent with previous research in offspring of GD15 poly(I:C) treated dams (Zuckerman & Weiner, 2005). It has been postulated that the deficit in PPI and enhanced sensitivity to methamphetamine observed in poly(I:C) offspring is due to changes in the dopaminergic system, particularly in subcortical brain regions (Vuillermot *et al.*, 2010; Meehan *et al.*, 2016). For example, extracellular levels of dopamine were increased in the nucleus accumbens of poly(I:C) offspring (Zuckerman & Weiner, 2003; Hadar *et al.*, 2015; Luchicchi *et al.*, 2016), but dopamine D2 receptor sensitivity did not differ between groups (Luchicchi *et al.*, 2016). In the nucleus accumbens, spontaneous firing rate of dopamine neurons was reduced (Hadar *et al.*, 2015) and tyrosine hydroxylase immunoreactivity (Meyer *et al.*, 2007) and levels of the dopamine metabolite, homovanillic acid (Winter *et al.*, 2009) were increased, adding further evidence of altered dopaminergic activity in the brains of poly(I:C) offspring.

Importantly, in contrast to spatial working memory, there were no sex differences in the effect of poly(I:C) on PPI or methamphetamine-induced locomotor hyperactivity. Previous poly(I:C) literature including both male and female rats has similarly found no significant sex differences in psychotomimetic drug-induced locomotor hyperactivity (Zuckerman & Weiner, 2005; Richtand *et al.*, 2011; Howland *et al.*, 2012; Richtand *et al.*, 2012; Vorhees *et al.*, 2012; Chou *et al.*, 2015; Vorhees *et al.*, 2015). This has implications for future studies deciding to use both sexes in experiments.

4.3 Limitations

It is important to mention the limitations of this study. Firstly, it is unclear whether our results are due to variability stemming from a litter effect or not. In this study, there were 10 litters used to generate the control offspring, and 12 litters generated the poly(I:C) offspring. We aimed to reduce a possible litter effect bias by using a maximum of 3 rats per sex in an experiment. As there were multiple studies being conducted using these offspring (i.e. experiments other than those presented here), in some cases, only one rat of a n=14 litter was used rather than using one rat per sex for each cohort. Future studies should carefully consider litter effects and the distribution of offspring per litter per experiment. Secondly, for the touchscreen experiments only, the rats were food-restricted. While this is necessary for increasing the motivation towards food rewards, it may have confounded results. It is possible that food restriction may have had a greater impact on one sex over the other. However, this is speculative and further studies are required to determine whether food restriction can, in a sex-specific manner, influence cognition. Finally, future studies should investigate the molecular basis of the effects observed in this study.

4.4 Implications and conclusion

Cognitive deficits, including working memory deficits, are core symptoms of schizophrenia (Sbisa *et al.*, 2017b). In a study assessing spatial working memory (using a sample comprising 79% males), schizophrenia patients were significantly less accurate than controls but only after a delay (Mazhari *et al.*, 2010). Overall men with schizophrenia have a worse prognosis than women, however there are many factors to consider, in particular hormone status (Gogos *et al.*, 2019). Few studies adequately address sex differences in cognition in patients with schizophrenia (Gogos *et al.*, 2019); but one carefully controlled study found no sex difference in spatial working memory (Roesch-Ely *et al.*, 2009), which is in contrast to the rat findings of the current study. Further research into sex differences in people with schizophrenia is required to disentangle the factors involved.

For the first time, we demonstrate spatial working memory impairment in poly(I:C) offspring of Long Evans rats treated intravenously at GD15, however, only in male rats. Further, no significant effect of MIA was seen in pairwise discrimination and reversal learning. Consistent with previous literature, poly(I:C) treatment caused reduction of PPI and

enhanced methamphetamine-induced locomotor hyperactivity, indicative of increased psychosis-like behaviour. Importantly, there were no sex differences in these effects. This research helps to further characterise the poly(I:C) model, which has emerged as a valuable tool in preclinical schizophrenia research, in addition to highlighting the differential effect MIA can have on male and female offspring, depending on the behavioural domain assessed.

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Author Contributions

AG and MvdB designed the study, analysed data and drafted the paper. AS and DW performed the experiments. AS contributed to writing the manuscript.

Data Accessibility

The data will be made available upon request from Andrea Gogos (andrea.gogos@florey.edu.au).

Conflict of Interest

The authors have no conflicts of interest to declare.

Abbreviations

ANOVA – Analysis of variance

GD - Gestational day

ITI - Inter-trial interval

MIA - Maternal immune activation

PPI - Prepulse inhibition

SEM - Standard error of the mean

TUNL - Trial-unique nonmatching-to-location

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	Male		Female	
% correct responding	Control	Poly(I:C)	Control	Poly(I:C)
TUNL learning week 6	68.7 ± 0.8	66.1 ± 1.3	63.5 ± 1.4 [#]	63.5 ± 1.5 [#]
Pairwise discrimination learning	75.0 ± 3.6	75.1 ± 2.2	77.6 ± 1.8	76.4 ± 1.9
Reversal learning	29.5 ± 2.6	34.8 ± 1.8	38.0 ± 1.8 [*]	36.7 ± 1.7

Table 1: Average percentage (%) correct responding over the last five days of TUNL training (week 6), the five days of pairwise discrimination learning and five days of reversal learning in male and female Long Evans rats that were offspring of poly(I:C)-treated dams or vehicle-treated dams. Data for TUNL learning were pooled over three separations. ^{*}*P* < 0.05 compared to male controls; [#]*P* < 0.05 for main effect of sex. Male control n=11, male poly(I:C) n=12, female control n=9, female poly(I:C) n=11.

	Male		Female	
% correct responding	Control	Poly(I:C)	Control	Poly(I:C)
TUNL learning week 6	68.7 ± 0.8	66.1 ± 1.3	63.5 ± 1.4 [#]	63.5 ± 1.5 [#]
Pairwise discrimination learning	75.0 ± 3.6	75.1 ± 2.2	77.6 ± 1.8	76.4 ± 1.9
Reversal learning	29.5 ± 2.6	34.8 ± 1.8	38.0 ± 1.8*	36.7 ± 1.7

Table 1: Average percentage (%) correct responding over the last five days of TUNL training (week 6), the five days of pairwise discrimination learning and five days of reversal learning in male and female Long Evans rats that were offspring of poly(I:C)-treated dams or vehicle-treated dams. Data for TUNL learning were pooled over three separations. * $P < 0.05$ compared to male controls; # $P < 0.05$ for main effect of sex. Male control $n=11$, male poly(I:C) $n=12$, female control $n=9$, female poly(I:C) $n=11$.

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