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Maternal immune activation during pregnancy alters the behavior profile of female offspring of Sprague-Dawley rats

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Abstract

Sex differences are documented in psychiatric and neurological disorders, yet most preclinical animal research has been conducted in males only. There is a need to better understand of the nature of sex differences in brain disease in order to meet the needs of psychiatric patients. We present the behavior profile of adult female offspring produced using a maternal immune activation model where pregnant rats receive an immune stimulant and the offspring typically show various abnormalities consistent with psychiatric illnesses such as schizophrenia and autism. The results in female offspring were compared to a previously published cohort of their male siblings (Lins et al. 2018). We examined prepulse inhibition, sociability, MK-801 induced locomotor activity, crossmodal object recognition, and oddity discrimination; behaviors relevant to the positive, negative, and cognitive symptoms of schizophrenia. No between-treatment differences in PPI or locomotor activity were noted. Tactile memory was observed in the control and treated female offspring, visual recognition memory was deficient in the polyI:C offspring only, and both groups lacked crossmodal recognition. PolyI:C offspring were impaired in oddity preference and had reduced preference for a stranger conspecific in a sociability assay. Systemic maternal CXCL1, IL-6, and TNF-a levels at 3 h post polyI:C treatment were determined, but no relationship was found between these cytokines and the behavior seen in the adult female offspring. Overall, female offspring of polyI:C-treated dams display an array of behavior abnormalities relevant to psychiatric illnesses such as schizophrenia similar to those previously reported in male rats.

Significance Statement

Sex differences are documented in mental illness and include differences in disease prevalence, symptom presentation, and response to treatment. Despite this, the majority of animal research has been conducted in males only. This study demonstrates the effects of maternal inflammation in pregnancy on long-term behavior outcomes in female offspring, revealing a behavior profile similar to male counterparts. We use a uniquely broad behavior testing battery to show that female offspring from inflammation-exposed pregnancies display an array of abnormal behaviors related to symptom domains of schizophrenia, similar to their male littermates. Maternal cytokine concentrations did not correlate with the severity of these behavior changes suggesting other factors may better indicate long-term disease risk in the offspring.

Key Words

sex differences, behavior, psychiatric illness, mental illness, sociability, recognition memory, prepulse inhibition

Introduction

Adverse events in utero and early life are linked to the development of psychiatric illness. Inflammation during pregnancy is associated with increased risk of psychiatric illnesses including autism, schizophrenia, and major depression in the offspring (Patterson, 2011; Jiang et al., 2016; Brown and Meyer, 2018; Gustafsson et al., 2018). The relationship between inflammation and psychopathology is often studied with models of maternal immune activation (MIA) where an immune stimulant such as polyinosinic:polycytidylic acid (polyI:C) is administered to pregnant rodents (Piontkewitz et al., 2012; Brown and Meyer, 2018). Offspring of treated dams display behavioral and neuropathological profiles consistent with psychiatric illness in humans (Brown and Meyer, 2018). The majority of MIA studies focus on the male offspring or lack consideration of sex as a biological variable despite policies by the National Institute of Health and other grant funding agencies that require the examination of sex as a factor in biomedical research (Clayton and Collins, 2014; Coiro and Pollak, 2019). This is particularly concerning for studies of MIA given the sex differences noted in the human psychiatric disorders associated with MIA as a risk factor (Klein and Corwin, 2002; Arad et al., 2017; Brown and Meyer, 2018; Coiro and Pollak, 2019).

Previous studies of the effects of MIA during pregnancy in rats has resulted in extensive knowledge of behavior effects in male offspring. Maternal treatment with polyI:C during gestation results in male offspring with reduced working memory span capacity, dysregulated fear responses, and impaired associative (object-in-place) and crossmodal memory while simple object recognition and object-location memory are largely unaffected, although impaired novel context recognition has also been reported (Wolff and Bilkey, 2010; Wolff et al., 2011; Sangha

et al., 2014; Ballendine et al., 2015; Murray et al., 2017). Other studies on adult male offspring from polyI:C-treated pregnancies have shown reduced levels of GAD67 in the dorsal hippocampus which appears to coincide with a loss of hippocampal-frontal coherence and correlate with prepulse inhibition (PPI) deficits (Dickerson et al., 2010, 2013, 2014; Wolff and Bilkey, 2010). PPI has been studied extensively in MIA rat models, yet the results are mixed with several studies showing deficits, no effect, or indicating the timing and type of inflammatory agent determines PPI outcomes (Fortier et al., 2004, 2007, Wolff and Bilkey, 2008, 2010; Ballendine et al., 2015; Hadar et al., 2015). Other studies with offspring of both sexes or sex unspecified report mixed results on PPI as well (Howland et al., 2012; Klein et al., 2013; Van den Eynde et al., 2014; Vorhees et al., 2015). Further MIA studies including male and female rat offspring report altered behaviors such as spontaneous hypolocomotion (Van den Eynde et al., 2014), latent inhibition (Zuckerman and Weiner, 2003, 2005; Zuckerman et al., 2003), and reduced startle (Van den Eynde et al., 2014). Other studies show no change in spontaneous locomotion but reduced sensitivity to the hyperlocomotive effects of amphetamine treatment (Bronson et al., 2011) or hyperlocomotion following amphetamine (Zuckerman et al., 2003; Vorhees et al., 2012) or MK-801 (Zuckerman and Weiner, 2005; but see also Howland et al., 2012). In some tasks, male rat offspring of MIA dams show greater impairments in tasks such as operant conditioning-based set shifting and earlier onset of latent inhibition deficits, reflecting the earlier onset of abnormal developmental trajectories (Piontkewitz et al., 2011a; Zhang et al., 2012; Patrich et al., 2016). While male MIA rats have impaired object-in-place memory, neither control nor MIA females perform this task, possibly reflecting sex-specific differences in baseline task performance (Howland et al., 2012; Ballendine et al., 2015). In a related rat model

of MIA, inflammation induced in lactating dams, resulting in the development of distinct sex-dependent phenotypes in the suckling offspring, where the females offspring displayed a depressive phenotype and male offspring displayed a psychiatric phenotype (Arad et al., 2017). Taken together, the complicated and often conflicting results from these studies demonstrate the need for sex by treatment analyses in future MIA research (Kentner et al., 2019; Coiro and Pollak, 2019).

The present study aims to contribute to the necessary evaluation of sex effects in the MIA model by highlighting female offspring behavior outcomes in tasks related to the symptoms of schizophrenia and analyzing these results in conjunction with previously published results in their male littermates. The male MIA offspring from the same cohort display hyperlocomotion following MK-801 administration, reduced sociability, impaired visual recognition memory, impaired oddity preference, altered set shifting, and facilitated reversal learning. Using a prospective design, we showed that these behavioral changes did not correlate with acute elevations in a selection of maternal serum cytokines collected 3 h post polyI:C treatment (Lins et al., 2018). We hypothesized that behavior abnormalities would be less severe or absent in early adulthood in accordance with previous literature (Piontkewitz et al., 2011a; Zhang et al., 2012; Patrich et al., 2016). We also correlated behavior of the female offspring with the acute cytokine concentrations from prospectively collected maternal blood and other measurements related to polyI:C treatment to assess relationships between acute maternal cytokine levels, other treatment effects, and offspring behavior including an oddity discrimination task not previously examined in female rats (Lins et al., 2018).

Methods

Animals

Timed-pregnant Sprague-Dawley rats (n=43, Charles River, Quebec) arrived at the animal holding facility on gestational day 7 (GD7). Primiparous dams were mated between 8 – 10 weeks of age and the presence of a vaginal plug considered GD1. Sires were a minimum of 10 weeks of age at time of mating and their specific breeding history (number of matings, successful pregnancies, mating design, or time between matings) was not guaranteed by the provider. Upon arrival, pregnant dams were housed individually in standard ventilated (395 x 346 x 227 mm) polypropylene cages. Food (Purina Rat Chow) and water were available ad libitum. The colony room is temperature, but not humidity, controlled (21°C) and maintained on an automatic 12/12 h light/dark cycle with lights on at 07:00 h. Dams were undisturbed until they received treatment on GD15. Behavior testing was conducted on adult female offspring (total n=71). All procedures were carried out during the light phase and were conducted in accordance with the Canadian Council on Animal Care guidelines for humane animal use and were approved by the University of Saskatchewan Animal Research Ethics Board.

Maternal Treatments and Blood Samples

Maternal treatment followed previously established protocols in Long-Evans and Sprague Dawley rats (Howland et al., 2012; Zhang et al., 2012; Sangha et al., 2014; Lins et al., 2016; Paylor et al., 2016; Lins et al., 2018; Figure 1A). Additional information is included to improve the scientific rigor of the MIA model as discussed by Kentner and colleagues (2019). These details also apply to our recent companion publication which tested male rat offspring from the same litters as described here (Lins et al. 2018).

Dam baseline weight (mean = 346.5 g) and rectal temperature were recorded on GD15 immediately prior to anesthesia with isoflurane (5% induction, 2.5% maintenance, for approximately 3 minutes) to receive a single intravenous (i.v.) tail vein injection of 0.9% saline or polyI:C (4 mg/kg, high molecular weight, InVivoGen, San Diego, CA, USA, thawed from storage at -20 °C). Dams were anesthetized a second time (as above, approximately 10 minutes) 3 h following initial treatment and a blood sample (<1.5 mL total and <6% total blood volume) was collected using a sterile catheter (BD Insyte™ Autogaurd™, 24 GA 0.75 IN 0.7 x 19 mm, REF 381412) from the opposite tail vein used to inject polyI:C or saline. Warm saline was administered once following initial treatment (3 mL), and a second time after blood collection (equal in volume to the collected blood sample). Blood samples coagulated at room temperature for 1 h then centrifuged at 10,000xG for 5 min and serum was stored at -80 °C until analysis. ELISAs for CXCL1 (GROα/KC; R&D Systems, Minneapolis, MN), CXCL2 (GROβ/MIP-2; R&D Systems), IL-6 (PeproTech, Rocky Hill, NJ), and TNF-α (PeproTech) were performed according to the manufacturer's instructions and these results are published elsewhere (Lins et al., 2018).

Dams had weight and rectal temperature measured 8, 24 and 48 h post treatment and then were undisturbed for the remainder of their pregnancy. Treatment was administered to n=43 dams, but 8 developed hypothermia and were euthanized within 48 h of treatment. Four additional dams experienced body temperature below 36°C but lacked additional indicators of severe sickness or suffering and these were given access to a warming pad on their home cage until their temperature returned to normal (within 24 h). 2 dams did not produce viable litters and 2 litters had no female offspring. Ultimately, offspring from a total of n=31 litters were

included (n=16 polyI:C treated dams and n=15 saline treated dams, Table 1). On postnatal day (PND) 1, litters were weighed, sexed, and culled to a maximum of 10 (4 females where possible). Standard husbandry included cage changes twice per week with one additional cage change during PND14-21. Prior to weaning, all cage changes, feeding, and monitoring of pups was performed by a single investigator to minimize disturbances. On PND23, pups were weaned and housed in same-sex sibling groups of 2 or 3 in standard housing as previously described with a PVC tube for enrichment.

Behavioral Testing

Behavior tests were conducted according to published protocols. One or two female offspring per litter were included in each test, except PPI where all available females were included (Table 2a-c). To control for the inherent relationships between siblings, effects from littermates were averaged and one value per litter was used (Zorrilla, 1997; Lazic, 2013). Estrous phase was determined daily between the hours of 07:00 and 08:30 prior to behavior testing. A single investigator used lavage with a p200 pipette and 20 μL of sterile physiological saline to collect cells from the vaginal wall for immediate visual examination with a light microscope. Proestrous was defined by the presence of uniform nucleated cells, while unnucleated cornified squamous cells were characteristic of estrous, densely packed leukocytes indicated metestrous, and scattered leukocytes alongside nucleated cells indicated diestrous (Hubscher et al., 2005). Estrous determination began 5 days prior to behavior testing and continued throughout experimentation. All rats displayed a typical 4-5 day cycle. Additional handling included exposure to investigators and emphasized picking up and moving the rats

until these motions could be carried out with ease, as well as habituation to transport between the housing and testing locations. All animal work occurred during the light phase (07:00-19:00 hrs) with the majority of behavior testing performed between 08:30-17:00. Testing began at 8 weeks of age (young adulthood) and was completed by 15 weeks of age. The order of testing was Prepulse Inhibition (PPI), Crossmodal Object Recognition (CMOR), Sociability, Oddity Discrimination, and finally MK-801 induced locomotor activity (Figure 1B). Ethanol (40%) was used to clean all behavior testing equipment between rats.

PPI: PPI measures the percent attenuation of motor response to a startling tone when the tone is preceded by a brief prepulse (Figure 2A; Lins et al., 2018). Two SR-LAB startle boxes (San Diego Instruments, San Diego, CA, USA) were used. Each session had constant background noise (70 dB) and began with 5 min of acclimatization, followed by 6 pulse-alone trials (120 dB, 40 ms). Pulse-alone (6), prepulse + pulse (36) and no stimulus (6) trials were then presented in a pseudorandom order, followed by 6 additional pulse-alone trials. Prepulse + pulse trials began with a 20 ms prepulse of 3, 6, or 12 dB above background (70 dB). Prepulse–pulse intervals (time between the onset of the prepulse and the 120 dB pulse) were short (30 ms) or long (80 ms). The inter-trial interval varied randomly from 3 to 14 s (Meyer et al., 2009; Howland et al., 2012; Ballendine et al., 2015).

Sociability Task: The testing apparatus was a rectangular arena (150 x 40 cm) of black corrugated plastic divided into three compartments, one middle start compartment (30 x 40 cm) and two ‘stranger’ compartments on either side (60 x 40 cm, see Figure 3A; Henbid et al., 2017; Lins et al., 2018). The walls dividing the middle compartment from the stranger compartments were clear Plexiglas (extend 12 cm from each wall leaving a 16 cm opening

allowing travel between compartments) and removable black opaque barriers which, when inserted, prevented entry into the stranger compartments. Each stranger compartment contained a circular mesh cage (18 cm diameter, 20 cm height) with hinged lid (3/4" plywood, painted matte black). The height of the cage was extended 20 cm with vertical metal rods to discourage climbing. The task began with 10 min habituation with the barriers removed. The test rat was then contained in the middle section with the barriers in place and a stranger rat was placed in one of the mesh cages. The barriers were removed, and the test rat explored for an additional 10 min. Video recording and locomotor activity tracking was done with EthoVision software, and videos were manually scored with a stopwatch by a trained investigator blind to treatment status, and the opaque cage roof obscured the location of the stranger rat. Stimulus exploration was scored when the test rat directly approached (watching, contacting, sniffing, or circling) each of the cages, with the face of the rat oriented toward the cage at a maximum distance of 2 cm. All stranger rats were sex, age, and treatment matched to the test rat (Bitanihirwe et al., 2010; Henbid et al., 2017).

MK-801 Induced Locomotor Activity: The apparatus was a square arena (40 x 40 x 60 cm) made of black corrugated plastic (Figure 3D; Lins et al., 2018). A camera mounted to the ceiling recorded all activity and EthoVision software was used to track activity. Rats were tested 4 at a time, with each rat placed in 1 of 4 separate arenas for 30 min of habituation. Immediately following, rats were administered MK-801 (0.1 mg/kg; i.p.) and placed back into the arena for an additional 120 min. Activity was recoded with Noldus Ethovision XT 11.5 software.

Visual, Tactile, and Crossmodal Object Recognition: This task uses spontaneous exploratory behavior to assess visual memory, tactile memory, and visual-tactile sensory integration

(Winters and Reid, 2010; Jacklin et al., 2012). The testing apparatus was a Y-shaped maze with 1 start arm and 2 object arms (10 × 27 cm) made of white corrugated plastic (Figure 4; Lins et al., 2018; Paylor et al., 2018). A white plastic guillotine-style door separated the start arm from the object arms, and Velcro at the distal end of the object arms fixed objects in place. A removable, clear Plexiglas barrier could be inserted in front of the objects. A tripod positioned above the apparatus held a video camera that recorded the task activity. Rats were habituated to the apparatus twice for 10 min. Lighting alternated during habituation between white light (during visual phases) and red light (during tactile phases) for 5 min each with the order counterbalanced, and the clear barriers were in place for one day of habituation and removed for the other with order counterbalanced. Test days consisted of a 3 min sample phase with two identical copies of an object attached with Velcro to the maze, a 60 min delay, and then a 2 min test phase with a third copy of the original object and a novel object placed in the maze. Rats began each phase in the start arm; the guillotine door was opened and closed once the rat entered the object arms. This task consisted of 3 distinct tests performed on 3 separate days in the following sequence: tactile memory (day 1; Figure 4A), visual memory (day 2; Figure 4C) and crossmodal memory (day 3; Figure 4E). Red light illuminated the tactile phases allowing the rats' behavior to be recorded while preventing the rats' visual assessment of the objects and the removal of the clear barriers allowed for tactile exploration. White light was used during visual phases, but clear Plexiglas barriers in front of the objects prevented tactile exploration. CMOR had a tactile sample phase (red light, no barriers) and a visual test phase (white light, clear barriers). Recognition memory was defined as significantly greater exploration of the novel object than the familiar object. Behavior recordings were manually scored with a

289 stopwatch by investigators blind to the treatment status of the rats and identity of the objects
290 (Winters and Reid, 2010; Ballendine et al., 2015).
291 Oddity Discrimination: The testing apparatus was a square arena (60 x 60 x 60 cm) constructed
292 of white corrugated plastic with Velcro in each of the 4 corners. Two days of habituation to the
293 arena (10 min sessions) preceded the test day. On test day, 3 identical objects made of glass or
294 plastic and one different, or 'odd' object were fixed to the Velcro locations (Figure 5; Lins et al.,
295 2018) and the rats' activity were recorded for 5 min using a video camera mounted to the
296 ceiling. Object exploration times were manually scored using a stopwatch by an investigator
297 blind to the treatment status of the rats (Bartko et al., 2007a). Object examination was counted
298 when a rat's face was oriented toward the object at a maximum distance of 2 cm.
299
300 Statistical Analyses
301 A between-subjects design was used, and analyses were conducted with independent
302 samples t-tests, one sample t-tests, and ANOVAs using Statistical Package for the Social Science
303 version 22 (IBM, Armonk, NY). Outliers were defined as having a performance metric falling
304 more than 2 standard deviations from the mean and were removed from analysis on a case by
305 case basis. Outliers were identified and removed prior to calculating litter averages to prevent
306 excessive exclusion of data points. 1 saline and 2 polyI:C rats were removed from visual
307 recognition memory, but all litters remained represented. 2 polyI:C litters were excluded from
308 analysis for startle to the P120 tone, 1 polyI:C litter was removed from the analysis for PPI 30
309 ms Interval and PPI 80 ms Interval. No litters or individuals were removed from the Oddity,
310 Sociability, or Locomotor task analyses. Estrous phase was incorporated into analysis as a

311 covariate, however no consistent patterns were observed, potentially due to a low n of rats in
312 each phase. Sphericity violations were accounted for using the Greenhouse-Geisser adjustment
313 and degrees of freedom were adjusted when Levene's Test was violated. The use of one- and
314 two-tailed tests is specified for each task. Relationships between maternal serum cytokine
315 concentrations and long term offspring outcomes were determined using bivariate correlations
316 followed by a Benjamini-Hochburg adjustment to control for multiple comparisons (Benjamini
317 and Hochberg, 1995). All data are presented as group means ± standard error of the mean
318 (SEM) and asterisks indicate a significant difference between groups with a 95% confidence
319 interval ($p<0.05$). The pound symbol (#) is used to indicate a significant difference from a
320 chance result.
321
322 **Results**
323 The acute effects of saline and polyI:C treatment on this cohort of pregnant dams and
324 neonatal pups have been published previously (Lins et al., 2018). Briefly, dams treated with
325 polyI:C had reduced body weight compared to saline when followed up at 8, 24, and 48 h post
326 treatment, but no significant change in body temperature at the same timepoints. Maternal
327 serum collected 3 h post treatment was analyzed with ELISA for concentrations of cytokines
328 CXCL1, CXCL2, IL-6, and TNF-α. CXCL1 and IL-6 were significantly elevated in polyI:C-treated
329 dams. On PND1, the average offspring mass (males and females pooled) from polyI:C-treated
330 litters was significantly less than controls but there was no difference in litter size between the
331 groups (Lins et al., 2018).
332

Maternal polyI:C treatment failed to significantly affect startle or PPI

Startle responses to acoustic stimuli were assessed by measuring startle alone and prepulse inhibition in saline (n=15 litters) and polyI:C female offspring (n=15 litters). Startle to the 120 dB pulses alone decreased during the session (Figure 2B; main effect of Time: $F_{(1.35,36.45)}$=26.26, p<0.001) but no Treatment or interaction was present. For prepulse trials with a 30 ms (short) interval, a main effect of Prepulse Intensity on PPI (Figure 2C; $F_{(2,56)}$=40.33, p<0.001) was found with no effect of Treatment. Overall, PPI was greater at 12 dB compared to 3 and 6 dB (p<0.001). For trials with an 80 ms (long) prepulse-pulse interval, a main effect for Prepulse Intensity was found (Figure 2D; $F_{(2,56)}$=89.37, p<0.001) for PPI, but no effect of Treatment and no interaction. Overall, PPI increased with louder prepulses.

PolyI:C offspring have sociability deficits

Both groups of female offspring (n=15 saline litters, n=15 polyI:C litters) displayed a significant preference for the cage containing an unfamiliar rat compared to the empty cage when analyzed using a within-subjects design (saline: $t_{(14)}$=14.18, p<0.001; polyI:C: $t_{(14)}$=8.11, p<0.001). When the results were compared between treatment groups, female polyI:C rats had a significantly lower discrimination ratio (DR, calculated as $Exploration_{Stranger}$ - $Exploration_{Empty}$ / $Exploration_{Total}$; Figure 3B; $t_{(28)}$=2.61, p<0.05) and spent significantly more time exploring the empty cage compared to the saline controls (Figure 3C; $t_{(28)}$=-2.59, p<0.05). There was no difference in total exploration times (p>0.05).

Both groups of offspring increase locomotor activity following MK-801 administration

Locomotor data comparing female polyI:C (n=13 litters) and saline (n=11 litters) offspring were analyzed with a mixed repeated measures ANOVA (Figure 3E). Results revealed a main effect of Time (Figure 3E; $F_{(2.47,62.24)}$=62.24, p<0.001) but no treatment effect and both groups displayed increased locomotion after MK-801 administration.

PolyI:C offspring perform tactile, but not visual, object recognition memory and neither group display crossmodal recognition

All CMOR data is presented as a discrimination ratio (DR; $exploration_{novel}$ – $exploration_{familiar}$ / $exploration_{total}$) for the first minute of the test phase. One-tailed single sample t-tests compared each group's exploration to chance (DR of 0). Both groups demonstrated significant tactile object recognition memory (Figure 4B; saline: $t_{(14)}$=3.00, p<0.01; polyI:C $t_{(14)}$=11.53, p<0.001). PolyI:C females did not perform above chance for visual memory (polyI:C: $t_{(13)}$=0.49, p>0.05), although saline offspring showed significant preference for the novel object ($t_{(15)}$=2.72, p<0.05). In the crossmodal phase, both groups of female rats failed to show a preference for the novel object (saline females: $t_{(14)}$=0.46, p>0.05; polyI:C females: $t_{(14)}$=1.71, p>0.05). There were no differences in total object exploration times between groups in any of the sample and test phases (Table 3).

PolyI:C-treated offspring have reduced oddity preference compared to saline offspring

Saline (n=15 litters) and polyI:C (n=14 litters) offspring both explored the odd object at a greater than chance level (Figure 5B; saline: $t_{(14)}$=5.27, p<0.001; polyI:C: $t_{(13)}$=2.66, p<0.05) when analyzed with a single sample t-test against a value of 25%. When the groups were compared

directly, saline offspring spent a significantly greater % exploration with the odd object compared to polyI:C offspring (Figure 5B; $t_{(27)}$=2.24, p<0.05). There was no difference in total exploration time between saline (97.53±4.08 s) and polyI:C rats (98.94±4.79 s, $t_{(27)}$=-0.23, p<0.05).

Correlations between measurements taken of the dams during pregnancy and offspring outcomes

Measurements taken during pregnancy were correlated with long-term behavior outcomes in the female offspring. Serum cytokine concentrations of CXCL1, CXCL2, IL-6 and TNFα were determined from blood samples collected from the dams 3 h post treatment and analyzed with ELISA. Additional effects of treatment were determined through monitoring with weight and rectal temperature measurements taken 8, 24, and 48 h post treatment (Lins et al., 2018). These data were correlated to behavior of the offspring using bivariate correlations. Maternal weight changes following treatment (anesthesia with saline or polyI:C administration and blood sampling) was the only variable associated with offspring behavior. Greater weight loss in polyIC-treated dams 8 h post treatment was associated with reduced startle response in their female offspring during the initial tone-alone trials (r=0.623, p<0.05, B-H p<0.05), and this was not seen in the saline group (r=0.44, p>0.05). Weight loss in the saline dams at 24 h post treatment was correlated to lower %PPI at the 80 ms interval (r=-0.555, p<0.05, B-H p<0.05). Saline dam weight loss 8 h after treatment was related to the DR in the sociability task (r=0.716, p<0.01, B-H p<0.05). The importance of these relationships is difficult to gauge due to no significant effects of saline treatment on weight, and the lack of treatment effects in startle or

399 PPI. Previous studies have also shown mixed results regarding maternal weight changes and
400 offspring behavior outcomes (Wolff and Bilkey, 2010b; Vorhees et al., 2012). Dedicated studies
401 will be necessary to determine the reliability and potential importance of these results.
402
403 **Male and female offspring show similar behavioral profiles in response to MIA**
404 The data presented in this paper were further analyzed in conjunction with the male
405 littermates from Lins and colleagues (2018) with Sex and Treatment as factors using 2x2
406 factorial ANOVAs. It should be noted that this analysis necessitates including 2 values per litter
407 (for each sex) which violates the assumption that subjects are independent because littermates
408 are inherently related. No main effect of Treatment ($F_{(1,60)}$=0.59, p>0.05) or Sex ($F_{(1,60)}$=0.25,
409 p>0.05), and no Sex by Treatment interaction ($F_{(1,60)}$=0.11, p>0.05) was found for 30 ms interval
410 PPI. For 80 ms interval PPI trials, no main effect of Treatment ($F_{(1,56)}$=0.10, p>0.05) or Sex
411 ($F_{(1,56)}$=0.82, p>0.05) was found, but a Sex by Treatment interaction was shown ($F_{(1,56)}$=4.07,
412 p<0.05). A Tukey HSD post hoc test revealed saline females had lower PPI than saline males
413 (p<0.05). Main effects of Treatment (polyI:C offspring have a lower DR than saline offspring;
414 $F_{(1,58)}$=9.55, p<0.01) and Sex (female offspring have a lower DR than male offspring; $F_{(1,58)}$=4.87,
415 p<0.05) were found for sociability, with no significant interaction ($F_{(1,58)}$=0.13, p>0.05). Tactile
416 object recognition memory did not differ by Treatment ($F_{(1,59)}$=0.30, p>0.05) or Sex ($F_{(1,59)}$=1.06,
417 p>0.05); however, but a significant Sex by Treatment interaction was revealed ($F_{(1,59)}$=4.25,
418 p<0.05). Tukey HSD post hoc testing failed to reveal any significant differences between
419 individual groups. Visual object recognition memory was not affected by Treatment
420 ($F_{(1,56)}$=3.60, p>0.05) or Sex ($F_{(1,56)}$=0.21, p>0.05), and no interaction was present ($F_{(1,55)}$=0.003,

421 p>0.05). Crossmodal object recognition memory was not affected by Treatment ($F_{(1,58)}$=0.17,
422 p>0.05) or Sex ($F_{(1,58)}$=0.01, p>0.05) and there was no interaction between these factors
423 ($F_{(1,58)}$=3.98, p>0.05). A main effect of Treatment ($F_{(1,55)}$=19.30, p<0.001, polyI:C offspring
424 explore the odd object less than saline offspring) was found for oddity, in the absence of a main
425 effect of Sex ($F_{(1,55)}$=0.03, p>0.05) or Sex by Treatment interaction ($F_{(1,55)}$=1.88, p>0.05).
426 Locomotor activity was not analyzed for Sex by Treatment interactions due to known
427 differences in MK-801 metabolism and the use of different doses in males and females (Andiné
428 et al., 1999).
429
430 **Discussion**
431 The adult female offspring of rat dams that received an immune stimulant during
432 pregnancy displayed various behavior abnormalities compared to the offspring of saline-treated
433 dams. The polyI:C-treated offspring had reduced sociability, impaired visual discrimination, and
434 lack of preference for an odd object compared to offspring from control litters. Both treatment
435 groups displayed heightened locomotor activity in response to MK-801 administration and
436 tactile recognition memory was intact in both groups. Neither group of offspring demonstrated
437 crossmodal memory, and there were no treatment effects on PPI. These results complement a
438 companion paper that assessed the male offspring (Lins et al., 2018), and by directly analyzing
439 sex by treatment interactions where possible, we shown that MIA during pregnancy had similar
440 effects on both sexes of offspring.
441 A significant limitation of this study is the use of timed-pregnant dams. Several studies
442 show an impact of travel stress on the dams and offspring. For example, Moriyama and

443 colleagues (2013) examined the effect of transport stress on seizure susceptibility in the
444 offspring and found an increase in variability in those transported during gestation; however,
445 maternal care behavior had a greater impact than transport stress on seizure susceptibility
446 (Moriyama et al., 2013). We have previously reported no observed changes to maternal
447 behavior following polyI:C administration (Zhang et al., 2012), but we did not assess this directly
448 in this cohort or strain. Shipment stress also increases susceptibility to the valproate-induced
449 developmental toxicity model of autism (Ogawa et al., 2007; Kuwagata et al., 2009). We are
450 unable to confirm if shipment stress had a similar impact in our study. Despite these limitations,
451 many comparable studies on development and gestational adverse events have relied on the
452 use of timed-pregnant dams (Lodge and Grace, 2001; Du and Grace, 2013, 2016a, 2016b; Van
453 den Eynde et al., 2014; Ballendine et al., 2015; Lins et al., 2018). Recently, Kentner and
454 colleagues (2019) highlighted that consideration of all MIA protocols will enable comprehensive
455 understanding of their impacts on offspring outcomes. Thus, we believe our results are of
456 value.
457
458 **Lack of sex-specific effects of polyI:C treatment on behavior of the offspring**
459 We previously reported that male polyI:C offspring from this cohort displayed greater
460 startle to the 120 dB tone at the end of the PPI protocol compared to saline males. Although
461 the effect in the males was small and limited to a single parameter, the female data presented
462 here shows no effect of MIA on any measure of PPI and acoustic startle response. The effects of
463 MIA on PPI in rodent models are mixed with many studies showing PPI impairments in the
464 offspring of immune challenged rats (Borrell et al., 2002; Romero et al., 2007; Wolff and Bilkey,

2010, 2008, Dickerson et al., 2010, 2013, 2014; Howland et al., 2012; Klein et al., 2013; Ballendine et al., 2015; Hadar et al., 2015) and mice (Ozawa et al., 2006; Smith et al., 2007) including impairments seen in both sexes (Meyer et al., 2009; Howland et al., 2012; Basta-Kaim et al., 2015). Other studies show no effects of MIA on PPI, similar to our observations (Missault et al., 2014; Van den Eynde et al., 2014; Vorhees et al., 2015; Lins et al., 2018). Sex effects in PPI in general have been reviewed and the influence of female sex hormone fluctuations have been studied (Kumari, 2011). High estrogen phases of the menstrual cycle have been associated with lower PPI, although this is not consistently observed (Swerdlow et al., 1997; Jovanovic et al., 2004; Kumari et al., 2008). Additionally, PPI disruption by a $5HT_{1A}$ agonist can be prevented by administration of exogenous estrogen and progesterone in rats which may imply a protective role of sex hormones against PPI disruption (Gogos and Van den Buuse, 2004). The results from the present study do not support the claim of a strong influence of estrous phase on PPI performance; however, it should be noted that estrous was not controlled for and the ability of the present study to detect an effect may be underpowered.

In the sociability task, polyI:C treated male offspring spent less time exploring a same-sex, unfamiliar conspecific compared to saline controls (Lins et al., 2018). We observed a different pattern of reduced sociability in the female polyI:C offspring, indicated by a significantly lower discrimination ratio compared to the saline offspring which was driven by significantly more time exploring the empty cage on the opposite side of the apparatus. The social exploration data is presented as a comparison between the saline and polyI:C offspring (comparing the relative degree of social preference), while others have presented the data as a within-subjects comparison to report either the presence or absence of social preference

(Silverman et al., 2010). We believe comparing treatment groups allows the detection of subtle behavior differences that could be missed in instances where stimuli with a substantial difference in salience (such as an unfamiliar rat versus an empty cage) result in high discrimination ratios, and this natural preference would need to be abolished to show a treatment effect. Natural preference for social stimuli versus objects is documented in rodents (Lee and Green, 2016). Presenting the data as a between groups comparison also allows direct evaluation alongside previously published sociability data from our lab, including that of the male littermates from this cohort (Henbid et al., 2017; Lins et al., 2018). The use of single sample comparisons may be best suited to tasks that are challenging for control animals to complete, such as the complex visual discrimination tasks including visual and crossmodal recognition memory where visual stimuli are less salient and the resulting DRs tend to be lower (Winters and Reid, 2010). Both strategies of data analysis are common in behavior literature, and factors such as strength or salience of stimuli and task difficulty should be considered when representing data. Overall, both male and female polyI:C offspring display a deficit in sociability compared to controls, but this presents in a subtly different manner depending on sex and may be related to previously observed differences in PFC development (Piontkewitz et al., 2011b; Paylor et al., 2016), The direct significance of MIA-induced developmental trajectory differences to the aberrant social behavior observed here remains to be determined.

The effects of MK-801 administration on locomotor activity have been reported in previous studies with mixed results (Zuckerman and Weiner, 2005; Howland et al., 2012; Vorhees et al., 2012; Missault et al., 2014). The male siblings in this cohort were significantly affected by a dose of 0.2 mg/kg (i.p) indicated by heightened locomotor activity which was not

seen in the control males (Lins et al., 2018). The females in this paper were given a lower dose of 0.1 mg/kg comparable to other studies (Andiné et al., 1999; Howland et al., 2012; Zhao et al., 2013). Unfortunately, both the saline and polyI:C females showed increased locomotion which confounds the ability to discern whether prenatal polyI:C treatment affected sensitivity to MK-801.

In the CMOR task, both saline and polyI:C females demonstrated object recognition in the tactile phase of the test, similar to what was seen in males of the same cohort (Lins et al., 2018) and Long Evans males (Ballendine et al., 2015). PolyI:C treated offspring were impaired in the visual phase, and neither group performed significantly different from chance exploration in the crossmodal phase, a notable distinction from the crossmodal memory exhibited by saline-treated males (Lins et al., 2018). Previous studies on MIA offspring have shown reduced discrimination in object memory behavior tasks in females and lower discrimination ratios are common in the crossmodal task, possibly reflecting task difficulty (Howland et al., 2012; Ballendine et al., 2015; Marks et al., 2016). Visual and crossmodal memory depend on the perirhinal cortex while the posterior parietal cortex is necessary for tactile memory, suggesting there may be regionally specific deficits as a result of MIA (Winters and Reid, 2010; Jacklin et al., 2016).

Oddity preference and perception have been assessed in several tasks using rats and mice (Bussey et al., 2005; Bartko et al., 2007a, 2007b; Cowell et al., 2010; Cloke et al., 2016; Marks et al., 2018; Paylor et al., 2018); however, to our knowledge this is the first study to assess this oddity task in female rats. Improved understanding of the nature of oddity discrimination is relevant for the successful management of cognitive impairment in conditions

such as schizophrenia, a symptom domain highly related to patient functional outcomes (Cloke et al., 2016). Prenatal polyI:C treatment affected females in the same manner as males with a significant reduction in oddity preference compared to saline offspring (Lins et al., 2018). The successful performance of oddity preference depends on multisensory integration similar to CMOR, yet distinct in that visual and tactile associations can be formed simultaneously and there is no mnemonic demand in the oddity task (Cloke et al., 2016). Multisensory integration is disrupted by NMDA receptor antagonism using ketamine in the orbitofrontal cortex and reversed with $\alpha_4\beta_2$ nicotinic acetylcholine in a $GABA_A$ dependent mechanism (Cloke et al., 2016) and abnormalities in these brain regions and receptor types may be good candidates to explore in future studies of MIA and oddity preference.

The degree to which these behavioral effects replicate or contradict previous data varies. The MIA literature displays a lack of reproducibility, which may be due to variety of protocols used. Procedural variations in model species and strain, timing of inflammatory insult, inflammatory agent, and dose and route of administration have the potential to alter experimental outcomes. Other details such as rodent housing (bedding type, pathogen-free status, temperature, etc.), parental age, maternal experience, food and water quality, cage companions, and age at weaning, which are not commonly reported, may influence outcomes and reduce reproducibility of the model (Kentner et al., 2019; Smolders et al., 2018). The basic protocol used here is relatively common in MIA literature, yet very similar protocols yield contrasting behavior responses; for example, hypo- versus hyperlocomotion in an open field (Van den Eynde et al., 2014; Lins et al., 2018). Comparison in this case is complicated by the use of spontaneous vs. drug-enhanced locomotor paradigms and the collection of maternal blood

553 samples (Van den Eynde et al., 2014; Lins et al., 2018). Indeed, distince neuopathological
554 alterations were noted with microglia activation in MIA offspring found in one study (Van den
555 Eynde et al., 2014), while previous work from our group has found no changes in microglia in
556 offspring generated in our laboratory (Paylor et al., 2016). These results support the notion that
557 enhanced reporting of such variables is warranted, and dedicated future studies should assess
558 the effects of such procedural differences directly (Goldstein et al., 2014; Careaga et al., 2018;
559 Kentner et al., 2019; Mac Giollabhui et al., 2019).
560
561 **Implications for sex differences in the maternal immune activation model**
562 MIA caused by polyI:C administration resulted in altered behavior in female offspring in
563 multiple behavior tasks including sociability, visual and crossmodal memory, and oddity
564 preference. Overall, the present data do not provide strong evidence for sex differences in
565 response to polyI:C treatment. Inflammation in pregnancy relates to the etiology of sexually
566 dimorphic disorders, notably schizophrenia and autism (Davis and Pfaff, 2014; Goldstein et al.,
567 2014; Patel et al., 2018). The lack of overt sex differences observed in the present study suggest
568 the MIA model in rats may be limited in this respect, though others have shown more
569 promising results in this regard (Piontkewitz et al., 2011a; Zhang et al., 2012).
570

Table and Figure Legends

Table 1: Summary of dams' treatment, adverse events and litter data. 8 dams were euthanized within 48 h of polyI:C administration because they developed low body temperature and showed sickness behaviors beyond what is acceptable as outlined in our Humane Intervention Protocol. One litter per treatment included male, but no female offspring, resulting in exclusion from the final count of litters included in this manuscript. The 2 saline-treated control dams that did not produce litters showed no evidence of pregnancy. Viable offspring count per dam includes all surviving offspring of both sexes present on PND1 prior to culling to a maximum of 10, excludes dams that did not give birth, and is presented as $\mu \pm$ SEM. All additional data presented on the dams only includes those that produced viable offspring.

Table 2: Summary detailing the number and litters of female offspring tested in each behavior task. All female offspring from n=31 litters completed PPI, while all other tasks included 1 or 2 offspring per litter. The unexpected death of 1 polyI:C rat with no female littermates reduced the number of litters tested to 15. Locomotor activity n was reduced due to some rats being diverted to concurrent research. The number of offspring per litter in each task is further summarized below. Behavior scores for littermates were averaged for a single value per litter. **[a]** Offspring included in behavior testing. **[b]** Number of litters with n=5 or fewer offspring included in PPI. **[c]** Number of litters with n=1 or n=2 offspring in CMOR, Sociability, and Oddity. **[d]** Number of litters with n=1 or n=2 offspring tested in MK-801 Induced Locomotor Activity.

Table 3: Exploration times (s) for each phase of the CMOR task, presented as $\mu \pm$ SEM.

Table 4: Summary of the effects of MIA on female offspring alongside the male offspring from the same cohort in a previously published companion paper (Lins et al., 2018). '↑' indicates heightened response or facilitation while '↓' indicates diminished response or impaired performance in comparison to a control group. A '–' symbol indicates no significant change. Percent change was calculated as a comparison to the equivalent control group ((saline – polyI:C / saline) x 100). Male locomotor data was calculated from the total distance travelled after MK-801 administration. For consistency, both male and female sociability percent change was calculated using the discrimination ratio data, although it should be noted the DR was a non-significant (n.s.) effect in the males and they instead spent significantly less time (s) exploring the social stimulus than controls.

Figure 1: [A] Schematic detailing the time line of maternal treatment and initiation of offspring behavior testing. Schematic has been published previously (Lins et al., 2018). **[B]** Flow chart depicting the order of the behavior test battery.

Figure 2: [A] Schematic illustrating a startle response to a 120 dB tone (top panel) versus the typical reduction in startle reactivity when a prepulse of 3, 6, or 12 dB precedes the startling tone (bottom panel). Schematic has been published previously (Lins et al., 2018). **[B]** Startle reactivity decreased over the course of the PPI testing protocol and polyI:C offspring had significantly higher reactivity at the "after" timepoint ($p<0.05$, indicated by an asterisk, *). **[C]** There were no differences between groups in % PPI for the short (30 ms) prepulse-pulse

615 interval but % PPI increased with increasing prepulse intensity where the 12 dB prepulse had
616 higher PPI than 2 or 6 dB prepulses. **[D]** There were no differences between groups in % PPI for
617 the long (80 ms) prepulse-pulse interval but % PPI increased with increasing prepulse intensity
618 (3 dB < 6 dB < 12 dB, $p<0.05$, indicated by asterisks, *).
619
620 **Figure 3: [A]** Schematic representing the black, three-chambered arena used to conduct the
621 sociability task. The chambers on either side of the center start chamber contain identical
622 holding cages, one of which would contain a social stimulus (an age, sex, and treatment
623 matched stranger rat) while the test rat was free to explore. Schematic has been published
624 previously (Henbid et al., 2017; Lins et al., 2018). **[B]** When the exploration data is presented as
625 a discrimination ratio, both groups show significant preference for the stranger rat; however,
626 polyI:C offspring show significantly less preference when compared to saline offspring. **[C]**
627 There was no significant difference between groups in total exploration or exploration of the
628 social stimulus, although polyI:C rats spent more time exploring the non-social stimulus than
629 saline rats. **[D]** Schematic of the black, square arena where rats' activity was monitored before
630 and after administration of MK-801. Schematic has been published previously (Lins et al., 2018).
631 **[E]** Graph displaying locomotor activity as distance travelled per 10 min time bin. Both groups
632 had elevated locomotor activity following MK-801 administration, but there was no effect of
633 maternal treatment.
634
635 **Figure 4:** Schematic of the Y-maze arena for the CMOR task. In each sample phase there are
636 two identical objects located at each arm of the Y-maze while the test phase uses a third,

identical copy of the object from the sample phase plus a novel object. **[A]** The Y-maze assembled to conduct the tactile phase in red light conditions where the rat is able to explore objects via touch. **[B]** Both groups of offspring display robust novelty preference in the tactile phase with novel object exploration significantly greater than chance levels. **[C]** The Y-maze assembled for the visual phase which is conducted in white light conditions with the addition of a clear, plexiglass window to prevent tactile exploration of the objects, limiting the rats to visual observation. **[D]** Saline offspring demonstrated visual memory with novel object exploration significantly greater than chance but polyI:C offspring did not perform above chance levels. **[E]** The Y-maze assembled for the crossmodal phase which has a tactile sample phase and visual test phase. **[F]** Both groups failed to display crossmodal recognition memory as novel object exploration was equal to chance. The pound symbol (#) indicates significant difference from chance exploration (DR=0, $p<0.05$) in a single sample t-test. Schematic has been published previously (Lins et al., 2018; Paylor et al., 2018).

Figure 5: [A] Schematic of the white square arena used to conduct the oddity discrimination task showing the arrangement of three identical objects and one different, or "odd" object. Schematic has been published previously (Lins et al., 2018). **[B]** Bar graph displaying the percent of total object exploration spent examining the odd object. PolyI:C offspring displayed significantly less oddity preference than saline offspring ($p<0.05$, indicated by an asterisk, *)

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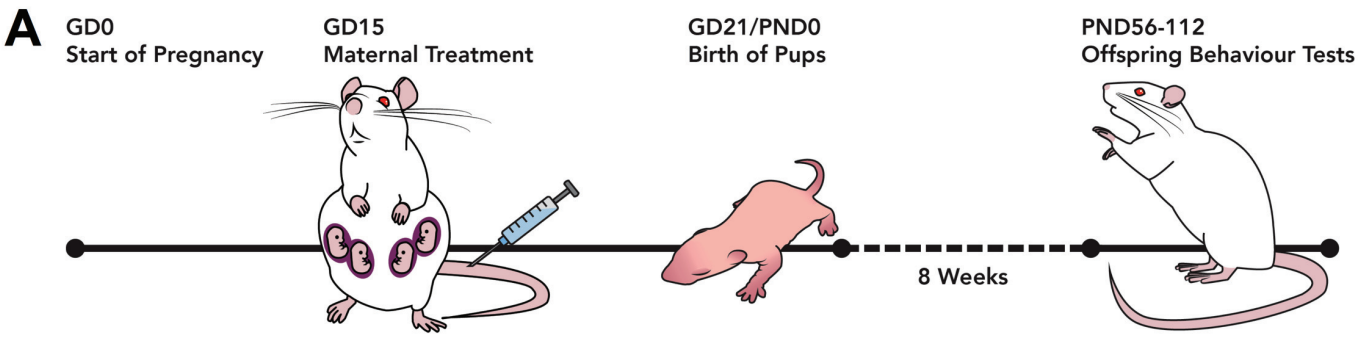
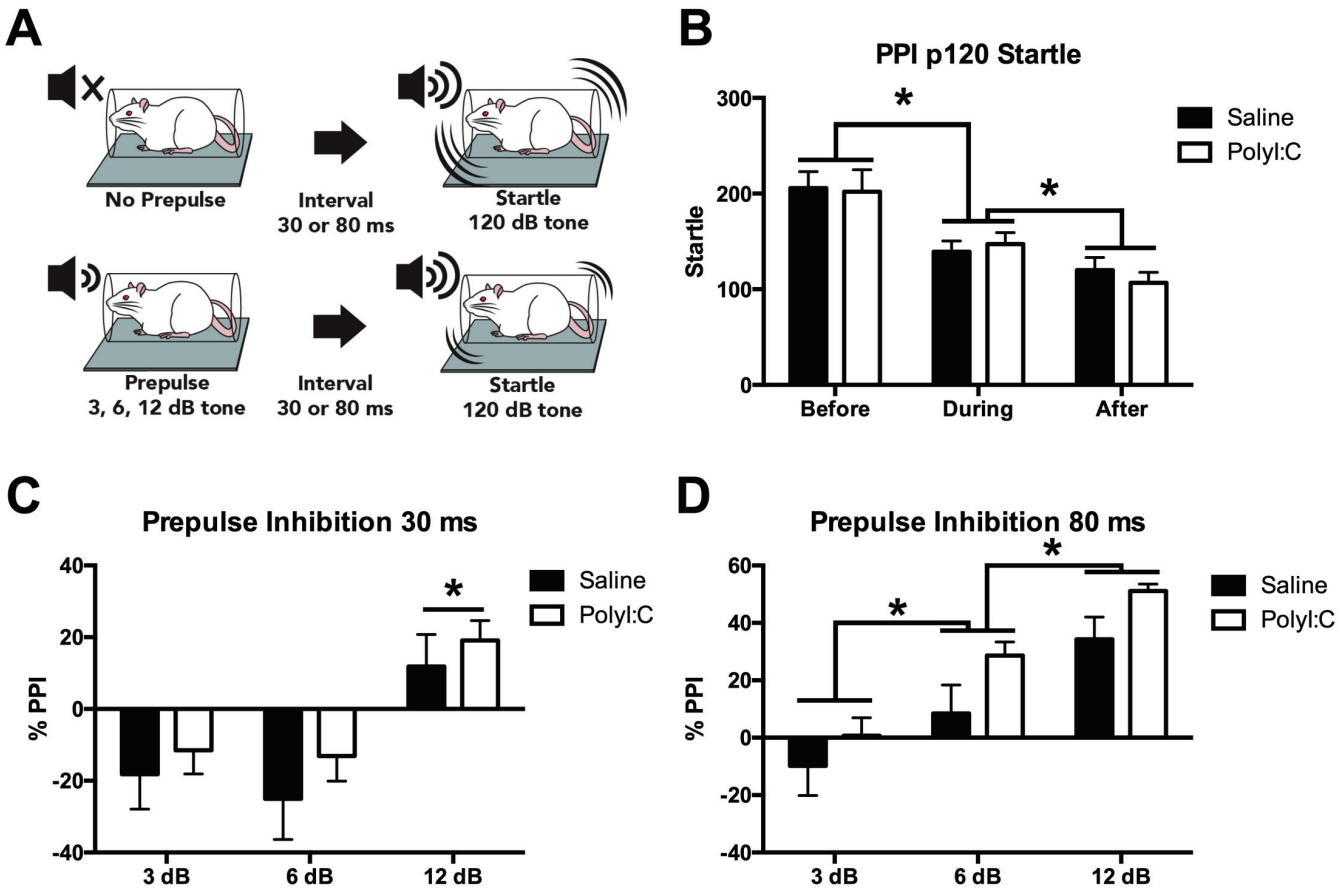
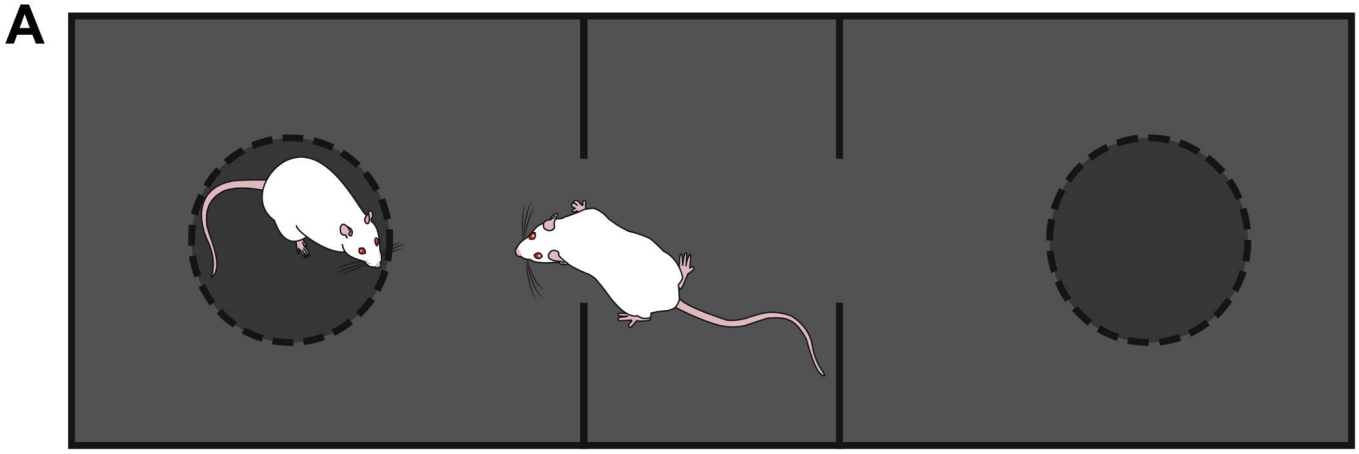
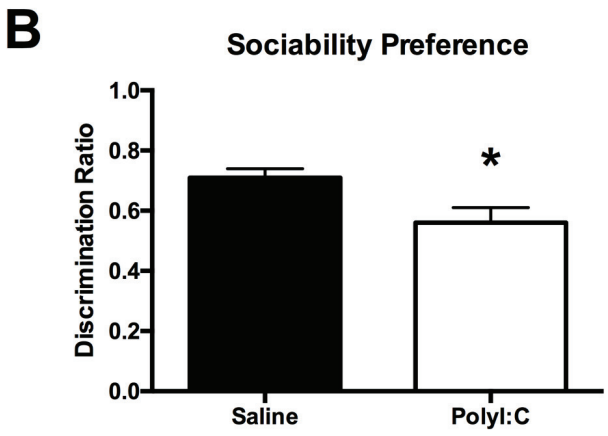


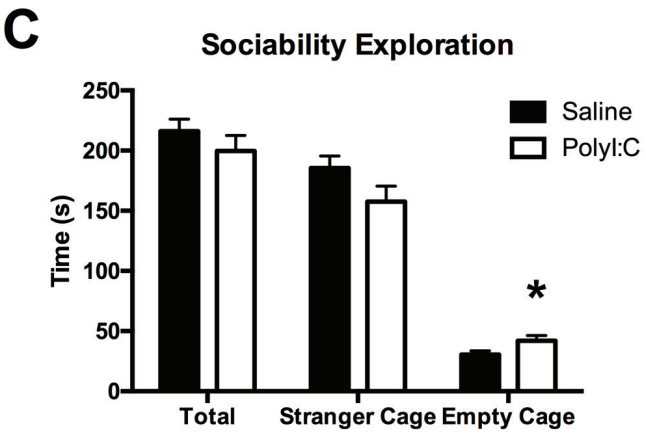
B

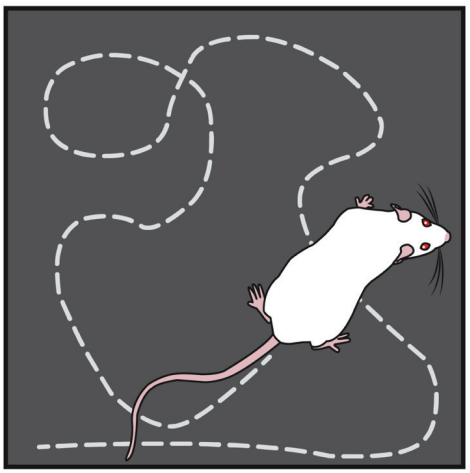
PPI → CMOR → Sociability → Oddity Discrimination → MK-801 induced Locomotor Activity

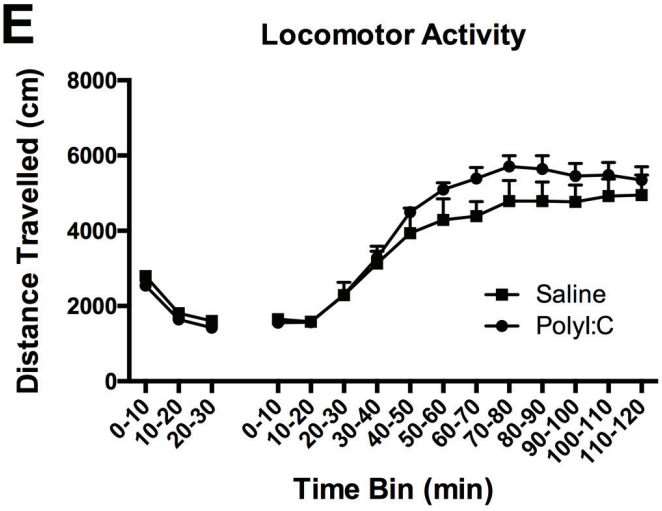


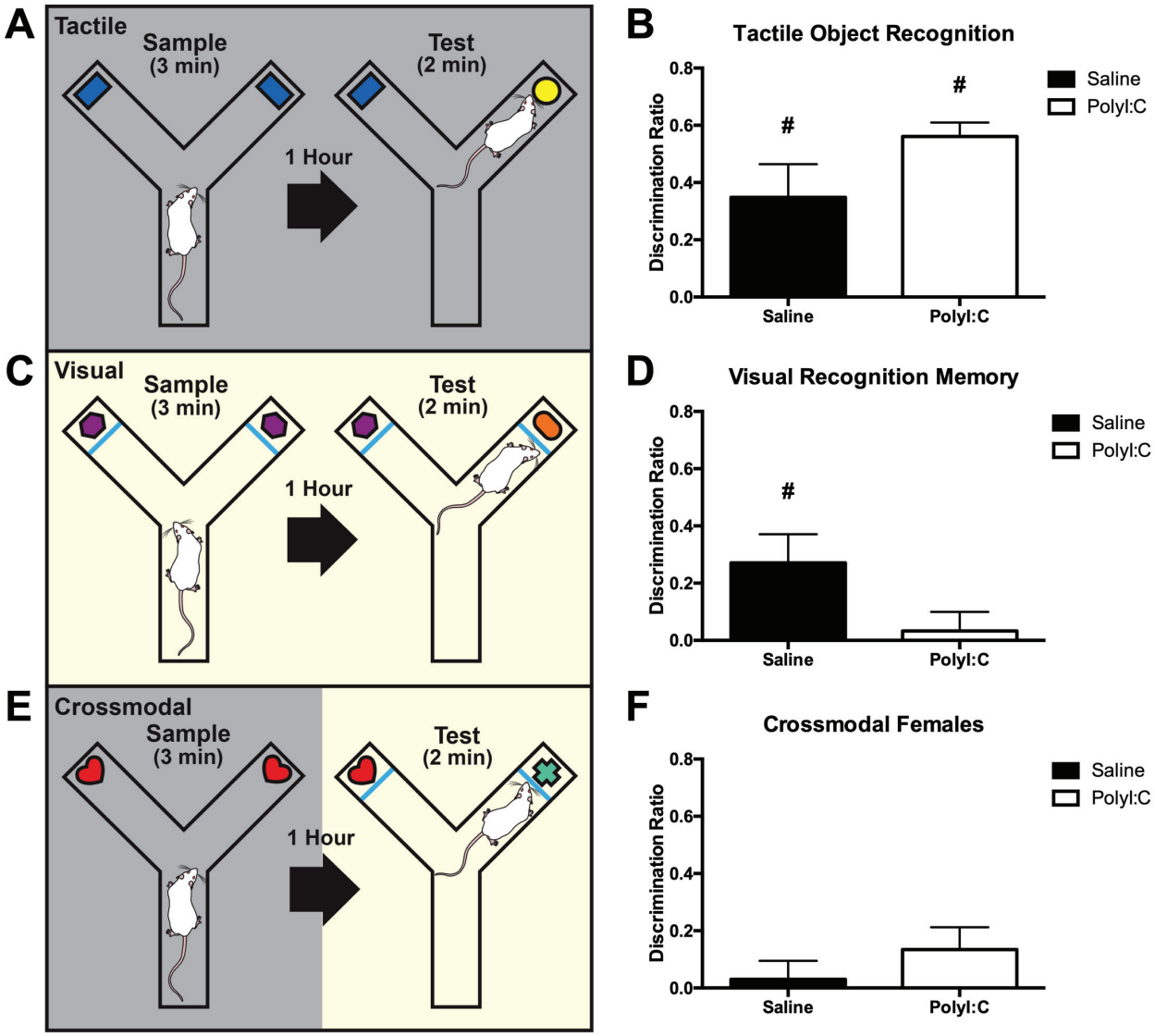












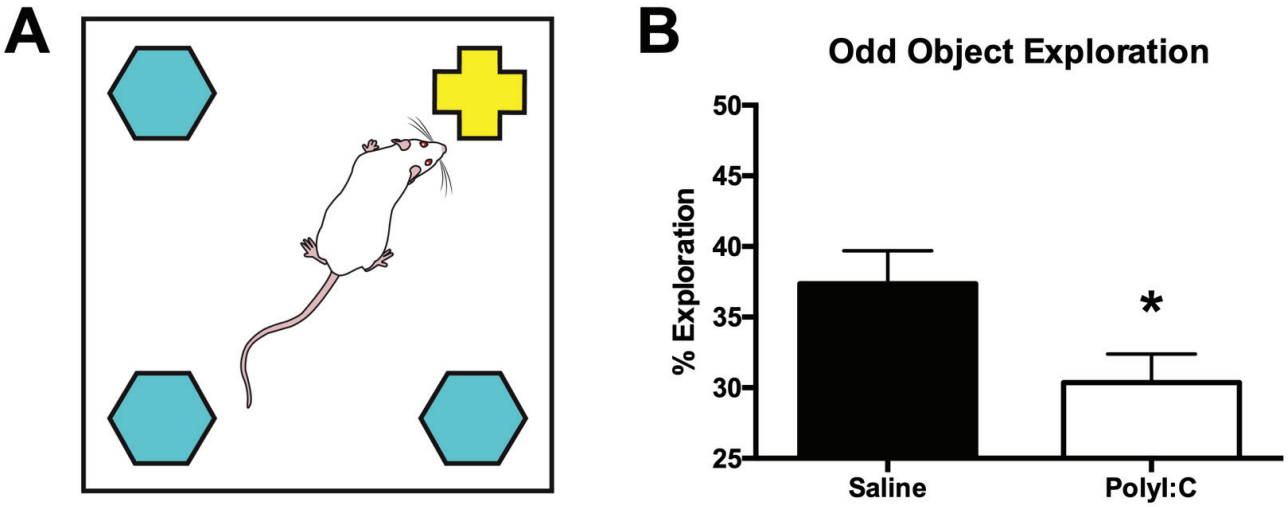


Table 1:

Treatment	*Total Dams Treated*	*Dams Euthanized*	*Dams w/ No Litter*	*Litters Included*	*Viable Offspring*
Saline	18	0	2 (+1 no ♀)	15	11.94 ± 0.76
PolyI:C	25	8	0 (+1 no ♀)	16	12.00 ± 0.81

Table 2:

a) Offspring included in behavior testing.

	Total Number		*PPI*		*CMOR*		*Sociability*		*Oddity Preference*		*Locomotor Activity*	
Treatment	Rats	Litters	Rats	Litters	Rats	Litters	Rats	Litters	Rats	Litters	Rats	Litters
Saline	36	15	36	15	22	15	22	15	22	15	14	11
PolyI:C	35	16	35	15	24	15	24	15	24	15	20	13

b) Number of litters with n=5 or fewer offspring included in PPI.

Treatment	*n=5*	*n=4*	*n=3*	*n=2*	*n=1*
Saline	1	5	1	0	8
PolyI:C	1	0	7	1	7

c) Number of litters with n=1 or n=2 offspring in CMOR, Sociability, and Oddity.

Treatment	*n=2*	*n=1*
Saline	7	8
PolyI:C	9	6

d) Number of litters with n=1 or n=2 offspring tested in MK-801 Induced Locomotor Activity.

Treatment	*n=2*	*n=1*
Saline	3	8
PolyI:C	7	6

Table 3:

Treatment	*Task Phase*	*Tactile*	*Visual*	*Crossmodal*
Saline	Sample	61.10 ± 2.49	6.41 ± 0.56	66.21 ± 4.73
	Test	36.71 ± 2.41	3.50 ± 0.61	3.36 ± 0.36
PolyI:C	Sample	60.32 ± 3.07	7.18 ± 0.83	58.61 ± 4.43
	Test	34.57 ± 3.42	2.72 ± 0.28	3.84 ± 0.27

Table 4:

Behaviour Test	*Males*	*Females*
Prepulse Inhibition	-	-
MK-801 Locomotion	↑ 47.72%	n.d.
Sociability	↓ 22.97% (n.s.)	↓ 21.13%
Tactile Memory	-	-
Visual Memory	↓ 90.33%	↓ 87.87%
Crossmodal Memory	↓ 93.71%	-
Oddity Preference	↓ 31.57%	↓ 18.69%

(-) = no significant change compared to controls
(n.d.) = not determined