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Maternal Immune Activation with the Viral Mimetic Poly:IC in Pregnant Rats

Thaísa Meira Sandini, Quentin Greba, Brittney Rose Lins, and John George Howland*

Department of Anatomy, Physiology, and Pharmacology, University of Saskatchewan, Saskatoon, Canada

*For correspondence: john.howland@usask.ca

[Abstract] Maternal immune activation (MIA) is increasingly well appreciated as an environmental risk factor for some psychiatric disorders. Administration of proinflammatory compounds such as the synthetic double-stranded RNA molecule polyinosinic-polycytidylic acid (polyI:C) to pregnant rodents results in the release of proinflammatory cytokines in the maternal circulation. Various behavioural and brain changes are produced in the offspring that are associated with psychiatric disorders such as autism and schizophrenia. This protocol describes the steps necessary for inducing MIA in pregnant rat dams, which will allow for investigations into the mechanisms in the dam and offspring that mediate the long-term effects of exposure to inflammation while in utero. Increasing our understanding of these mechanisms may provide new insights for the diagnosis, treatment, and prevention of psychiatric disorders. This protocol has been developed and improved over the years by various researchers in Dr. Howland's laboratory at the University of Saskatchewan.

Keywords: Psychiatric illness, Maternal infection, Cytokine, Animal model, Development, Autism, Schizophrenia

[Background] Epidemiological studies provide substantial evidence for an association between prenatal infections and increased risk for the development of some neuropsychiatric disorders, such as schizophrenia and autism, in the offspring (Brown and Meyer, 2018). Indeed, inflammation in pregnancy may alter normal development of the fetus thereby increasing the risk of the emergence of psychopathologies. Accurate identification of the mechanisms underlying these effects may advise early interventions. As a result, a number of research groups have developed models of MIA in rodents and non-human primates that offer a fruitful opportunity to examine the complex etiology of complex psychiatric disorders. Researchers have used these models to assess behavioural, pharmacological, and pathophysiological outcomes of MIA in the offspring (for reviews of this literature, see: Piontkewitz *et al.*, 2012; Reisinger *et al.*, 2015; Estes and McAllister, 2016; Careaga *et al.*, 2017; Brown and Meyer, 2018; Bergdolt and Dunaevsky, 2019; Gumusoglu and Stevens, 2019; Kentner *et al.*, 2019; Meyer, 2019). The protocol we describe below is adapted from Lins *et al.* (2018 and 2019) with some changes including our protocol for in-house timed pregnant breeding of rats. The use of a viral mimetic instead of infection with a virus is advantageous as the duration of the effect is better controlled and biosafety concerns are reduced. Some studies have shown similar effects on the offspring following inoculation of pregnant mice with the influenza virus thereby supporting the validity of the polyI:C model (Shi *et al.*, 2003).

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Materials and Reagents

1. 25 Gauge needles (BD PrecisionGlide™ needle, catalog number: 14-826AA)
2. Antiseptic isopropyl alcohol pad, sterile, medium (Alliance®, catalog number: 211-MM-05507)
3. Syringes (3 ml) (BD, Luer-Lok™ Tip, catalog number: B309657)
4. Virgin female Sprague-Dawley rats
5. Male Sprague-Dawley rats
6. Poly:IC HMW (InvivoGen, Catalog code: tlrl-pic-5)
7. Vaseline (Vaseline® Petroleum Jelly Original)
8. Sterile normal saline (0.9%) (Hospira®)

Equipment

1. Pipette 200 µl (Eppendorf Research® plus, catalog number: 3124000083)
2. Heating pads (Sunbeam®, Standard size, catalog number: 730AO-CAD-MASTER)
3. Beaker flask (300 ml)
4. Scale suitable for weighing rats
5. Thermometer
6. Rodent rectal temperature probe (Physitemp, model: BAT-12)
7. Hot plate (Thermolyne, Nuova II)
8. Isofluorane machine suitable for rats (ARVS)
9. Fume hood (Hamilton, SafeAire II, model: HMLAB027)
10. Light microscope (Boreal 2 Digital Compound Microscopes–HM Series)

Procedure

A. Breeding

1. Male and female rats (approximately 60 days old) are delivered to the vivarium, housed in same sex pairs, and given one week to acclimatize before experimentation. In our facility, male and female rats are housed in the same colony room in standard ventilated cages. Food and water are provided *ad libitum* throughout the experiment. The light-dark cycle is 12 h:12 h (lights on at 7 am) in our facility.
2. Handle female rats for 3 min on 3 consecutive days before breeding.
3. One day before breeding, separate male rats into individual cages.
4. Approximately 1 h before the start of the dark cycle, place female rats with a male rat of the same strain (ratio 2 females in each male cage) and leave overnight.
5. Approximately one hour after the light cycle begins (*i.e.*, 14 h after pairing), remove the female rats from the male's cage. Immediately collect a sample from the vagina of each rat with a sterile P200 pipette tip filled with 50-60 µl of sterile physiological saline. To achieve this, gently restrain

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each rat and pipette the saline into the vaginal canal, then retract the saline back into the pipette and eject onto a clean glass microscope slide. If the rat urinates, wait until urination stops before taking the sample.

6. Always use a new pipette tip for each rat.
7. Using a light microscope, check for the presence of spermatozoa (see Lucio *et al.*, 2013 [Figure 2A] for an example photomicrograph of rat spermatozoa).
   Important: It is not necessary to stain the slides, but it is necessary to visualize the cells collected before the solution dries.
8. If spermatozoa are present, the rat is considered pregnant. That day is then considered day 0 of gestation.
9. Place the pregnant rat in a new home cage and begin monitoring body weight and food intake. Care should be taken to minimize disturbances during pregnancy. In our protocol, body weight is monitored weekly when the cages are changed. Specific experimental considerations may necessitate more frequent monitoring of the pregnant dams.
10. Rats that are not suspected to be pregnant should be placed back in their home cage (*i.e.*, separated from the male) until they are re-paired. There is some risk that rats believed not to be pregnant actually are. If possible, wait 1-2 weeks before re-pairing. Monitor body weight during this time as rats that are pregnant will show an increase in body weight during the first 14 days of pregnancy.
11. On the day that re-pairing is desired, proceed with Step A2 again.

B. Poly:IC preparation

1. Poly:IC is prepared according to the manufacture's instructions (poly:IC, High Molecular Weight (HMW), InvivoGen, San Diego, CA). Note that some researchers also use polyI:C sodium salt from Sigma Aldrich.
2. To improve the solubility of the polyI:C, we heat it up in a 300 ml beaker filled with distilled water (150 ml). Using a hot plate, heat the distilled water to a maximum of 65 °C and place the bottle containing the polyI:C solution in the distilled water for 10 min.
3. Shake the bottle containing the polyI:C carefully and slowly when done.
4. Allow the polyI:C to cool for 1 h at room temperature.
5. Prepare appropriate aliquots for the number of rats to be treated and store at 4 °C or -20 °C. The poly:IC solution is stable for 1 month at 4 °C and 1 year at -20 °C.

C. Poly:IC treatment

1. On gestational day (GD) 15, the pregnant rats are removed from their colony room one at a time, weighed, and rectal temperature is taken. Use vaseline to aid insertion of the rectal probe.
2. Place the rat inside the anesthesia induction chamber. Induce anesthesia with 5% isofluorane.

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3. Once the rat is anesthetized, remove the rat from the induction chamber and place into a fume hood on a heating pad with a nose cone over its face to maintain anesthesia (2.5% isofluorane is typically sufficient for maintenance).
4. Pinch the rat's toes to ensure that it is sufficiently anesthetized. In the absence of a reaction, treatment can proceed.
5. Before the injection, clean the tail with an antiseptic isopropyl alcohol pad and then locate one of the two lateral tail veins in the middle third of the tail. With the needle point orientated bevel up, insert just below the surface and into the vein. Inject the appropriate amount of poly:IC or sterile physiological saline (control group) with a 25 Gauge needle. When the needle is placed appropriately, the plunger will depress easily with no resistance. If resistance is felt, remove the needle and try again at a position higher on the tail or on the opposite tail vein. We typically administer 4 mg/kg of polyI:C (Lins *et al.*, 2018 and 2019).
6. Turn off the anesthesia and transfer the rat back into her home cage. The rat should be on a heating pad from the time she is placed under anesthesia to the time she completely wakes up. Once the rat is awake and can maintain balance, she can be returned to the colony room.
7. Our protocol typically involves monitoring weight and temperature 3, 8, 24 and 48 h following treatment. These measurements are taken quickly in a procedure room just outside the rats' colony room. Body temperature can be a key measure of sickness response in the MIA model. The effect of Poly:IC on body temperature is usually demonstrated as a biphasic response, with an early hyperthermia (3 h-6 h) followed by a delayed hypothermia. Regarding hypothermia, if a dam's body temperature is below 36 °C, it is necessary to provide access to a warming pad in their home cage for 24 h until their temperature returns to normal. In the case of persistent hypothermia, euthanasia may be necessary.
8. Pregnant rats deliver their litters naturally. Starting on gestational day 20, we check for litters twice per day during the light cycle. The day of birth is noted as postnatal day 0.
9. On postnatal day 1, remove dams and litters one at a time from the colony to a nearby procedure room. Before handling the pups, place the dam in a holding cage. While handling the pups as little as possible, count and sex them, and weigh as a group. We typically cull litters back to 10 pups with a roughly balanced sex ratio where possible.

Notes

1. Variability in the published protocols for maternal immune activation exists (Kentner *et al.*, 2019). In an effort to improve reproducibility, a checklist for reporting methodological details has been developed and should be used in all future studies using maternal immune activation (Kentner *et al.*, 2019). Careful consideration must be given to sample sizes given that offspring from a given litter cannot be considered independent samples (see Kentner *et al.*, 2019 and references therein).

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2. Successful MIA can be observed with the increased maternal proinflammatory cytokines (such as CXCL1, IL-6, IL-1β TNF-α) after poly:IC treatment (reviewed in Bergdolt and Dunaevsky, 2019). Also, previous studies have observed some behavioral phenotypes such as prepulse inhibition deficits in MIA offspring (Dickerson *et al.*, 2010; Yee *et al.*, 2011; Giovanoli *et al.*, 2016; Meehan *et al.*, 2017), impairments in sociability and cognitive domains (Vorhees *et al.*, 2015; Mattei *et al.*, 2017; Lins *et al.*, 2018). Furthermore, some studies also reported hypolocomotive behavior in poly:IC treated offspring (Van den Eynde *et al.*, 2014). Another relevant behavioral phenotype observed in MIA offspring is increased locomotor activity induced by amphetamine, an indirect dopamine receptor agonist, or dizocilpine (MK-801), an NMDA receptor antagonist (Meyer *et al.*, 2008; Vorhees *et al.*, 2015; Lins *et al.*, 2018).
3. Variability in methods for MIA induction include differences in animal strain, doses of polyI:C, time of treatment (early, mid, or late gestation), route of administration (intraperitoneal, sub-cutaneous, intra-venous), and source of polyI:C (Meyer *et al.*, 2006 and 2008; Careaga *et al.*, 2018; Mueller *et al.*, 2019; Kowash *et al.*, 2019; Kentner *et al.*, 2019). As a result of these different induction protocols, some variablitlies in behavioral response can be observed. For example, previous studies showed impaired pre-pulse inhibition (PPI) in polyI:C offspring (reviewed in Meyer and Feldon, 2009, Mattei *et al.*, 2014) while others did not (Lins *et al.*, 2018, 2019; Van den Eynde *et al.*, 2014). Our previous data also demonstrated that polyI:C offspring showed heightened sensitivity to MK-801 (Lins *et al.*, 2018); this finding replicates previous studies which report hyperlocomotion following MK-801 (Zuckerman and Weiner, 2005).

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Competing interests

Research in the Howland laboratory is funded by peer reviewed research grants from government agencies and charities. The authors have no financial or other competing interests to declare.

Ethics

Experimental protocols and the use of animals were approved by the University of Saskatchewan Animal Research Ethics Board and conformed to the guidelines of the Canadian Council on Animal Care. Research conducted was approved under protocol 20080107 which has been active from 2008 to the present time.

bio-protocol www.bio-protocol.org/e3817 Bio-protocol 10(22): e3817. DOI:10.21769/BioProtoc.3817

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