Early neurodevelopmental and behavioural consequences of maternal immune activation at GD12.5 in Wistar rats

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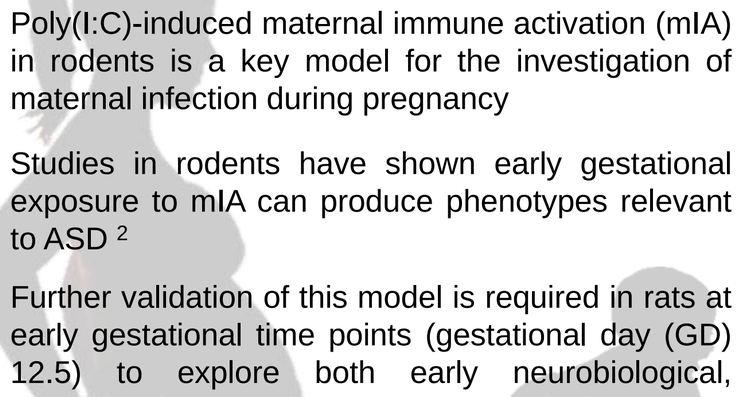
Introduction

- Viral infection in pregnancy has been associated with increased risk for the development of autism spectrum disorder (ASD) [1]
- Poly(I:C)-induced maternal immune activation (mIA) in rodents is a key model for the investigation of maternal infection during pregnancy
- Studies in rodents have shown early gestational exposure to mIA can produce phenotypes relevant to ASD [2]
- Further validation of this model is required in rats at early gestational time points (gestational day (GD) 12.5) to explore both early neurobiological, neurodevelopmental and behavioural changes in male and female offspring

Aims

- Provide a comprehensive evaluation of the effects of poly(I:C) treatment at GD12.5 in rats
- Explore early neurodevelopmental changes within this model
- Perform behavioural phenotyping

Methods



- **Behavioural testing:** All offspring from poly(I:C)- and saline-treated dams were tested in the open field test (OFT)
- **qPCR:** mRNA expression in frontal cortex (FC) was normalised to SDHA and GAPDH housekeeping genes and presented as relative expression from a control (Rest analysis 2009, Qiagen)
- **Statistics:** For comparisons between offspring of poly(I:C)- and saline-treated pups, a nested-ANOVA was performed with litter treated as a random variable. The homoscedasticity (Levene's test) and normality (Shapiro-Wilk test) of each data set were tested. For comparison of mean values unpaired T-test or Mann-Whitney test was used when parametric analysis was not applicable

Results: Maternal response

10 mg/kg poly(I:C) induces a variable immune response in pregnant Wistar rats at GD12.5

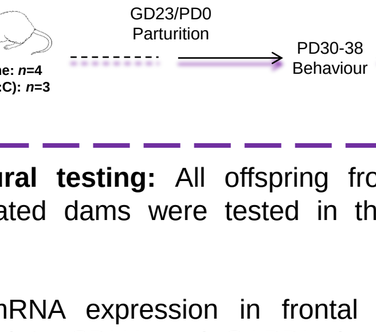
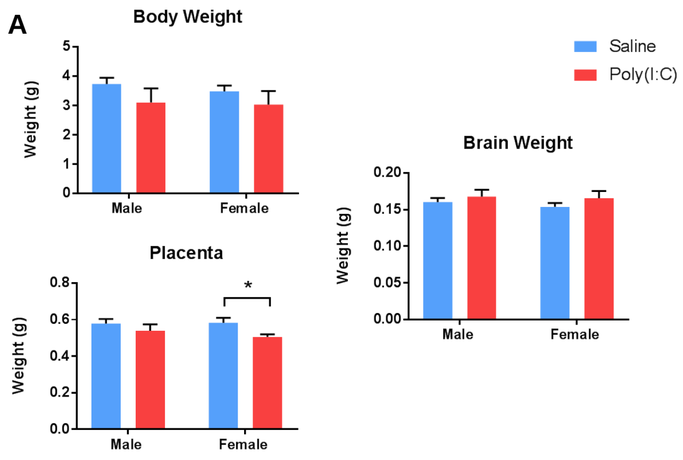


Figure 1. A subset of the Wistar rats treated with poly(I:C) showed a detectable level (125 pg/ml) of IL-6 (*n*=9/15). Data are presented as mean ± SEM, *n*=15-18 dams (some data points below detectable level).

Results: GD21

mIA reduces placenta weight in female offspring only with no effect on body or brain weight

Morphometric analysis



qPCR

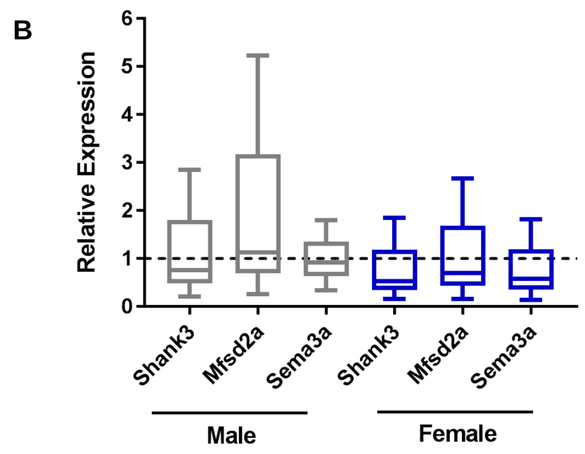
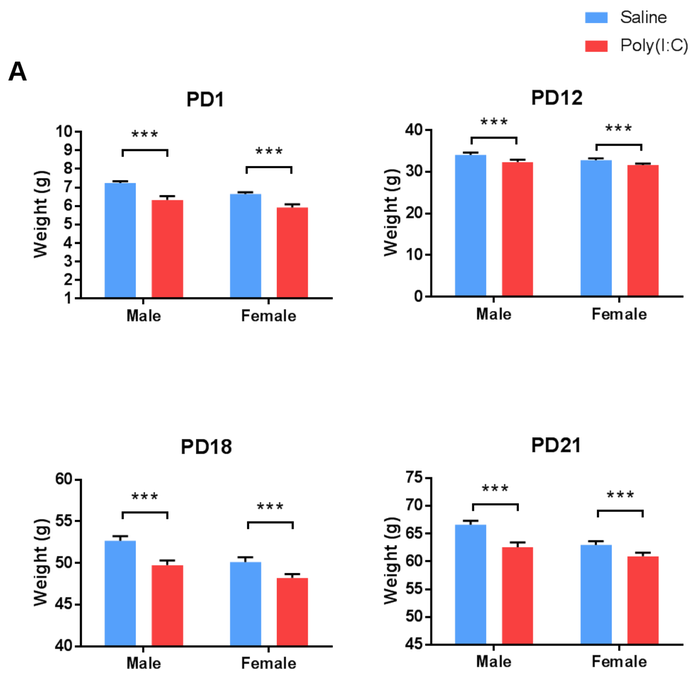


Figure 2. A) 10 mg/kg poly(I:C) produced no change to offspring body weight or brain weight at GD21. Placenta weight was significantly reduced in female offspring. **B)** No significant difference was found in expression of Shank3, Mfsd2a or Sema3a at GD21 in the frontal cortex male or female offspring. Data presented as mean ± SEM; *n*=12/sex/treatment; **p*<0.05. (Shank3, synaptic scaffold protein, Mfsd2a (blood brain barrier (BBB) integrity), Semaphorin-3a (axonal guidance)).

Results: PD21

mIA induces a reduction in body weight in male and female offspring throughout early development

Morphometric analysis



mIA induces a reduction in brain weight in male offspring at PD21

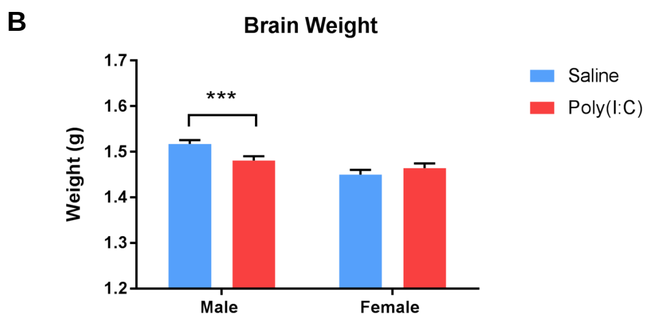


Figure 3. A) 10 mg/kg poly(I:C) produced a significant reduction to offspring body weight at all postnatal time points tested. **B)** A significant reduction in brain weight was found in male but not female offspring at PD21. Data presented as mean ± SEM; *n*=12-16/sex/treatment; ****p*<0.001.

Results: PD21

mIA alters expression of Shank3 and Mfsd2a in a sex-dependent manner in frontal cortex (FC) at PD21

qPCR

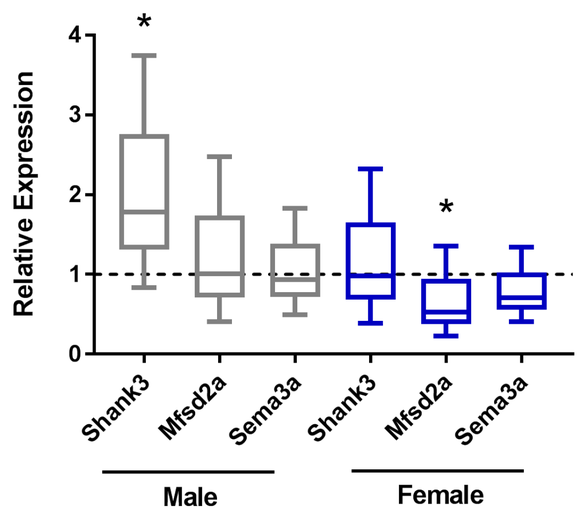


Figure 4. 10 mg/kg poly(I:C) on GD12.5 induced an increase in Shank3 gene expression in male offspring in the frontal cortex. In female offspring the expression of Mfsd2a was significantly decreased at PD21. No change was seen in Sema3a in either sex. Data presented as mean ± SEM; *n*=12-16/sex/treatment **p*<0.05. (Shank3, synaptic scaffold protein, Mfsd2a (blood brain barrier (BBB) integrity), Semaphorin-3a (axonal guidance)).

Results: Behavioural phenotyping

mIA increases anxiety-like behaviour in male adolescent offspring in the OFT

Open field test

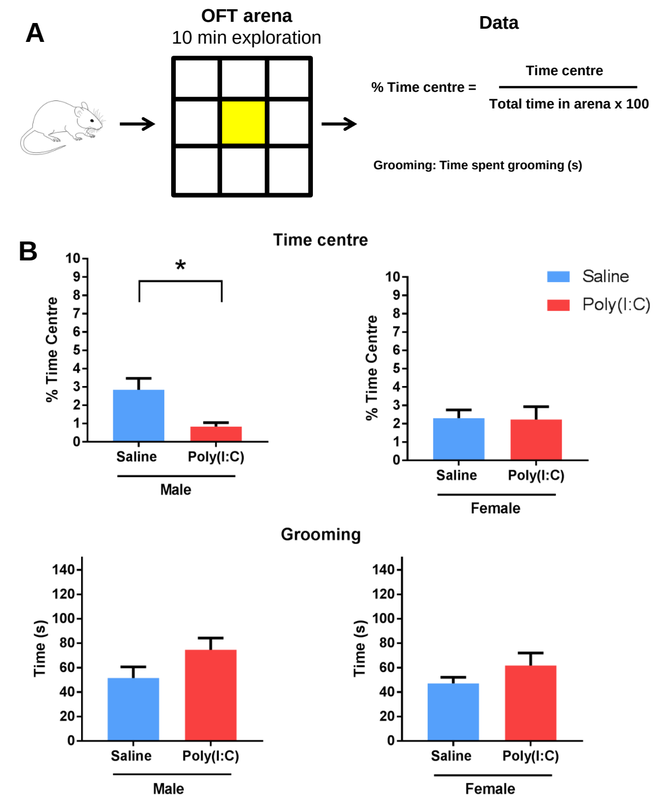


Figure 5. A) OFT protocol performed in Wistar rats. **B)** 10mg/kg poly(I:C) significantly increased anxiety-like behaviour at PD30 in male offspring only. Both male and female offspring showed an increase in time spent grooming. This effect did not reach significance. Data are presented as mean ± SEM; *n*=12-16/treatment, **p*<0.05.

Conclusions

mIA at GD12.5 in Wistar rats resulted in…

- a significant reduction in female placenta weight but no change in other morphometric parameters or gene expression at GD21
- significant treatment- and sex-dependent changes in gene expression in the FC related to synaptic scaffold protein Shank3 and marker of BBB integrity, Mfsd2a
- significant treatment- and sex-dependent changes in brain weight at PD21
- the appearance of a subtle anxiety-like phenotype in male offspring but not in any other behavioural parameter measured (social interaction, play or communication and repetitive behaviour)

This study provides an in depth longitudinal evaluation of this mIA model. This is the first study to use GD12.5 mIA in Wistar rats for investigation of NDDs.

References

[1] Knuesel, et al., (2014) Maternal immune activation and abnormal brain development across CNS disorders. Nat. Rev. Neurol.

[2] Reisinger, et al., (2015) The Poly(I:C)-induced maternal immune activation model in preclinical neuropsychiatric drug discovery. Pharmacol. Therapeutics

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