Phenotypic effects of maternal immune activation and early postnatal milieu in mice mutant for the schizophrenia risk gene neuregulin-1

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Abstract

Risk for schizophrenia is likely to involve gene × environment interactions. Neuregulin 1 (NRG1) is a schizophrenia risk gene, hence any interaction with environmental adversity, such as maternal infection, may provide further insights into the basis of the disease. This study examined the individual and combined effects of prenatal immune activation with Poly I:C and disruption of the schizophrenia risk gene NRG1 on the expression of behavioural phenotypes related to schizophrenia.

NRG1 heterozygous (NRG1 HET) mutant breeding pairs were time-mated. Pregnant dams received a single injection (5 mg/kg i.p.) of Poly I:C or vehicle on gestation day 9 (GD9). Offspring were then cross-fostered to vehicle-treated or PolyI:C-treated dams. Expression of schizophrenia-related behavioural endophenotypes was assessed at adolescence and in adulthood.

Combining NRG1 disruption and prenatal environmental insult (Poly I:C) caused developmental stage-specific deficits in social behaviour, spatial working memory and PPI. However, combining Poly I:C and cross-fostering produced a number of behavioural deficits in the open field, social behaviour and PPI. This became more complex by combining NRG1 deletion with both Poly I:C exposure *and* cross-fostering, which had a robust effect on PPI.

These findings suggest that concepts of gene × environment interaction in risk for schizophrenia should be elaborated to multiple interactions that involve individual genes interacting with diverse biological and psychosocial environmental factors over

early life, to differentially influence particular domains of psychopathology, sometimes over specific stages of development.

Introduction

On a background of established genetic risk, epidemiological studies have indicated an association between maternal bacterial and viral infections during pregnancy and higher incidence in adult offspring of psychosis in general, and of schizophrenia in particular (Brown and Derkits, 2010; Brown, 2011). It has been suggested that elevation of pro-inflammatory cytokines in the maternal host in response to infection is a key factor in fetal brain maldevelopment, thought to be fundamental to the pathobiology of schizophrenia (Meyer et al., 2006a; Smith et al., 2007). Further support for the neuroinflammation hypothesis of schizophrenia comes from studies reporting increased peripheral levels of selected pro-inflammatory cytokines (interleukin-6 [IL-6], IL-12, tumor necrosis factor-α [TNF-α], IL-1β, and interferon-γ [IFN-γ]) in schizophrenia patients experiencing their first psychotic episode or an acute relapse; these are normalised following antipsychotic treatment (Miller et al., 2011).

Polyriboinosinic-polyribocytidilic acid (Poly I:C), a synthetic analogue of double-stranded RNA, stimulates the production and release of selected pro-inflammatory cytokines (Meyer and Feldon, 2010; Giovanoli et al., 2013). In the rodent prenatal Poly I:C model, administration of Poly I:C to pregnant dams causes elevations in maternal serum cytokines that are accompanied by emergence in adulthood of behavioural and neural phenotypes related to those evident in schizophrenia (Meyer et al., 2006b). Subsequent studies demonstrated that the effects of maternal immune challenge during gestation between early (gestational day [GD] 9) and late (GD17) periods in mice are dissociable in terms of fetal brain cytokine responses to maternal inflammation and their pathological consequences in terms of

brain and behaviour (Meyer et al., 2008a; Bitanihirwe et al., 2010; Meyer et al., 2008b).

Interactions between genetic risk and environmental stressors in early stages of life appear important in the development of schizophrenia (Cirulli et al., 2009; Nestler and Hyman, 2010; van Os et al., 2010). Among several candidate genes of current interest, case-control and family-based association studies have confirmed a role for neuregulin-1 (NRG1) in risk for schizophrenia (see Gong et al., 2009, for meta-analysis). Several studies have also reported an association between NRG1 and disrupted neuroinflammatory processes in schizophrenia. Interaction between NRG1 and IL-1β is associated with increased risk for schizophrenia, as well as earlier age at onset (Hänninen et al., 2008). Additionally, a missense mutation within the transmembrane region of NRG1 increases activation of pro-inflammatory cytokines such as IL-6, IL-8 and TNF-α in patients with schizophrenia (Marballi et al., 2010). Furthermore, neonatal exposure to influenza virus infection is associated with decreased expression of NRG1 Type III isoform transcripts in the medial prefrontal cortex of adult C57BL6 mice (Asp et al., 2010).

Studies using mutant mice may inform on relationships between candidate risk genes and specific, experimentally controlled environmental interventions, i.e. gene (G) × environment (E) interactions. We employed mice with heterozygous deletion of the transmembrane domain of NRG1 to study the interaction between NRG1 genotype and prenatal immune challenge, *via* administration of Poly I:C to pregnant dams, on the expression of behavioural phenotypes related to schizophrenia.

Materials and Methods

Animals and Housing

Heterozygous NRG1 TM-domain mutant mice were constructed, bred and genotyped as described previously (Stefansson et al., 2002; O'Tuathaigh et al., 2007; Lai et al., 2010). Mice were housed in groups of two to five per cage and maintained at 21±1°C, with a constant humidity of 45-65%, on a 12 hr light dark cycle [08.00 on: 20.00 off] with *ad libitum* access to food and water. These studies were approved by the Research Ethics Committee of the Royal College of Surgeons in Ireland. They were conducted under licence from the Department of Health and Children in accordance with Irish legislation and the European Communities Council Directive 86/609/EEC for the care and use of experimental animals, and from the Environmental Protection Agency in relation to the contained use of genetically modified organisms.

Experimental Design

Timed Mating. This procedure was as previously described (Meyer et al., 2005); see **Supplementary Materials and Methods** for details.

Prenatal Treatment. Poly I:C was dissolved in 0.9% physiological saline. On GD9, pregnant dams received intraperitoneal (i.p.) injection of either 0.9% physiological saline or Poly I:C (5.0 mg/kg) (Meyer et al., 2005). Mice were then either returned to the home cage for the duration of their pregnancy, or sacrificed for measurement of maternal serum cytokines (see **Maternal Serum Cytokines** below).

Postnatal cross-fostering. This procedure was employed, as a control measure, to determine if an effect of maternal immune activation on maternal behaviour influences offspring, independent of effect of maternal immune activation on offspring (Schwendener et al., 2009). The day on which pups were born was considered postnatal day 0 (PND0). Pups were cross-fostered between PND0 and

PND2. Whole litters were removed from the original mothers. Pups were gently mixed with the bedding of the new cage and left for a period of 10 min. Pups were then introduced to the new cage containing a surrogate mother. Pups from either saline-treated or Poly I:C-treated pups were cross-fostered to either a saline-treated or Poly I:C-treated dam.

Maternal Serum Cytokines. Three hours after Poly I:C or saline administration, mice were sacrificed by decapitation and trunk blood collected. Blood was then allowed to clot at room temperature for 1 hr, centrifuged at 900 rpm and serum removed.

Multiplex Cytokine Immunoassay. Levels of IL-10, TNF-α, mouse keratinocyte-derived chemokine (mKC), IL-6 and IFN-γ were analysed using a multiplex cytokine immunoassay (MSD® 96-Well MULTI-SPOT kit, Meso Scale Discovery, Maryland, USA), as described previously (Desbonnet et al., 2012); see **Supplementary Materials and Methods** for details.

Figures 1A and 1B provide an overview of the experimental design.

Behavioural Assessments

An important validation criterion for any preclinical neurodevelopmental model of schizophrenia is the capacity to mimic the maturational delay between the early neurodevelopmental insult and the emergence of its relevant consequences in early adulthood, in the manner characteristic of the clinical condition. Therefore, the effects of prenatal Poly I:C administration on sociability and social novelty preference and spontaneous exploratory activity in the open field test were tested, in that order, at adolescence (PND35-45) and at adulthood (3 months; PND90-135). Phenotypic studies conducted in NRG1 hypomorphs have demonstrated that novelty-induced

hyperactivity and social interaction deficits have been the most consistently observed schizophrenia-linked phenotypes in this mutant line (Stefansson et al., 2002; O'Tuathaigh et al., 2007; Desbonnet et al., 2012). Additionally, Y-maze spontaneous alternation and prepulse inhibition were assessed at adulthood (PND90-135). At adulthood, there was at least a 1-week interval between each behavioural test. Table 1 provides a breakdown of group sizes for experimental mice which underwent behavioural assessments at both adolescence and adulthood.

Sociability and Social Novelty Preference: Social approach/avoidance behaviour was assessed using a paradigm for social interaction as described previously (O'Tuathaigh et al., 2007; Desbonnet et al., 2012).

Prepulse Inhibition [PPI]: Testing of PPI was performed in a startle chamber (SR-LAB, San Diego Instruments, San Diego, CA, USA) as described previously (Desbonnet et al., 2012).

Spontaneous Alternation: Spatial working memory was assessed using the continuous variant of the spontaneous alternation procedure, as described previously (O'Tuathaigh et al., 2007; Desbonnet et al., 2012).

Novel Open Field: Exploratory activity in a novel environment was assessed by placing each mouse in the center of an open arena [white perspex sides and base: 30 × 30 × 20 cm] for 10 min. Distance moved and velocity of movement were recorded using Ethovision videotracking (Ethovision®, Noldus Inc., Wageningen, The Netherlands). Using this technology, a central zone and four corner zones were demarcated; time spent in and number of entries into each zone were measured.

For assessment of sociability and social novelty preference, PPI, and spontaneous alternation, full details are given in **Supplementary Materials and Methods**.

Statistics

Multivariate analysis of variance (ANOVA) was applied to assess group differences across the data, using SPSS 15.0. Repeated measures ANOVA was performed to analyse data for sociability and social novelty preference, startle habituation and % PPI for each pulse intensity (100, 110 and 120dB). Where the data were not normally distributed, analyses were conducted following square root transformation. Statistically significant effects in each ANOVA were followed with lower-level ANOVA and/or post hoc comparisons. A p value of less than 0.05 was considered significant.

Results

Maternal Serum Cytokines

Administration of Poly I:C to pregnant dams on GD9 resulted in a marked increase in maternal serum protein levels at 3 hr post injection. Cytokine levels were increased for IL-10 [$F_{1,24} = 46.05$, $p < .001$], TNF-α [$F_{1,25} = 15.27$, $p < .001$], IL-8 [$F_{1,23} = 22.71$, $p < .001$], IL-6 [$F_{1,24} = 9.65$, $p < .001$] and IFN-γ [$F_{1,23} = 4.40$, $p < .05$]; there were no effects of genotype or treatment × genotype interactions (Fig. 2).

Gestational Length and Litter Size

Administration of Poly I:C reduced gestational length by 3.4 % [$F_{1,21} = 2.23$, $p < .05$], but had no effect on litter size.

Body Weight

Administration of Poly I:C had no effect on body weight of offspring at PND35 or PND90. Reduction in body weight at PND35 was observed in WT but not in NRG1 HET offspring cross-fostered to surrogate dams who received Poly I:C, relative to offspring cross-fostered to surrogate dams who received saline [effect of cross-fostering, $F_{1,76} = 4.29$, $p < .05$; cross-fostering × genotype interaction, $F_{1,76} = 4.98$, $p < .05$]; no effect was evident at PND90 (see **Figure S1**).

Sociability

At PND35, as expected, mice spent more time in the chamber containing *Stranger 1* than in the opposite, empty chamber [effect of chamber, $F_{1,136} = 20.02$, $p < .001$; no effects of genotype or dam-Poly I:C; no interactions with genotype] (Fig. 3A). Preference for *Stranger 1* was reduced in male offspring cross-fostered to a

saline-treated surrogate and in female offspring cross-fostered to a Poly I:C-treated surrogate [chamber × cross-fostering × sex interaction, $F_{1,136} = 12.43$, $p < .001$] (see **Figure S2**). Independently, preference for *Stranger 1* was reduced in offspring of saline-treated dams that were cross-fostered to a saline-treated surrogate and in offspring of Poly I:C-treated dams that were cross-fostered to a Poly I:C-treated surrogate [chamber × cross-fostering × dam-Poly I:C interaction, $F_{1,136} = 4.55$, $p < .05$; no chamber × cross-fostering × dam-Poly I:C × sex interaction] (see Fig. 3B).

At PND90, as expected, preference for *Stranger 1* endured [effect of chamber, $F_{1,136} = 4.73$, $p < .05$] but effects of cross-fostering were no longer evident [no main effect of cross-fostering or interactions between cross-fostering and dam-Poly I:C, genotype or sex]. Rather, preference for *Stranger 1* was now reduced by maternal Poly I:C treatment in WT but not in NRG1 HET offspring [chamber × dam-Poly I:C × genotype interaction, $F_{1,132} = 8.24$, $p = .005$]; time spent with *Stranger 1* was reduced [$p < .05$] in WT offspring of dams treated with Poly I:C relative to WT offspring of dams treated with saline (Fig. 3C).

Social Novelty Preference

At PND35, while offspring of dams treated with saline spent more time in the chamber containing *Stranger 2* than in the opposite chamber containing the now familiar *Stranger 1*, as expected, offspring of dams treated with Poly I:C showed no such preference [chamber × dam-Poly I:C interaction, $F_{1,136} = 4.57$, $p < .05$] (Fig. 4A). There were no other effects of, or interactions with, dam-Poly I:C, genotype, cross-fostering or sex.

At PND90, preference for *Stranger 2* was reversed to preference for *Stranger 1* in NRG1 HET offspring [chamber × genotype interaction, $F_{1,132} = 5.47$, $p = .02$]

(Fig. 4B). This effect occurred primarily in NRG1 HET offspring of dams who were cross-fostered to surrogates treated with saline, with no such effect evident in offspring cross-fostered to surrogates treated with Poly I:C [chamber × genotype × cross-fostering interaction, $F_{1,132} = 10.31$, $p = .002$] (Fig. 4C). There were no effects of, or interactions with, dam-Poly I:C or sex.

Open Field

Distance travelled. At both PND42 and PND90, distance travelled was not altered by Poly I:C treatment of dams, genotype or cross-fostering [no main effects on ANOVA].

Time spent in outer zone. At PND42, time spent in the outer zone *vs* the centre zone was not altered by Poly I:C treatment of dams, genotype or cross-fostering [no main effects on ANOVA] (Fig. 5A & **Figure S3**). Time in the outer zone was increased in (i) saline offspring cross-fostered to a Poly I:C-treated surrogate relative to a saline-treated surrogate and (ii) in Poly I:C offspring cross-fostered to a saline-treated surrogate relative to saline offspring cross-fostered to a saline-treated surrogate [cross-fostering × dam-Poly I:C interaction, $F_{1,48} = 5.01$, $p < .05$] (see **Figure S3**). Entries into the outer zone were decreased in Poly I:C offspring cross-fostered to a saline-treated surrogate relative to saline offspring cross-fostered to a saline-treated surrogate [cross-fostering × dam-Poly I:C interaction, $F_{1,48} = 4.62$, $p < .05$] (see **Figure S4**). Entries into the centre zone were decreased in saline offspring cross-fostered to a Poly I:C-treated surrogate relative to saline offspring cross-fostered to a saline-treated surrogate [cross-fostering × dam-Poly I:C interaction, $F_{1,48} = 7.15$, $p < .01$] (see **Figure S4**).

At PND90, NRG1 HET offspring time spent less time in the outer zone (with more time spent in the centre zone) [effect of genotype, $F_{1,31} = 12.34$, $p < .001$; no genotype × dam-Poly I:C interaction] (Fig. 5B), but made more entries into the outer zone compared to WT offspring [effect of genotype, $F_{1,31} = 11.22$, $p < .002$; no genotype × dam-Poly I:C interaction] (see **Figure S5**). Entries into the centre zone were increased in NRG1 HET compared to WT offspring; this effect was more pronounced in NRG1 HET offspring from Poly I:C treated dams than WT [effect of genotype, $F_{1,31} = 6.51$, $p < .02$; genotype × dam-Poly I:C interaction, $F_{1,31} = 6.55$, $p < .02$] (see **Figure S5**).

Time spent in corners. At PND42, time spent in corners was not altered by Poly I:C treatment of dams, cross-fostering or genotype [no main effects on ANOVA] (Fig. 5A & **Figure S3**). However, corner entries were increased in NRG1 HET offspring compared to WT offspring [effect of genotype, $F_{1,48} = 5.32$, $p < .05$; no genotype × dam-Poly I:C interaction] (see **Figure S3**).

At PND90, time spent in corners was unrelated to genotype (Fig. 5B). Corner time was reduced in offspring of Poly I:C-treated dams cross-fostered to a saline-treated surrogate compared to offspring of saline-treated dams cross-fostered to a saline-treated surrogate [cross-fostering × dam-Poly I:C interaction, $F_{1,31} = 13.09$, $p < .001$] (see **Figure S3**). Corner entries were not altered by Poly I:C treatment of dams, genotype or cross-fostering.

Prepulse Inhibition

Startle Response / Habituation and Prepulse Inhibition: Assessment of startle responsivity before and after trial blocks showed habituation at pulse-alone intensities of 110 dB (effect of trial block: $F_{1, 48} = 3.96$, $p = .05$) and 120 dB (effect of

trial block: $F_{1, 48} = 41.69$, $p < .01$) but not at 100 dB. At both the 110 and 120 dB pulse-alone intensities, no effect of genotype, dam-Poly I:C, cross-fostering, or sex was observed in relation to startle responsivity or habituation (all $p > .05$). At 100 dB, cross-fostering impacted upon startle habituation, with cross-fostered offspring demonstrating reduced habituation (cross-fostering × trial interaction: $F_{1, 48} = 4.55$, $p < .05$).

Across all groups, % PPI increased with prepulse intensity (4, 8 and 16dB) at pulse intensities of 110dB [effect of prepulse intensity, $F_{2,122} = 2.99$, $p = .05$] and at 120dB [effect of prepulse intensity, $F_{2,122} = 3.86$, $p < .05$].

Pulse Intensity 110dB. PPI was disrupted in NRG1 HET [effect of genotype, $F_{1,47} = 7.10$, $p < .02$]. Increase in PPI with prepulse intensity was disrupted in the offspring of Poly I:C-treated dams [prepulse × dam-Poly I:C interaction, $F_{2,94} = 3.58$, $p < .05$] (Fig. 6A); these effects were observed to a greater extent in male than in female offspring [dam-Poly I:C × sex interaction, $F_{1,47} = 5.42$, $p < .05$].

While cross-fostering was without effect on PPI among offspring of saline-treated dams, cross-fostering disrupted the increase in PPI with prepulse intensity among offspring of Poly I:C-treated dams [prepulse × dam-Poly I:C × cross-fostering interaction, $F_{2,94} = 3.25$, $p < .05$] (Fig. 6A). In WT, cross-fostering to a Poly I:C-treated surrogate was without effect on PPI in offspring of either saline-treated or Poly I:C-treated dams; however, in NRG1 HET, cross-fostering to a Poly I:C-treated surrogate disrupted PPI in offspring of saline-treated dams but facilitated PPI in offspring of Poly I:C-treated dams [dam-Poly I:C × cross-fostering × genotype interaction, $F_{1,47} = 6.51$, $p < .02$] (Fig. 6A).

Pulse Intensity 120dB. PPI was disrupted in NRG1 HET [effect of genotype, $F_{1,47} = 6.32$, $p < .02$] (Fig. 6B). While cross-fostering was without effect on PPI

among offspring of saline-treated dams, cross-fostering disrupted the increase in PPI with prepulse intensity among offspring of Poly I:C-treated dams [prepulse × dam-Poly I:C × cross-fostering interaction, $F_{2,94}$ =3.59, $p < .05$].

Spontaneous Alternation

Alternation was reduced in offspring of dams treated with Poly I:C [effect of dam-Poly: I:C, $F_{1,67} = 4.77$, $p < .05$; no effect of genotype or dam-Poly I:C × genotype interaction] (Fig. 7). Alternation was reduced in female but not in male NRG1 HET offspring [genotype × sex interaction, $F_{1,67} = 6.48$, $p < .05$], with this effect being diminished in offspring of dams treated with Poly I:C [genotype × sex × dam-Poly I:C interaction, $F_{1,67} = 4.81$, $p < .05$]; among females, alternation was reduced in WT but not NRG1 HET offspring of dams treated with Poly I:C relative to saline (Fig. 7). Alternation was not influenced by cross-fostering [no main effect of cross-fostering or interactions between cross-fostering and dam-Poly I:C, genotype or sex].

Total maze arm entries were increased in female but not male NRG1 HET offspring [genotype × sex interaction, $F_{1,67} = 5.10$, $p < .05$], more so in female NRG1 HET offspring that had been cross-fostered to a Poly I:C-treated surrogate [genotype × sex × cross-fostering interaction, $F_{1,67} = 5.20$, $p < .05$]; among females, entries were increased in NRG1 HET offspring that had been cross-fostered to a Poly I:C-treated surrogate relative to NRG1 HET offspring cross-fostered to a saline-treated surrogate and WT offspring cross-fostered to either surrogate [data not shown]. Arm entries were also increased in female but not male offspring of saline-treated dams that had been cross-fostered to a Poly I:C-treated surrogate rather than to a saline-treated surrogate [cross-fostering × dam-Poly I:C × sex interaction, $F_{1,67} = 6.27$, $p < .02$; no interaction with genotype, data not shown].

Discussion

Independent and interactive effects of NRG1 genotype, prenatal Poly I:C and postnatal cross-fostering were assessed in terms of four behavioural models related to schizophrenia: social behaviour, PPI, spatial working memory, and locomotor activity/anxiety in the open field. As most previous studies have involved a single experimental manipulation, the approach adopted here was first to identify any overall effects of genotype [NRG1; G] and prenatal environmental manipulation [maternal Poly I:C treatment; E], and then to determine any interactions [G × E] between them, for each behavioural model.

Crossfostering was also adopted in this study as an important control measure for any potential postnatal effects of genotype and/or treatment on the phenotype of the offspring. There is a growing body of evidence to show that the early-life stress of cross-fostering in rodents induces long-lasting behavioural abnormalities reminiscent of psychological disorders (Priebe et al., 2005; Lu et al., 2009; Weiss et al., 2011; Holloway et al., 2013). Previous studies involving this prenatal immune challenge model have shown that adoption of prenatal control mice to immune-challenged surrogate mothers was sufficient to induce specific pharmacological and neuroanatomical abnormalities relevant to schizophrenia in the fostered offspring (Meyer et al., 2008b). In the present examination of NRG1 × prenatal immune challenge interactions, our data suggests that immunological stress during pregnancy may affect postpartum maternal factors in such a way that being reared by an immune-challenged surrogate mother may independently be associated with emergence of schizophrenia-related phenotypes. Additionally, it may interact in an additive or synergistic manner with other genetic or environmental manipulations to contribute to the development of distinct forms of psychopathology in adult life.

These factors [NRG1, maternal PolyI:C treatment, crossfostering to immune stressed *vs* non-stressed surrogate] are considered collectively, as summarised in Table 2.

As expected, maternal exposure to Poly I:C produced a marked increase in maternal serum cytokines; importantly, this response did not differ between the genotypes. Elevations in maternal cytokines are one of the key events triggering postnatal behavioural abnormalities (Buka et al., 2001). On the basis of the present study, abnormalities in offspring can be attributed to either maternal treatment of dams with Poly I:C, and not to any disruptions in maternal immune response consequent to loss of NRG1. Gestational length in NRG1 HET was very slightly reduced (-3%) by maternal exposure to Poly I:C; some studies have reported that pre-term birth is also a risk factor for schizophrenia (Ichiki et al., 2000; Vuillermot et al., 2010).

Though maternal Poly I:C was without effect on sociability in either WT or NRG1 HET offspring in adolescence, in adulthood maternal Poly I:C reduced sociability in WT but not in NRG1 HET offspring. This indicates a G [NRG1] × E [maternal immune activation] interaction by which reduction in NRG1 attenuates the action of maternal Poly I:C to disrupt sociability in offspring ontogenically over some period between adolescence and adulthood. This observation is reminiscent of the developmental trajectory of schizophrenia, in which symptoms become evident following adolescence to achieve diagnostic import in young adulthood. It has been reported previously that functional and structural abnormalities emerge progressively in the offspring of Poly I:C-treated dams through to young adulthood (Meyer et al., 2008a; Vuillermot et al., 2010; Piontkewitz et al., 2011; Shepherd et al., 2012). This type of G × E interaction in social behaviour has also been observed in autism research; the combination of a risk gene for autism, tuberous sclerosis protein 2, and

Poly I:C ameliorated the behavioural deficits in sociability caused by Poly I:C alone (Ehninger et al., 2012).

There were no G × E interactions in relation to social novelty preference in either adolescence or adulthood. However, when performance of WT and NRG1 offspring in this phase of the test is analysed according to the treatment of the foster mother, reversal of social novelty preference to preference for the familiar conspecific in NRG1 HET occurred only in offspring cross-fostered to a saline-treated surrogate and was absent in those cross-fostered to a Poly I:C-treated surrogate, an effect that only emerges in adulthood (Fig. 4C). This suggests that cross-fostered to a Poly I:C surrogate negates the effect of loss of NRG1 to reverse social novelty preference, and does so ontogenically over some period between adolescence and adulthood.

Disruption of PPI has been observed in the offspring of rodent dams subjected to a variety of immune challenges (Smith et al., 2007; Ozawa et al., 2006; Fortier et al., 2007; Makinodan et al., 2008; Cardon et al., 2010; De Miranda et al., 2010). However, some studies have failed to replicate this effect (Ozawa et al., 2006). In the present study, we find the PPI deficit following Poly I:C treatment of dams to be more prominent in male than in female offspring. Here, we also find that PPI is disrupted in NRG1 HET offspring. This replicates some previous findings in these same TM NRG1 mutants (Stefansson et al., 2002; Desbonnet et al., 2012) and in type III NRG1 mutants (Chen et al., 2008). Although there are no synergistic effects of gene deletion and prenatal infection on PPI, this may be attributable to the fact that the existing magnitude of PPI disruption in NRG1 HET mutants masks any potential NRG1 × prenatal stress interactions on sensorimotor gating; a similar putative masking effect has been observed for NRG1 × adolescent social stress effects on PPI levels (Desbonnet et al., 2012).

Maternal Poly I:C treatment has been reported to disrupt aspects of exploratory behaviour in offspring when given in early/mid-pregnancy (Meyer et al., 2006a; Smith et al., 2007; Meyer et al., 2005; Shi et al., 2003) and in mid/late-pregnancy (Fortier et al., 2007; De Miranda et al., 2010; Chen et al., 2008); no such effect was reported when Poly I:C was given late in pregnancy (Bitanihirwe et al., 2010). Here, administration of Poly I:C on GD9 was not associated with any alteration in overall activity in terms of total distance moved (Meyer et al., 2005).

NRG1 HET have been reported to show a hyperactive phenotype in novel environments (O'Tuathaigh et al., 2006; van den Buuse et al., 2009). While no such effect was evident here in terms of total distance moved, NRG1 HET showed an increase in entries into the corner zone in adolescence and an increase in entries into both outer and centre zones in adulthood, with a shift to less time spent in the outer and more time spent in the centre zone; this would suggest some overall increase in activity in association with progressive reduction in anxiety. This illuminates the ontogeny of anxiolysis that has been reported previously in NRG1 HET during young adulthood (Desbonnet et al., 2012; Boucher et al., 2007; O'Tuathaigh et al., 2008) and elaborates the need for phenotypic studies to be conducted on a longitudinal basis (Piontkewitz et al., 2011).

Heterozygous deletion of NRG1 in offspring was associated with alteration to the effect of maternal Poly I:C on those offspring in terms of an increase in entries into the centre zone in adulthood, which may reflect disruption to anxiety-related behaviour; no such increase was evident in WT offspring of Poly I:C-treated dams or NRG1 HET offspring of saline-treated dams. This indicates a G × E interaction by which NRG1 acts normally to inhibit this effect of maternal immune activation on offspring.

In the present study, maternal treatment with Poly I:C on GD9 resulted in impaired immediate spatial working memory in terms of reduced % alternation in the Y-maze. Impairment in spatial working memory in female but not male NRG1 HET was less evident in offspring of dams treated with Poly I:C. This indicates a sex-specific G × E interaction whereby loss of NRG1 attenuates the action of maternal Poly I:C to disrupt spatial working memory in female offspring.

In conclusion, the profile of relationships reported here (Table 2), involving the schizophrenia risk gene NRG1 and prenatal infection, an established environmental factor induced by Poly I:C injection during early gestation, alongside the postnatal cross-fostering manipulation, indicate the complexity of G × E interactions that regulate behavioural phenotypes relevant to psychotic illness. The present findings highlight the importance of considering prenatal environmental events when examining the contributions of genetic defects to specific domains of psychopathology and associated pathobiology, and also suggest that developmental stage and gender are influential factors that deserve greater consideration in models of G × E interaction in schizophrenia.

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Supplementary information is available at the *Neuropsychopharmacology* website.

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Table/Figure Legends

Figure 1. Experimental timeline for (A) maternal cytokine analysis and (B) behavioural assessments in male (M) and female (F) WT and NRG1 HET mutants in relation to gestational day (GD) and postnatal day (PND).

Figure 2. Acute effects of Poly I:C (5.0 mg/kg) treatment on gestational day 9 on maternal serum cytokines (IL-10, TNF-α, IFN-γ, IL-8, IL-6) in WT and NRG1 HET pregnant dams. Data are mean cytokine levels ± SEM from serum collected 3 hr after either saline or Poly I:C; *$p < .05$, **$p < .01$, ***$p < .001$ *vs* saline. n = 6-8 per genotype and per treatment condition.

Figure 3. Effect on sociability at **(A)**, **(B)** postnatal day (PND) 35 and **(C)** PND90 in offspring of Poly I:C (5.0 mg/kg) treated dams in WT and NRG1 HET. Data are mean times ± SEM spent in the empty chamber *vs* chamber containing *Stranger 1*.
(B) Poly I:C treatment of dams in relation to genotype resulted in four groups: WT offspring of saline-treated dams (WT – Sal); WT offspring of Poly I:C-treated dams (WT - Poly I:C); NRG1 HET offspring of saline-treated dams (NRG1 HET - Sal); and NRG1 HET offspring of Poly I:C-treated dams (NRG1 HET - Poly I:C); *$p < .05$ *vs* empty chamber; $^{\#}p < .05$ *vs Stranger 1* WT offspring of saline-treated dam (WT – Sal). n = 5-8 per genotype / treatment /cross-fostering / sex group as indicated in Table 1.

Figure 4. Effect on social novelty preference at **(A)** postnatal day (PND) 35 in offspring of saline *vs* Poly I:C (5.0 mg/kg)-treated dams, and at **(B)** PND90 in WT and

NRG1 HET mice. Data are mean times ± SEM spent in the chamber containing *Stranger 2 vs* chamber containing (now familiar) *Stranger 1*; * $p < .05$ *vs* saline-treated *Stranger 1*. # $p < .05$ *vs* WT *Stranger 1*. **(C)** Effect on social novelty preference at PND90 of cross-fostering in WT and NRG1 HET offspring. Data are mean times ± SEM spent in the chamber containing *Stranger 2 vs* chamber containing (now familiar) *Stranger 1*. Cross-fostering in relation to genotype resulted in four groups: WT offspring cross-fostered to a saline-treated surrogate (WT – Cross-Sal); NRG1 HET offspring cross-fostered to a saline-treated surrogate (NRG1 HET – Cross-Sal); WT offspring cross-fostered to a Poly I:C-treated surrogate (WT – Cross-Poly I:C); and HET offspring cross-fostered to a Poly I:C-treated surrogate (NRG1 HET – Cross-Poly I:C); ***$p < .001$ *vs Stranger 1*; # $p < .05$ *vs Stranger 1* WT offspring cross-fostered to a saline-treated surrogate (WT – Cross-Sal). n = 5-8 per genotype / treatment /cross-fostering / sex condition as indicated in Table 1.

Figure 5. Effect on open field behaviour at **(A)** postnatal days (PND) 42 and **(B)** PND90 of Poly I:C (5.0 mg/kg) treatment of dams in WT and NRG1 HET offspring. Data are mean time spent in indicated zone ± SEM over the 10 min session. Poly I:C treatment of dams in relation to genotype resulted in four groups: WT offspring of saline-treated dams (WT - Saline dam); WT offspring of Poly I:C-treated dams (WT - Poly I:C dam); NRG1 HET offspring of saline-treated dams (HET - Saline dam); and NRG1 HET offspring of Poly I:C-treated dams (HET - Poly I:C dam). Centre zone: ***$p < .001$ *vs* WT offspring. Outer zone: ***$p < .001$ *vs* WT offspring. n = 5-8 per group as indicated in Table 1.

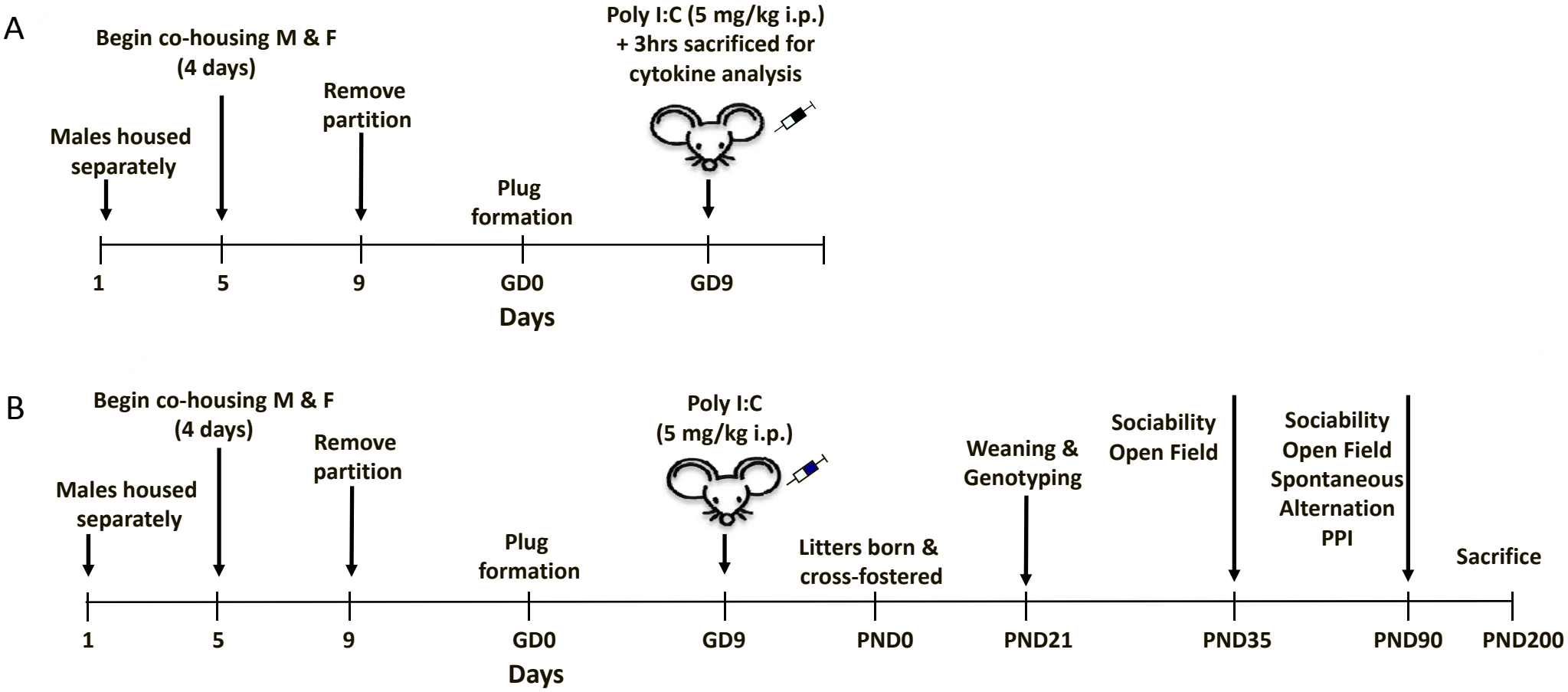
Figure 6. Prepulse inhibition **(**PPI) in WT and NRG1 HET offspring of Poly I:C (5.0 mg/kg)-treated dams following cross-fostering, at pulse intensity 110 dB **(A)** and 120 dB **(B)** . Data are mean % PPI ± SEM for prepulses of 4, 8 and 16 dB. Poly I:C treatment of dams and cross-fostering resulted in four groups: offspring of saline-treated dams cross-fostered to a saline surrogate (Saline – Cross-Sal); offspring of Poly I:C-treated dams cross-fostered to a saline treated surrogate (Poly I:C – Cross-Sal); offspring of saline-treated dams cross-fostered to a Poly I:C-treated surrogate (Saline – Cross-Poly I:C); and offspring of Poly I:C-treated dams cross-fostered to a Poly I:C-treated surrogate (Poly I:C – Cross-Poly I:C); $^{\#}$ $P < 0.05$ *vs* WT offspring of Poly I:C (5.0 mg/kg)-treated dams, cross-fostered to a saline-treated surrogate (WT, Poly I:C – Cross-Sal); ∞ $P < 0.05$ *vs* WT; $^{\beta}$ $P < 0.05$ *vs* corresponding offspring of saline-treated dams (Saline). $^{\Delta}$ $P < 0.05$ *vs* corresponding offspring of Poly I:C-treated dams cross-fostering to saline-treated surrogates (Poly I:C – Cross-Sal). n = 5-8 per group as indicated in Table 1.

Figure 7. Effects on spontaneous alternation of Poly I:C (5.0 mg/kg) treatment of dams in WT and NRG1 HET offspring. Data are mean % alternation ± SEM over the 10 min session. Effect of Poly I:C treatment of dams in male and female WT and NRG1 HET offspring; * $p < .05$ *vs* female WT offspring of saline-treated dams; $^{\#}$ $p <$ .05 NRG1 HET *vs* WT. n = 5-8 per group as indicated in Table 1.

Table 1: Group sizes for experimental mice subjected to behavioural testing.

Table 2: Behavioural effects of Poly I:C and cross-fostering in NRG1 mutants: G, effect of NRG1 mutation; E_1, effect of Poly I:C treatment; E_2, effect of cross-fostering;

+[a], effect greater in males than females; +[b], effect greater in females than males; Adol, effect in adolescence; Adult, effect seen in adulthood.


A
Begin co-housing M & F
(4 days)
Remove
partition
Poly I:C (5 mg/kg i.p.)
+ 3hrs sacrificed for
cytokine analysis
Males housed
separately
Plug
formation
1
5
9
GD0
GD9
Days
B
Begin co-housing M & F
(4 days)
Remove
partition
Poly I:C
(5 mg/kg i.p.)
Sociability
Open Field
Sociability
Open Field
Spontaneous
Alternation
PPI
Weaning &
Genotyping
Males housed
separately
Plug
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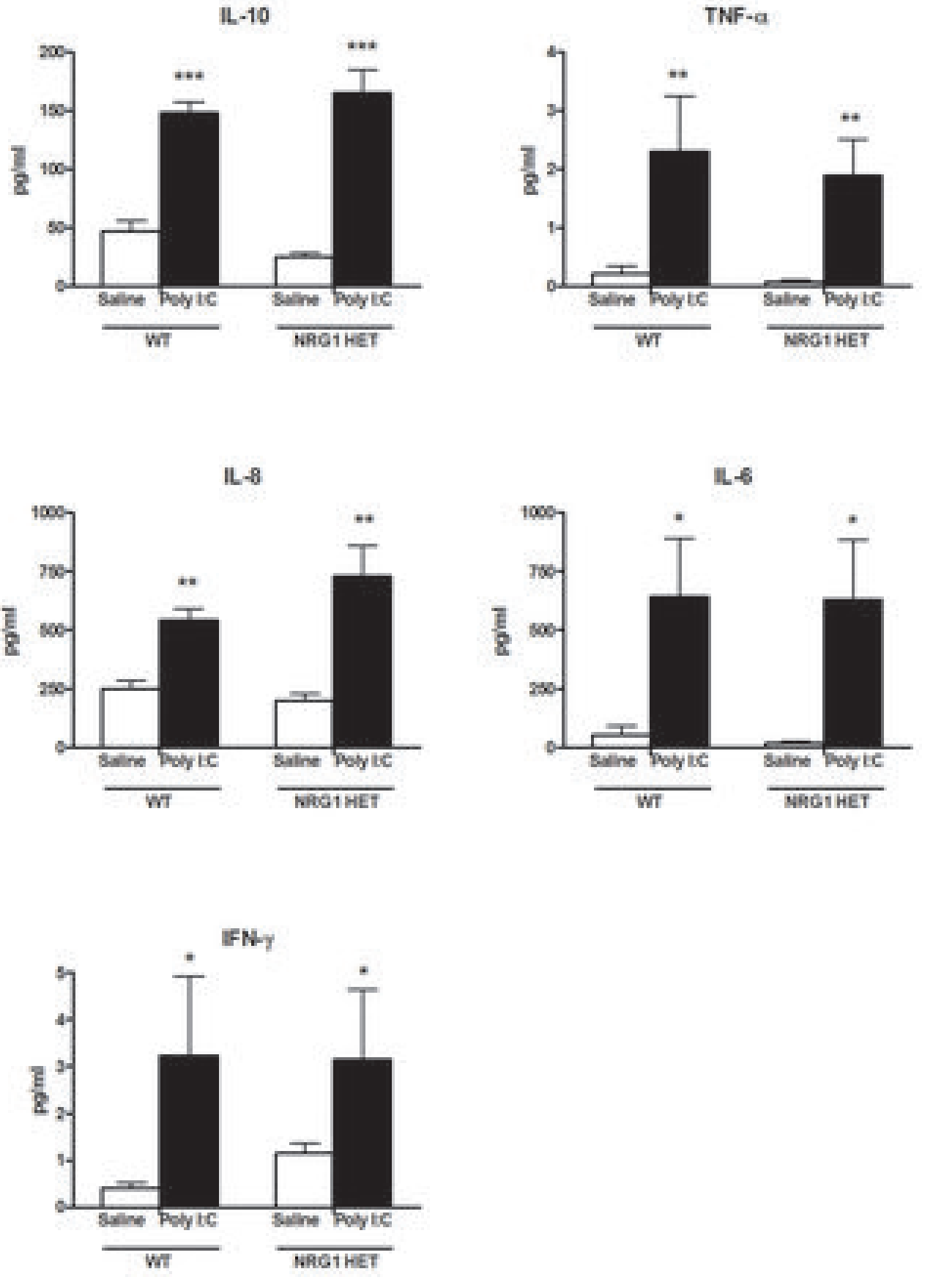
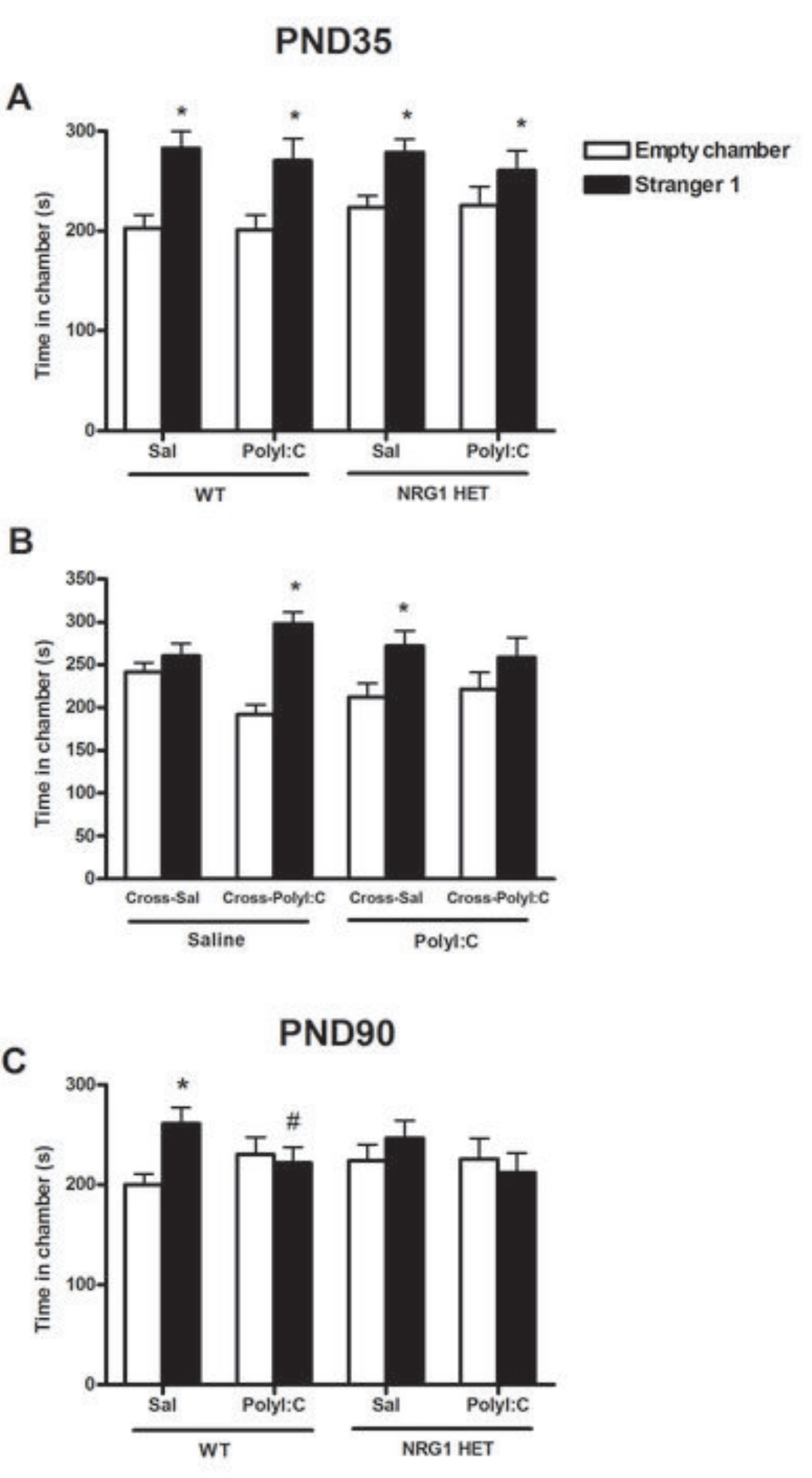

IL-10
TNF-α
IL-8
IL-6
IFN-γ
pg/ml
Saline
Poly I:C
WT
NRG1 HET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WT
NRG1 HET
B
Time in chamber (s)
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Cross-Sal
Cross-Polyl:C
Cross-Sal
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Saline
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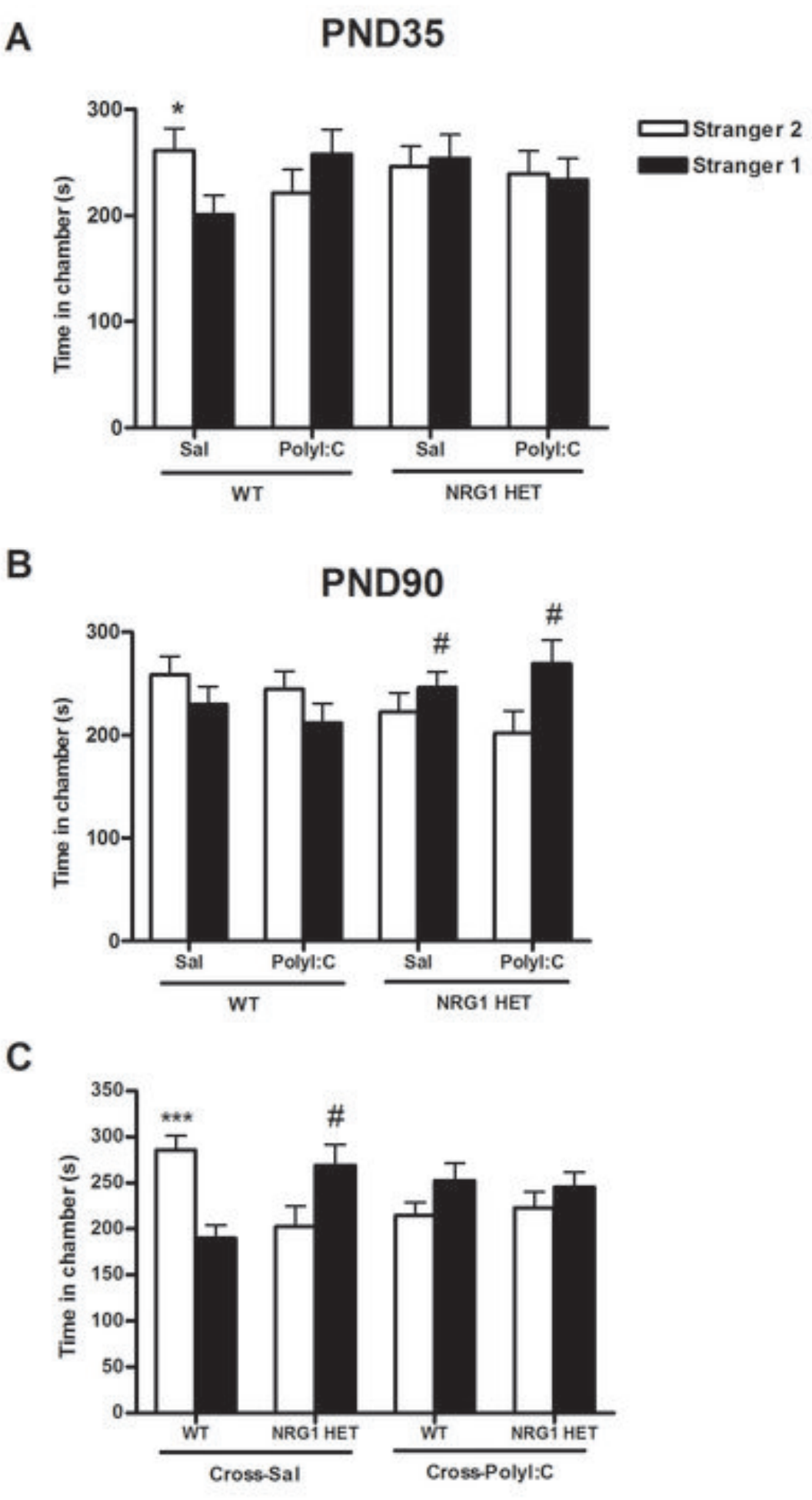
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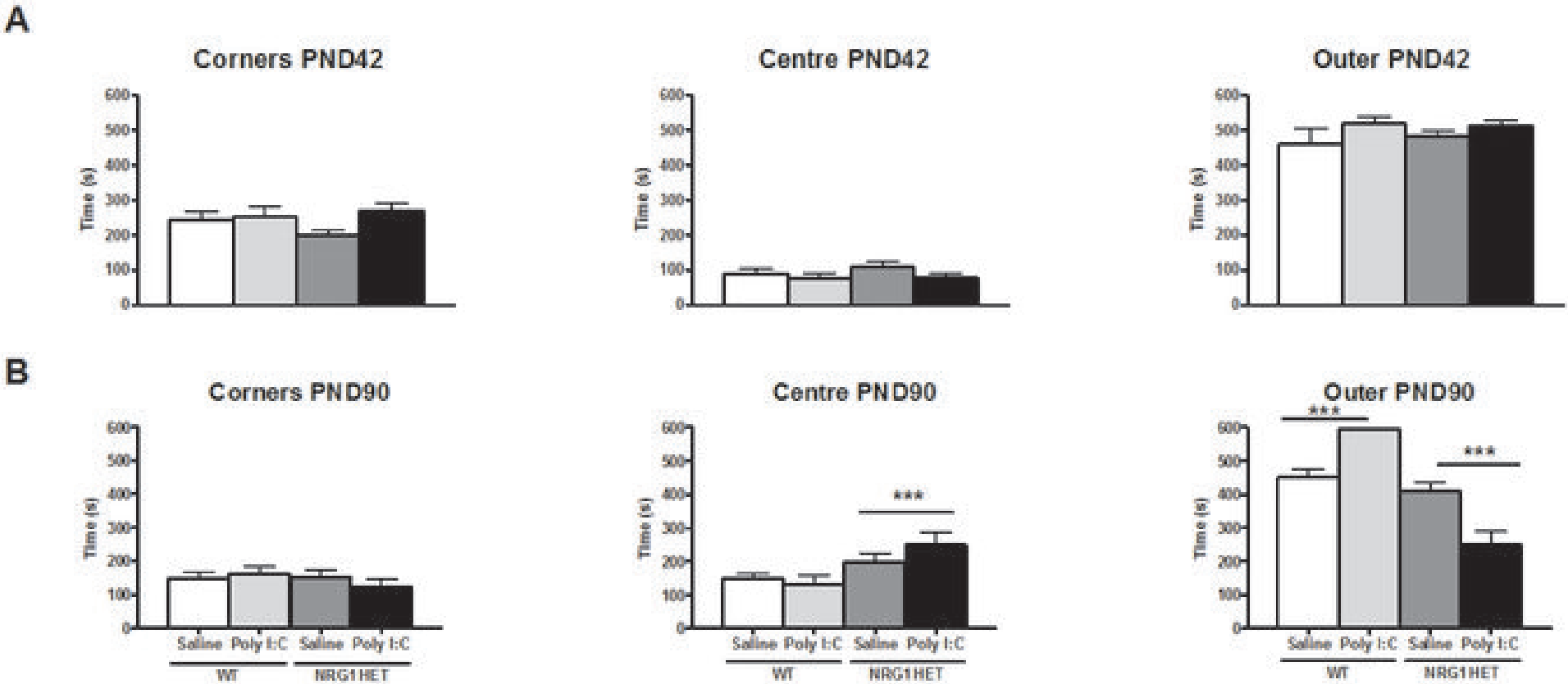
A
Corners PND42
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Saline
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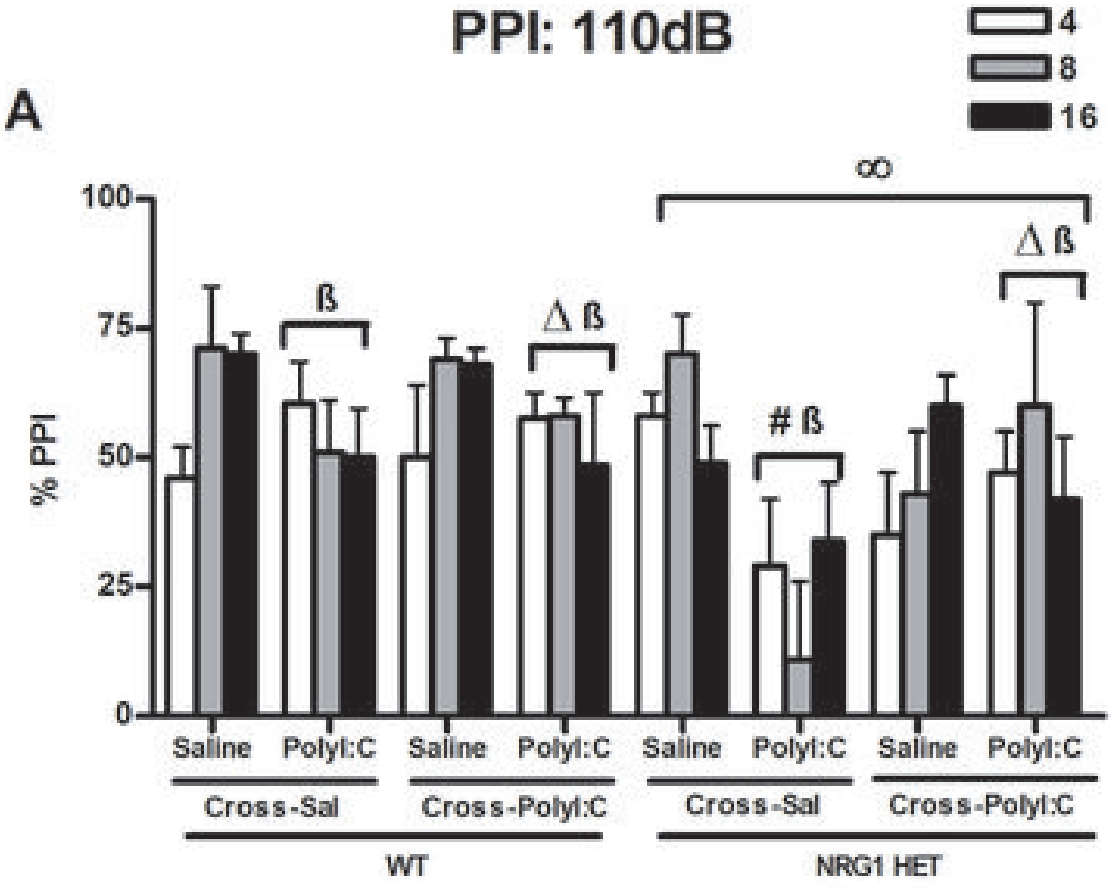
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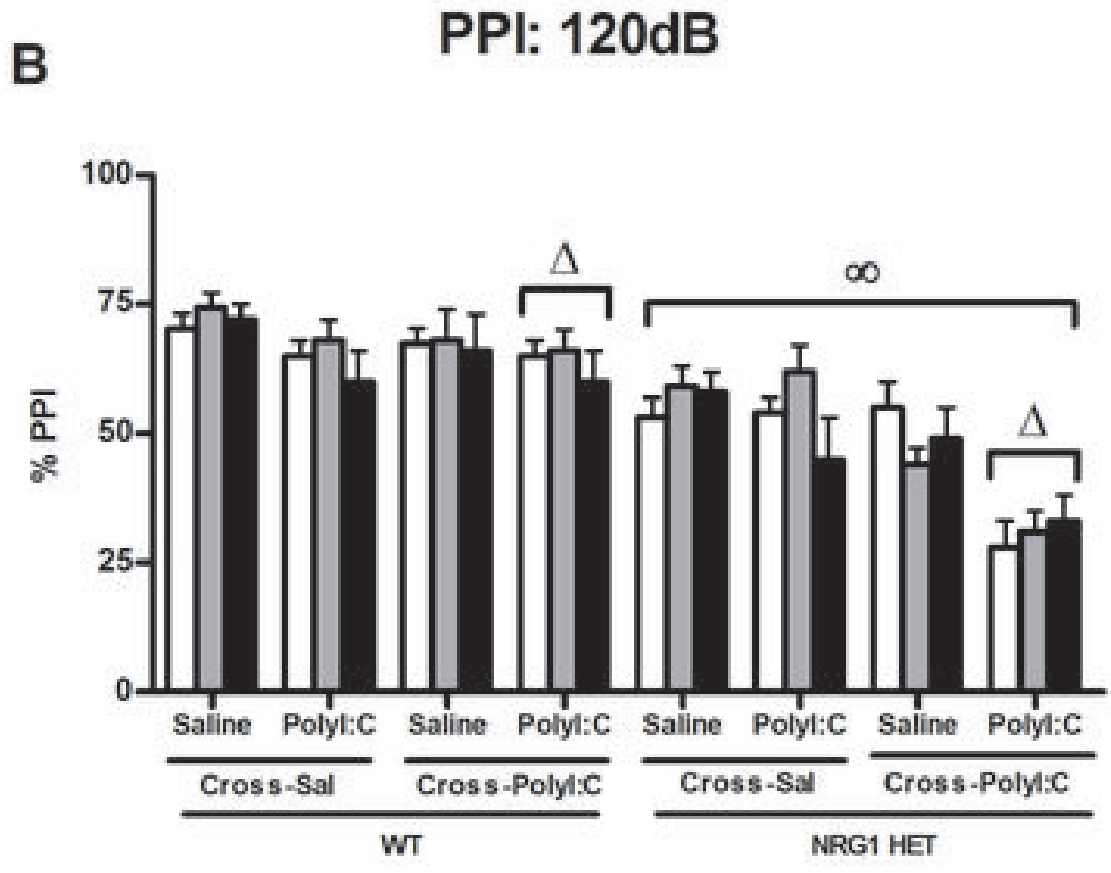
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Saline
Polyl:C
Saline
Polyl:C
Cross-Sal
Cross-Polyl:C
Cross-Sal
Cross-Polyl:C
WT
NRG1 HET

	Genotype			
	WT		NRG1 HET	
Cross-fostering condition	**Maternal immune stress condition**			
	Sal	Poly I:C	Sal	Poly I:C
Sal	5 ♀5 ♂	5 ♀5 ♂	6 ♀6 ♂	7 ♀5 ♂
Poly I:C	5 ♀5 ♂	5 ♀4 ♂	5 ♀8 ♂	5 ♀7 ♂

Phenotype	G	E_1	E_2	G × E_1	G × E_2	E_1 × E_2	G × E_1 × E_2
Open field							
Distance moved	–	–	–	–	–	–	–
Time outer	+[Adult]	–	–	–	–	+[Adol]	–
Time corners	–	–	–	–	–	+[Adult]	–
Time centre	–	–	–	–	–	–	–
Entries outer	+[Adult]	–	–	–	–	+[Adol]	–
Entries corners	+[Adol]	–	–	–	–	–	–
Entries centre	+[Adult]	–	–	+[Adult]	–	+[Adol]	–
Social behaviour							
Sociability	–	–	+[Adol]	+[Adult]	–	+[Adol]	–
Social novelty preference	+[Adult]	+[Adult]	–	–	+[Adult]	–	–
Y-maze							
Spontaneous alternation	+[b]	+	–	+[b]	–	–	–
Total arm entries	+[b]	–	–	–	+[b]	+[b]	–
Prepulse inhibition							
PPI pulse 110	+	+[a]	+[a]	–	–	+	+