Immune activation in lactating dams alters sucklings’ brain cytokines and produces non-overlapping behavioral deficits in adult female and male offspring: A novel neurodevelopmental model of sex-specific psychopathology

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Abstract

Early immune activation (IA) in rodents, prenatal through the mother or early postnatal directly to the neonate, is widely used to produce behavioral endophenotypes relevant to schizophrenia and depression. Given that maternal immune response plays a crucial role in the deleterious effects of prenatal IA, and lactation is a critical vehicle of immunological support to the neonate, we predicted that immune activation of the lactating dam will produce long-term abnormalities in the sucklings. Nursing dams were injected on postnatal day 4 with the viral mimic poly-I:C (4 mg/kg) or saline. Cytokine assessment was performed in dams’ plasma and milk 2 h, and in the sucklings’ hippocampus, 6 h and 24 h following poly-I:C injection. Male and female sucklings were assessed in adulthood for: a) performance on behavioral tasks measuring constructs considered relevant to schizophrenia (selective attention and executive control) and depression (despair and anhedonia); b) response to relevant pharmacological treatments; c) brain structural changes. Maternal poly-I:C injection caused cytokine alterations in the dams’ plasma and milk, as well as in the sucklings’ hippocampus. Lactational poly-I:C exposure led to sex-dimorphic (non-overlapping) behavioral abnormalities in the adult offspring, with male but not female offspring exhibiting attentional and executive function abnormalities (manifested in persistent latent inhibition and slow reversal) and hypodopaminergia, and female but not male offspring exhibiting despair and anhedonia (manifested in increased immobility in the forced swim test and reduced saccharine preference) and hyperdopaminergia, mimicking the known sex-bias in schizophrenia and depression. The behavioral double-dissociation predicted distinct pharmacological profiles, recapitulating the pharmacology of negative/cognitive symptoms and depression. In-vivo imaging revealed hippocampal and striatal volume reductions in both sexes, as found in both disorders. This is the first evidence for the emergence of long-term behavioral and brain abnormalities after lactational exposure to an inflammatory agent, supporting a causal link between early immune activation and disrupted neuropsychodevelopment. That such exposure produces schizophrenia- or depression-like phenotype depending on sex, resonates with notions that risk factors are transdiagnostic, and that sex is a susceptibility factor for neurodevelopmental psychopathologies.

1. Introduction

Epidemiologic studies show that early exposure to environmental adversities such as infection increases the risk for many adult-onset psychopathologies including schizophrenia and depression (Bale et al., 2010; Brown, 2011; Goodwin, 2011; Kneeland and Fatemi, 2013; Koponen et al., 2004; Machon et al., 1997). The
epidemiological association in humans has been supported by animal studies demonstrating that early-life infection/immune activation, either prenatal (corresponding to first and second trimester of human pregnancy (Clancy et al., 2007; Semple et al., 2013)) or early postnatal (corresponding to the third trimester of human pregnancy (Clancy et al., 2007; Semple et al., 2013)) produces in the offspring long-term behavioral, brain, and immune abnormalities relevant to schizophrenia and depression (Abazyan et al., 2010; Bilbo and Schwarz, 2012; Doosti et al., 2013; Fatemi et al., 2009; Garay et al., 2013; Harvey and Bokska, 2012; Khan et al., 2014; Meyer and Feldon, 2009; Meyer et al., 2005; Nawa and Takei, 2006; Piontkewitz et al., 2011a; Pletnikov et al., 2002; Rana et al., 2012; Romero et al., 2007; Zuckerman et al., 2003).

Studies of prenatal exposure have illuminated the crucial role of maternal immune response in the deleterious effects of infection/immune activation by demonstrating that induction of maternal cytokines leads to increased cytokine levels in the placenta/aminotic fluid and the fetal brain, where it likely interferes with normal development (Arrode-Bruses and Bruses, 2012; Bilbo and Schwarz, 2012; Garay et al., 2013; Gilmore and Jarskog, 1997; Gilmore et al., 2005; Kneeland and Fatemi, 2013; Meyer, 2013; Meyer et al., 2006; Urakubo et al., 2001). Surprisingly, although the mammary glands/milk link between mother and neonate in a similar manner to the placental link between mother and fetus, and lactation is a critical vehicle of immunological support to the developing neonate (Brandtzaeg, 2003; Field, 2005; Hosea Blewett et al., 2008; Field et al., 2003), so the observations span 3 periods, before, during, and after poly-C exposure. The observations were conducted in the vivarium by a “blind” observer who was able to view the rats from several angles without disturbing them. The following behaviors were scored: (1) mother off pups, (2) mother grooming/licking any pup or pups, (3) mother nursing pups in an arched-back posture, (4) mother nursing pups in a “blanket” posture in which the mother lays over the pups, (5) mother nursing pups in a passive posture in which the mother is lying either on her back or side while the pups nurse. The ongoing behavior of each dam was observed every 3 min for 5 s (20 observations per period X 4 periods a day = 80 observations per mother per day) and ticked on a checklist, and the total number of occurrences of each behavior were calculated (Caldji et al., 1998; Champagne et al., 2003; Myers et al., 1989)

2. Methods and materials

2.1. Animals and neonatal treatments

All experimental protocols conformed to the guidelines of the Institutional Animal Care and Use Committee of Tel Aviv University and NIH. Wistar rats (Tel-Aviv University Medical School) were kept under reversed cycle lighting (lights on: 1900–0700 h) with ad lib food and water. At about 3 months of age, female rats were extensively handled for 5 days and mated. Pregnant dams were housed individually, and the day of birth was defined as day zero. On postnatal day (PND) 4, whole litters were removed from the dams and placed in cages with clean bedding warmed with hot water bottles. While dams’ weight was recorded, their litters were culled to ten, composed of five females and five males when possible. Poly-I:C dissolved in saline (4 mg/kg/1 ml) or saline were injected intraperitoneally to the dams (lactational poly-I:C, LPIC) or subcutaneously (using a Hamilton syringe [10 μL; 30G needle], Hamilton Inc., Reno, NV) to pups (to whole litters; neonate poly-I:C, NPIC), and whole litters were returned to the mother. All injections were given between 15:00 and 17:00 h. Time away from the mother was less than 3 min per litter. On PND 21 pups were weaned and housed 2–4 to a cage by sex and litter, and maintained undisturbed until behavioral testing in adulthood at which time they were assigned to the experimental groups.

2.2. Maternal-neonatal interface

2.2.1. Maternal behavior

Dams and their pups were observed in their home cages on PNDs 2–7 for 60 min 4 times daily (06:00, 11:00, 16:00, 20:00). PNDs 2–7 were chosen because sickness behavior induced by systemic poly-I:C doses similar to that used in this study, lasts between 24 and 48 h (Cunningham et al., 2007; Gibney et al., 2013), so the observations span 3 periods, before, during, and after poly-C exposure. The observations were conducted in the vivarium by a “blind” observer who was able to view the rats from several angles without disturbing them. The following behaviors were scored: (1) mother off pups, (2) mother grooming/licking any pup or pups, (3) mother nursing pups in an arched-back posture, (4) mother nursing pups in a “blanket” posture in which the mother lays over the pups, (5) mother nursing pups in a passive posture in which the mother is lying either on her back or side while the pups nurse. The ongoing behavior of each dam was observed every 3 min for 5 s (20 observations per period X 4 periods a day = 80 observations per mother per day) and ticked on a checklist, and the total number of occurrences of each behavior were calculated (Caldji et al., 1998; Champagne et al., 2003; Myers et al., 1989)

2.2.2. Cytokine and corticosterone assessment in maternal milk and plasma

Two hours after poly-I:C/saline injection, dams were anesthetized [isoflurane (2.5% in 97.5% O2) followed by ketamine (100 mg/ml, i.p.) and xylazine (20 mg/ml, i.p.)] and injected s.c. with 4 IU oxytocin (Sigma, Israel) to stimulate milk flow. In order to minimize stress, pups were not separated from dams prior to milking resulting in a small amount of milk sufficient for the testing of no more than three substances. We chose corticosterone, and the cytokines IL-1β and IL-6, based on the known effects of poly-I:C (Cunningham et al., 2007; Gandhi et al., 2007; Kimura et al., 1994; Meyer et al., 2006), and their involvement in schizophrenia and depression (Dowlati et al., 2010; Khandaker et al., 2015). Milking was done manually. Milk samples were kept on ice until centrifuged at 14,000 rpm for 30 min at 4 °C. Immediately after milking, two ml of blood were withdrawn by cardiac puncture using...
EDTA-containing syringes (1.8 mg/1 ml blood). Samples were kept on ice until centrifuged at 2000g for 20 min at 4 °C. The aqueous fractions of the milk and plasma were collected and stored at –80 °C until assayed. Levels of corticosterone and cytokines were measured using commercially available ELISA kits (Assaypro or Quantikine R&D systems kits, respectively) according to the manufacturer’s instructions with the exception that milk samples were run undiluted. All standards and samples were run in duplicates.

2.2.3. Cytokine assessment in hippocampal tissue of sucklings
Sucklings were rapidly decapitated 6 h (PND4) or 24 h (PND5) following lactational poly-L-C exposure. Whole brain was extracted and placed in Leibovitz L-15 medium (Sigma, Israel) and both hippocampi were immediately dissected, rapidly frozen on dry ice and stored at –80 °C until further processing. Tissues were processed using the Bio-Rad cell lysis kit (Bio-Rad, California, USA) according to the manufacturers’ instructions. Total protein content was determined using the Bio-Rad protein protein (Bio-Rad) according to the manufacturer’s instructions and adjusted to 1000 μg/ml. Samples were then diluted 1:2 in Bioplex sample diluent (Bio-Rad, California, USA) containing 0.5% BSA (Sigma, Israel) and stored at –80 °C until the cytokine assay was carried out. IL-1β, IL-4, IL-6, TNF-α and IFN-γ concentrations were determined using a Bio-Plex rat cytokine group 1 5-plex Assay (Bio-Rad, California, USA) according to the manufacturer’s instructions.

2.3. Adult phenotyping
Adult phenotyping begun at PND 90 and included assessment of schizophrenia- and depression-phenotypic behaviors; assessment of structural brain changes using in vivo MRI; and pharmacological characterization.

2.3.1. Behavior
For a full description, see Supplement [1.2].

2.3.1.1. Latent inhibition (LI) in conditioned emotional response (CER) procedure with weak or strong conditioning. In the LI task, animals receiving nonreinforced stimulus preexposure show subsequently poorer conditioning to this stimulus compared to a novel (non-preexposed) stimulus, but this attentional bias to less effectively process old inconsequential stimuli is overcome with training, so that pre-exposed rats condition as efficiently as their non-pre-exposed counterparts, ceasing to display LI. Previous research has shown that pharmacological, physiological, and neurodevelopmental manipulations can produce two types of LI deficit depending on the status of LI in the non-manipulated controls: 1) disruption of LI when parameters of preexposure and conditioning that produce the LI effect in non-manipulated controls are used, or 2) abnormal persistence of LI when parameters of preexposure and conditioning that abolish the LI effect in non-manipulated controls are used. In psychological terms, disrupted LI is considered to reflect attentional/cognitive over-switching and distractibility relevant to positive symptoms, whereas persistent LI is considered to reflect attentional/cognitive perseveration relevant to negative/cognitive symptoms. (Weiner, 2003; Weiner and Arad, 2009). Paradigmatic manifestations are amphetamine-induced LI disruption and MK-801-induced LI persistence.

LI was conducted in a conditioned emotional response (CER) procedure with water as reinforcement as described previously (Gaisler-Salomón and Weiner, 2003; Zuckerman et al., 2003). After 5 days of handling and 5 days of training to drink in the experimental chambers, water deprived rats underwent four stages given 24 h apart: 1) Pre-exposure. With the bottle removed, pre-exposed (PE) rats received 40 tones (10 s; 80 dB, 2.8 kHz) 40 s apart, whereas non-pre-exposed (NPE) rats were merely confined to the chamber. 2) Fear conditioning. NPE and PE rats received either two (weak conditioning) or five (strong conditioning) tone-shock (0.5 mA, 1 s) pairings. 3) Lick retraining. 4) Test. Rats were allowed to complete 75 licks, whereupon the tone was presented, and the time to complete the next 25 licks was recorded. Intact animals show LI, namely, poorer fear conditioning (significantly shorter times to complete licks 76–100) of the PE compared to NPE rats, with two tone-shock pairings, whereas with five pairings, LI is abolished, so that PE animals condition as effectively as the NPE. The former allows demonstration of disrupted LI whereas the latter allows the demonstration of persistent LI (Barak et al., 2009a; Gaisler-Salomón et al., 2008; Gaisler-Salomón and Weiner, 2003). It is important to note that animals which show persistent LI with five conditioning pairings, also show LI with two conditioning pairings; therefore, presence of LI with two conditioning trials does not allow to conclude whether LI is unaffected by the experimental manipulation or is an undisclosed persistent LI, unless animals are tested with five conditioning trials.

2.3.1.2. Discrimination reversal (DR). In this task, animals associate responses to two different stimuli/locations with the presence or absence of reinforcement, and then must adapt their response when those contingencies reverse. Impaired reversal is considered to reflect impaired executive control, a cardinal neuropsychological deficit in schizophrenia (Barak and Weiner, 2011; Gilmour et al., 2013). DR was conducted in a T-maze submerged in a water pool. Animals were trained for 3 days on a left-right position discrimination with the choice of correct arm reinforced by escape onto an invisible platform. On day 4 they were retrained on the previously acquired discrimination, and then trained on the reversal of this discrimination, i.e., with the platform located in the opposite arm. In each session, animals were trained to a criterion of 5 consecutive correct responses.

2.3.1.3. Forced swim test (FST). In the FST rodents placed in an inescapable cylinder of water eventually stop active escape behaviors and develop immobility, and this behavioral passivity and quiescence, or “despair” (Porsolt et al., 1978), in response to uncontrollable stress is considered to mimic negative affect, a cardinal feature of depression (Berton et al., 2012; Cryan et al., 2005). FST was conducted as described previously (Weiner et al., 2003). Rats were placed individually in glass cylinders (height 50, diameter 20 cm) filled with water (23 ± 1 °C) for 15 min on day 1, and again for 5 min 24 h later (test). Duration of immobility (no movement other than what is necessary to keep the head above water) was recorded.

2.3.1.4. Saccharine preference test (SPT). The SPT is based on the natural preference of rodents for a sweet solution over water, and decreased preference is considered to mimic anhedonia (decreased ability to experience pleasure/reduced sensitivity to reward), a core affective state of depression (Berton et al., 2012). Rats were given free access to two 200 ml bottles containing tap water for 3 days (habituation period) followed by 3 days with free access to two bottles containing tap water or saccharin solution (0.1%; Sigma, Israel). Percentage saccharin preference (%) was calculated as 100 × saccharin consumption/total liquid consumption.

2.3.1.5. Amphetamine-induced activity (AIA). Increased locomotor response to amphetamine is considered to mimic the exacerbation of psychotic symptoms in response to amphetamine (Laruelle et al., 1996). AIA was measured as described previously (Piontkiewitz et al., 2011a). Rats were placed in camera-equipped dark grey boxes connected to a computer-run image analysis software that...
“grabbed” an image from each box every second. After 30-min habituation, they were injected with amphetamine (1 mg/kg/ml), and replaced into the boxes for 60 min. Percentage of pixels that went from dark to light or vice versa from 1 s to the next provided the measure of animal’s activity (activity counts). One-second activity values ranged from 0% to approximately 7.5%.

2.3.2. Pharmacological characterization

Pharmacological assessment was performed on LI with strong conditioning in lactational poly-I:C males and FST in lactational poly-I:C females. These tasks were used because they are well characterized pharmacologically in relation to cognitive/negative symptoms and depression, respectively (Cryan et al., 2005; Weiner and Arad, 2009). The following drugs were tested: the typical antipsychotic drug (APD) haloperidol (Johnson & Johnson, Belgium); an ampoule containing 5 mg haloperidol in 1 ml solvent containing 6 mg lactic acid diluted with saline to a dose of 0.1 mg/kg/ml; the atypical antipsychotic clozapine (Novartis, Switzerland; dissolved in 1 N acetic acid (15 μl/mg) and diluted to a dose of 2.5 mg/kg/ml); the NMDA receptor agonist glycine (Sigma-Aldrich, Israel; dissolved in saline to a dose of 800 mg/kg/3 ml); and the SSRI anti-depressant paroxetine (Unipharm, Israel; dissolved in sterile water (0.5 ml/7 mg) and diluted with saline to a dose of 7 mg/kg/ml. Drugs were administered intraperitoneally, 30 (clozapine, glycine and paroxetine) or 60 (haloperidol) min prior to pre-exposure and conditioning sessions of the LI procedure or prior to test session of the FST procedure. No-drug controls received the appropriate vehicle. The drugs were chosen on the basis of previous preclinical studies with FST and MK-801-induced persistent LI (Barak et al., 2009a; Black et al., 2009; Cryan et al., 2005; Dalla et al., 2010), as well as the clinical pharmacology of schizophrenia and depression (Attard and Taylor, 2012; Hirschfeld, 2012; Kandtowitz and Javitt, 2012; Kudlow et al., 2012; Melzter, 2012; Yang and Svensson, 2008), whereas the specific doses were chosen on the basis of their demonstrated effects on LI persistence and immobility in FST in our previous studies (Gaisler-Salomon et al., 2008; Gaisler-Salomon and Weiner, 2003; Weiner et al., 2003). Based on these studies, we expected reversal of persistent LI with clozapine and glycine but not haloperidol or paroxetine, and reduction of immobility with paroxetine and clozapine but not haloperidol or glycine.

2.3.3. In-vivo sMRI

Animals were scanned on a 7.0 T/30 cm (Bruker, Rheinstetten, Germany) with a volume coil for excitation and a rat quadrature coil for acquisition as described (Piontkewitz et al., 2011a,b). The scans were carried out under 1% isoflurane (Nicholas Piramal, UK) in 98% O2 anesthesia and lasted 30 min.

Coronal T2-weighted images of the brain were acquired with RARE sequence, repetition time = 3000 ms and echo time = 49 ms, RARE factor 8, 4 averages, field of view of 3 × 3 cm, matrix dimensions of 256 × 128 (zero filled to 256 × 256) and 18 coronal slices of 1 mm thickness without gap. The 18 coronal sections used for image analysis were taken perpendicular to a line connecting the superior end of the olfactory bulb with the anterior line of the cerebellum. The areas of the lateral ventricles (LV), hippocampus (HIP), prefrontal cortex (PFC), striatum (STR), and whole brain (WB) were obtained from the T2-weighted images with manual segmentation (Medical Image Analysis version 2.4, MATLAB®). LV, HIPP, PFC, STR and WB volumes were calculated by combining all slices where they appeared (approximately 2.20 mm to −4.52 mm; −2.2 mm to −6.2 mm; 1.2 mm to −4.2 mm; 2.2 mm to −1.2 mm; and 6.7 mm to −9.3 mm from Bregma, respectively) and multiplied by slice thickness (1 mm). The anatomical borders used to draw the contour around the four regions are described in Piontkewitz et al. (2011a). High intra-rater reliability (r_incs > 0.9) was obtained for all the MRI-derived volumetric assessments.

2.4. Experimental design

The study was conducted in four rounds (see Fig. 1). Adult phenotyping was conducted in three rounds. In Round 1, female and...
male rats that received lactational or neonate poly-I:C exposure underwent LI in CER with weak conditioning, DR, FST, AIA and sMRI. Since lactational poly-I:C rats had intact LI with weak conditioning, whereas neonate poly-I:C rats showed unusual pattern of LI disruption, in Round 2, lactational poly-I:C rats underwent LI with strong conditioning, to search for persistent LI, as well as a second depression-relevant task, SPT, whereas neonate poly-I:C rats underwent LI in a different procedure, two-way avoidance, to replicate the CER disruption pattern. Female and male offspring were used in all the experiments of rounds 1 and 2. In Round 3 pharmacological characterization was performed in lactational poly-I:C rats on persistent LI in males and FST in females. After the completion of adult phenotyping, we assessed in Round 4 maternal behavior, corticosterone/cytokine levels in the dams’ milk and plasma, and hippocampal cytokine levels in sucklings following lactational poly-I:C. The offspring used for adult phenotyping were derived from 30 poly-I:C (10 NPIC; 20 LPIC) and 26 saline (9 NPIC; 17 LPIC) litters. The sucklings were derived from 9 poly-I:C and 8 saline litters. To control for litter effects, no more than 1–2 rats from the same litter were included in any of the experimental groups. Assessments of the dams used 36 poly-I:C and 33 saline injected dams (See Tables S1, S2, and S3 for n/per group in each experiment).

2.5. Statistical analyses

Data were analyzed with two- (immune activation × sex for all behaviors except LI and imaging; immune activation × time for maternal behavior) or three- (immune activation × sex × preexposure for LI; immune activation × sex × time for hippocampal cytokine levels) way ANOVAs, with repeated-measurement factors where appropriate (stage [reversal], blocks [AIA], days [SPT], time [maternal behavior]). For AIA data, separate ANOVAs were used for spontaneous and AMPH-induced activity. Times to completelicks 76–100 in CER were logarithmically transformed. Significant interactions were followed by least significant difference (LSD) post hoc comparisons. Corticosterone and cytokine levels in dams’ plasma and milk were analyzed using t-tests.

3. Results

3.1. Effects of neonate poly-I:C exposure

3.1.1. Adult phenotyping

3.1.1.1. Behavior. Poly-I:C-injected neonates of both sexes failed to show LI in CER in adulthood, which was due to poor learning of the NPE rats, and the same pattern was obtained using avoidance procedure to measure LI, pointing out to a deficit in fear conditioning in these rats. Performance in the FST, reversal and AIA was unaffected (see Supplement for Results S2.1–2.4 and Discussion S3).

3.1.1.2. Structural brain changes. Poly-I:C-injected neonates of both sexes had smaller WB volumes than their same-sex controls but no differences in the volumes of the selected regions (see Supplement for Results S2.5 and Discussion S3).

3.2. Effects of lactational poly-I:C exposure

3.2.1. Adult phenotyping

3.2.1.1. Behavior. 3.2.1.1.1. LI in CER with weak or strong conditioning. Each LI experiment included eight groups in a 2×2×2 factorial design (immune activation × sex × pre-exposure). In both experiments, the eight groups did not differ in times to complete licks 51–75 prior to tone onset (p’s > 0.05; overall mean A period = 6.35 and 7.85 s).

3.2.1.1.2. DR. The experiment included four experimental groups in a 2×2 design with main factors of immune activation and sex. The four groups did not differ in the acquisition of the initial discrimination (Fig. 3 inset; ANOVA yielded no significant effects). Reversal slowed down all the groups, but in the poly-I:C offspring, a sex-specific impairment emerged, such that poly-I:C males required more trials to criterion compared to their sex-matched controls, whereas no difference was found in females (Fig. 3). ANOVA with a repeated measurement factor of stage (discrimination, reversal), yielded significant main effects of immune activation (F(1,26) = 6.3, p < 0.05) and stage (F(1,26) = 146.7, p < 0.0001), as well as significant interactions of immune activation × stage (F(1,26) = 4.89, p < 0.01) and preexposure × immune activation × stage (F(1,26) = 4.41, p < 0.05). Post-hoc comparisons confirmed significant difference between poly-I:C and saline-exposed males (p < 0.01), but not between poly-I:C and saline-exposed females.

3.2.1.1.3. FST. Lactational poly-I:C exposure did not affect immobility time in the male offspring compared to their sex-matched controls, but led to increased immobility time in female offspring compared to their sex-matched controls. ANOVA yielded a significant immune activation × sex interaction (F(1,46) = 7.31, p < 0.05). Post-hoc tests confirmed significant difference (p’s < 0.05) between immobility times of poly-I:C and saline exposed females but not males (Fig. 4).

3.2.1.1.4. SPT. The experiment included four experimental groups in a 2×2 design with main factors of immune activation and sex. Lactational poly-I:C exposure did not affect baseline water consumption, but led to sex-specific saccharine preference reduction whereby female, but not male, offspring had reduced preference compared to their sex-matched controls. ANOVA yielded a significant main effect of immune activation (F(1,28) = 18.3, p < 0.005), and a significant immune activation × sex interaction (F(1,28) = 20.96, p < 0.0001). Post-hoc comparisons confirmed significant difference (p < 0.01) in saccharine consumption between poly-I:C and saline females and no difference in males (Fig. 5).

3.2.1.1.5. AIA. The experiment included four experimental groups in a 2×2 design with main factors of immune activation and sex. Lactational poly-I:C exposure did not affect spontaneous activity (blocks 1–6; only significant effect of blocks, F(5,120) = 4.89, p < 0.05), but affected AIA (blocks 7–18) in a sex-dependent manner, with male offspring showing lower and female weak fear conditioning [lower lick suppression] of the PE as compared to the NPE group), was present in all saline- and poly-I:C-exposed offspring (Fig. 2A). ANOVA of the mean log times to complete licks 76–100 (after tone onset) of the eight groups yielded significant main effects of preexposure (F(1,54) = 8.69, p < 0.0001) and sex (F(1,54) = 51.13, p < 0.0001), reflecting higher suppression in females.

3.2.1.1.6. AIA. Poly-I:C-injected neonates of both sexes showed significantly reduced AIA compared to saline controls (Fig. 2B). ANOVA yielded a significant immune activation × sex interaction (F(1,5120) = 4.89, p < 0.05), but no difference between poly-I:C and saline-exposed females.
offspring showing higher AIA compared to their sex-matched controls. A $2 \times 2 \times 12$ ANOVA of blocks 7–18 (after amphetamine injection) yielded main effects of sex ($F_{(1,24)} = 195.49, p < 0.0001$), and blocks ($F_{(11,264)} = 44.5, p < 0.0001$), and interactions of immune activation $\times$ sex ($F_{(1,24)} = 22.9, p < 0.0001$), sex $\times$ blocks ($F_{(11,264)} = 26.37, p < 0.0001$), treatment $\times$ blocks ($F_{(11,264)} = 4.05, p < 0.0001$) and immune activation $\times$ sex $\times$ blocks ($F_{(11,264)} = 2.35, p < 0.01$). Post-hoc comparisons confirmed significant difference in activity scores between male poly-I:C and saline offspring on blocks 8–12, and between female poly-I:C and saline offspring on blocks 10–18 (all $p$'s $< 0.05$; Fig. 6).

3.2.1.2. Pharmacological dissociation between persistent LI and increased immobility in the FST. These experiments were designed to assess whether the two sex-specific phenomena are pharmacologically distinct as would be expected if they reflected different pathological domains.

3.2.1.2.1. Persistent LI in males. The experiment included twenty experimental groups in a $2 \times 2 \times 12$ design with main factors of pre-exposure, immune activation and drug (saline, haloperidol, clozapine, glycine, paroxetine). The 20 experimental groups did not differ in their times to complete licks 51–75 before tone onset (all $p$'s $> 0.05$; overall mean A period = 6.81 s). As before, with 5 conditioning trials, LI was absent in vehicle-treated controls, but present in vehicle-treated offspring exposed to lactational poly-I:C. Persistent LI in poly-I:C exposed offspring was reversed by clozapine and glycine but not by haloperidol. In addition, as was shown previously in intact animals [Barak and Weiner, 2009b; Black et al., 2009; Gaisler-Salomon and Weiner, 2003], all three drugs produced LI in controls. Paroxetine had no effect in controls as well as in poly-I:C offspring. These effects were supported by ANOVA of log times to complete licks 76–100 after tone onset, which
yielded a significant main effect of pre-exposure (F(1,93) = 43.68, p < 0.001) and significant immune activation × drug (F(4,457) = 8.99, p < 0.001) and pre-exposure × immune activation × drug (F(4,457) = 5.13, p < 0.001) interactions. Post hoc comparisons confirmed significant PE/NPE differences (p’s < 0.05) in vehicle-, haloperidol- or paroxetine-injected poly-I:C males as well as in control offspring injected with haloperidol, clozapine or glycine, but not in the other conditions (Fig. 7A).

3.2.1.2. FST in females. The experiment included ten groups in a 2 × 5 design with main factors of immune activation and drug (saline, haloperidol, clozapine, glycine, paroxetine). Adult female offspring exposed to lactational poly-I:C injected with vehicle spent more time immobile than their vehicle-injected saline exposed counterparts. Paroxetine and clozapine reduced immobility in both conditions. Haloperidol and glycine had no effect in poly-I:C exposed females and increased immobility in controls. ANOVA yielded significant main effects of immune activation (F(1,57) = 11.16, p < 0.01) and drug (F(4,457) = 54.77, p < 0.001), as well as a significant immune activation × drug interaction (F(4,457) = 2.96, p < 0.05). Post hoc comparisons confirmed significant differences (p’s < 0.01) between immobility times of vehicle-treated poly-I:C and saline offspring as well as of clozapine- and paroxetine-treated poly-I:C offspring (Fig. 7B).

3.2.1.3. Structural brain changes. Lactational poly-I:C-exposed rats of both sexes had smaller WB (Table S5), HIP (Fig. 8A-B) and STR volumes than their same-sex saline offspring as revealed in post hoc analyses.

3.2.2. Maternal-neonatal interface

3.2.2.1. Maternal behavior. The experiment included 2 groups of lactating dams (saline-injected: n = 9; poly-I:C-injected: n = 10). The groups did not differ in mean number of female or male pups, or pups per litter (p’s > 0.05, data not shown). ANOVA with maternal treatment × repeated measures factor of time (before, days 2–3; during, days 4–5; after, days 6–7 poly-I:C exposure) revealed that grooming/licking of the pups was higher in poly-I:C-injected dams but this was seen both before and after the injection (significant main effect of treatment, F(1,16) = 6.17, p < 0.05; treatment × time interaction not significant; Fig. 9A). Archedback nursing and time spent off the pups changed over time in both groups, with the former decreasing [main effect of time, F(2,34) = 17.43, p < 0.0001; Fig. 9B] and the latter increasing [main effect of time, F(2,34) = 9.26, p < 0.001; Fig. 9C]. No other differences in maternal behavior were revealed between poly-I:C and saline dams.

3.2.2.2. Maternal plasma and milk cytokines and corticosterone 2 h following poly-I:C injection. Poly-I:C injection led to a significant elevation of IL-1b, IL-6 and corticosterone in the plasma of the lactating dams [IL-1b: t(30) = 2.98, p < 0.01; IL-6: t(12) = 2.54, p < 0.05; corticosterone: t(27) = 3.39, p < 0.0001; Fig. 10A] as well as in
maternal milk [IL-1β: \( t_{(31)} = 4.35, p < 0.0001 \); IL-6: \( t_{(13)} = 4.84, p < 0.05 \); corticosterone: \( t_{(31)} = 3.20, p < 0.005 \) compared to saline injection; Fig. 10B].

3.2.2.3. Hippocampal cytokines in the sucklings 6 h and 24 h following maternal poly-I:C injection. The experiment included 8 groups in a 2 × 2 factorial design (immune activation × sex × time). Each cytokine was analyzed separately. There were no differences between the groups in hippocampal IL-1β or IL-4 levels. IL-6 and IFN-γ were elevated at both time points only in male poly-I:C offspring compared to their controls [IL-6: main effect of sex, \( F_{(1,55)} = 5.74, p < 0.05 \); main effect of immune activation, \( F_{(1,55)} = 4.63, p < 0.05 \); immune activation × sex interaction, \( F_{(1,55)} = 7.76, p < 0.01 \), and a significant difference in post hoc comparisons, \( p < 0.01 \); IFN-γ: sex × treatment interaction, \( F_{(1,56)} = 4.43, p < 0.05 \), and a significant difference in post hoc comparisons, \( p < 0.01 \); Fig. 11A and B]. Both male and female poly-I:C offspring had a lower hippocampal level of TNF-α 24 h, but not 6 h, following exposure to poly-I:C compared to saline offspring [treatment × time interaction, \( F_{(1,56)} = 6.78, p < 0.05 \) and significant differences in post hoc comparisons, \( p < 0.005 \); Fig. 11C].

4. Discussion

We provide the first demonstration that exposure of lactating dams to an immune stimulator leads to long-term behavioral and brain structural abnormalities in the nursing offspring of both sexes. Taken together with the well documented long-term effects of maternal immune challenge in pregnant dams, the present findings provide further support for a causal link between early maternal immune activation and disrupted neuropsychodevelopment, and extend the period of vulnerability to this risk factor to early postnatal period. Notably, the effects of lactational poly-I:C exposure were distinct from, and more extensive than, those of a direct poly-I:C injection to pups at the same dose and under identical conditions. The limited effect of neonate poly-I:C could be related to the depressed capacity of the neonatal innate immune system to produce pro-inflammatory cytokines in response to pathogens (Levy, 2007). Indeed, the regimens of neonate immune stimulation by means of direct poly-I:C or LPS injections typically consist of several days of injections (e.g., for poly-I:C, PNDs 2–6 (Hida et al., 2014; Ibi et al., 2009, 2010), and under such conditions, more extensive outcomes were obtained (Ibi et al., 2009), albeit not in all studies (Ibi et al., 2010) (see Supplement for results and discussion of neonate poly-I:C).

Remarkably, the behavioral endophenotypes emerging following lactational poly-I:C exposure are sexually dimorphic (i.e., non-overlapping), with male but not female offspring exhibiting cognitive impairment, and female but not male offspring exhibiting affective deficit. Specifically, male offspring of poly-I:C injected mothers but not their female littermates, exhibited abnormally persistent LI and slower reversal compared to controls, reflecting an impaired ability of poly-I:C exposed males to modify their behavior when previously learned stimulus-outcome relationships or rules are no longer relevant. Importantly, both persistent LI and slow reversal were not due to a learning deficit but rather to a selective deficit in cognitive flexibility since poly-I:C males did

Fig. 8. Brain structural changes in lactationally exposed adult male and female offspring. (A,C) Representative T2-weighted images at the level of the hippocampus (A) and striatum (C) from a saline (SAL) or poly-I:C (Poly)-exposed male offspring imaged at PND 120. (B,D) Mean ± SEM of hippocampus (HIP) and striatum (STR) volume of adult male and female offspring (n/group = 7). Asterisks denote a significant difference (\( * p < 0.05 \); \( ** p < 0.01 \)) between same-sex poly-I:C and saline offspring as revealed in post hoc analyses.
not differ from controls in the acquisition of fear conditioning and of the initial discrimination. The opposite pattern of sex-specific behavioral abnormalities emerged in the FST and SPT so that in these tasks, female but not male offspring were affected, with only females exhibiting increased immobility and decreased saccharine preference. Lactational poly-I:C also led to sex-specific abnormalities in response to dopaminergic stimulation, with hypo- and hyper-response to amphetamine in males and females, respectively. Low dopaminergic activity is well known to underlie behavioral and cognitive inflexibility (Kehagia et al., 2010), and is consistent with findings of persistent LI and retarded reversal in males, as dopaminergic blockade induces persistent LI (Feldon and Weiner, 1991; Weiner et al., 1997), as well as perseveration in reversal (De Steno and Schmauss, 2008; Izquierdo and Jentsch, 2012). Hyperdopaminergic response of females is less expected, but is consistent with findings showing that increase in phasic firing of VTA DA neurons (which mediates increased response to amphetamine), underlies a depressive phenotype after repeated social defeat stress in mice (Cao et al., 2010; Chaudhury et al., 2013).

While sex differences in various behaviors and pharmacological responses have been demonstrated in both intact and developmentally compromised rodents (Dalla et al., 2010; Joel and Yankelevitch-Yahav, 2014; Simpson and Kelly, 2012), to the best of our knowledge this is the first demonstration of doubly dissociated, non-overlapping behavioral phenotypes in the two sexes. Furthermore, since persistent LI and impaired reversal are considered relevant to cognitive inflexibility and impaired executive control in schizophrenia (Barak and Weiner, 2011), whereas increased immobility in the FST and reduced sweet solution preference are considered relevant to negative affect and reduced hedonic capacity characteristic of depression (Berton et al.,...
sex-specific behavioral phenotypes seen here may be seen as recapitulating the direction of the sex bias in schizophrenia and depression (Abel et al., 2010; Essau et al., 2010), or more in line with current thinking, as representing sex-biased domains of psychopathology (negative/cognitive vs affective) that can be trans-diagnostic or co-exist within the same disorder (Buckholtz and Meyer-Lindenberg, 2012; Insel et al., 2010). It should be noted in this context that the effect of sex found here is much more robust than in human psychopathologies, as here we have sex dichotomy (non-overlapping) rather than sex bias (overlapping). This may be because in laboratory animals, the genetic and environmental variables (age, stress, housing conditions, nutrition, drug exposure) that may interact with sex to affect brain and behavior, are strictly controlled, reducing the variability/heterogeneity of outcomes compared to human brains and behavior, thus allowing to reveal a “clean” effect of sex.

Our finding that the same neurodevelopmental insult led to schizophrenia- or depression-relevant “symptoms” is in line with the recently emphasized notion that risk factors are transdiagnostic (Buckholtz and Meyer-Lindenberg, 2012; Insel et al., 2010; Serretti and Fabbri, 2013; Smoller, 2013). However, how shared risk factors give rise to distinct psychopathologies remains perplexing. Our results show that sex can transform some common neurodevelopmental etiologies into distinct psychopathologies, in line with the notion that sex may constitute an important susceptibility factor for psychopathologies of developmental origin (Abel et al., 2010; Bale et al., 2010; McCarthy et al., 2012). Indeed, our results suggest that one reason for such sex-biasing is that neurodevelopmentally, males and females may be more prone to the development of cognitive and affective deficits, respectively, at least with some early risk factors (Walder et al., 2013).

Fig. 11. Hippocampal cytokines in the sucklings. Mean ± SEM levels of IL-6 (A), IFN-γ (B) and TNF-α (C) in hippocampi extracted from pups 6 h or 24 h following lactational saline or poly-I:C exposure (n per group = 8). *significant difference (p < 0.05) in cytokine levels between poly-I:C and saline exposed pups.

The relevance of the obtained sex-specific deficits to distinct domains of psychopathology was supported by the distinct pharmacological profiles of persistent LI and immobility in FST, which were consistent with pre-clinical and clinical pharmacology of negative/cognitive symptoms (Attard and Taylor, 2012; Barak and Weiner, 2011; Kantrowitz and Javitt, 2012; Meltzer, 2012; Yang and Svensson, 2008) and depression (Cryan et al., 2005; Hirschfeld, 2012; Kudlow et al., 2012). Specifically, increased immobility was alleviated by paroxetine and clozapine, in line with the known antidepressant activity of SSRIs (Hirschfeld, 2012; Kudlow et al., 2012) and atypical APDs (Chen et al., 2011) but not by haloperidol which lacks anti-depressant activity. This pattern of action of the three drugs on immobility has been reported in numerous FST experiments (Borsini et al., 1985; Gorka and Janus, 1985; Redrobe et al., 1998; Renard et al., 2001; Sanchez and Meier, 1997; Weiner et al., 2003). Glycine was ineffective in reducing immobility in poly-I:C offspring or in controls, indicating that NMDA receptor activation is devoid of anti-depressant activity. This is consistent with the dramatic anti-depressant action of NMDAR antagonists like ketamine (Li et al., 2015; Zarate et al., 2006; Zhang et al., 2014).

Persistent LI was unaffected by paroxetine, consistent with the inefficacy of antidepressants in schizophrenia (Ballon and Stroup, 2013), but reversed by glycine, in line with studies suggesting a beneficial influence of NMDA receptor agonists on cognitive/negative symptoms (Ermilov et al., 2013; Goff et al., 1995; Heresco-Levy et al., 1999; Kantrowitz and Javitt, 2012; Kantrowitz et al., 2016; Krystal et al., 2003; Yang and Svensson, 2008; Singh and Singh, 2011), as well as by clozapine but not by haloperidol, in line with reports suggesting higher efficacy for clozapine than haloperidol (Agid et al., 2010; Attard and Taylor, 2012; Leucht et al., 2013; Meltzer, 2012; Miyamoto et al., 2012; Takeuchi et al., 2013). The pharmacological profile of lactational poly-I:C-induced persistent LI in males is identical to that previously found for MK801-induced persistent LI (Agid et al., 2010; Attard and Taylor, 2012; Leucht et al., 2013; Meltzer, 2012; Miyamoto et al., 2012; Takeuchi et al., 2013).
many site studies, the only stage III trial was not successful (Beck et al., 2016). Likewise, the superiority of clozapine is continuously debated, with some experts endorsing its superiority (Leucht et al., 2013; Miyamoto et al., 2012), while others not (Fusar-Poli et al., 2015). This translational gap requires caution in interpreting the pharmacological profile of persistent LI as having relevance to cognitive/negative symptoms.

We used paroxetine and glycine expecting to pharmacologically differentiate between persistent LI and increased immobility (by sex). Considering the known mechanisms of action of these two compounds reveals a possible difference in the underlying neurotransmitter dysfunction in lactational poly-1C-exposed males and females. Glycine is an obligatory co-agonist (with glutamate) for NMDA receptor activation. Administration of glycine increases the availability of glycine at modulatory site on the NMDA receptors, enhancing NMDA receptor neurotransmission (Black et al., 2009; Cummings and Popescu, 2015). Thus, the efficacy of glycine in lactational poly-1C-exposed males suggests a hypofunction of glutamateergic neurotransmission via NMDA receptors in these animals, as has been postulated for schizophrenia (Coyle, 1996; Javitt and Zukin, 1991; Lencz and Malhotra, 2015; Marsman et al., 2013; Mouchlianitis et al., 2016). Regarding paroxetine, the main action of SSRIs is increasing extracellular serotonin concentrations, and promoting serotoninergic neurotransmission (Kohler et al., 2016). The efficacy of paroxetine in lactational poly-1C exposed females suggests that serotonin levels in these animals are low, in line with an underlying serotonin deficiency believed inherent to major depression (Kambeitz and Howes, 2015; Kohler et al., 2016; Morissette and Stabl, 2014). Taken together with sex-specific dopaminergic dysfunction as reflected in the effects of amphetamine on activity, the pharmacological outcomes with glycine and paroxetine further support the presence of sex-specific neurotransmitter dysfunctions following lactational poly-1C exposure that are likely to underlie the sex-specific behavioral deficits.

Unlike the sex-specificity of the behavioral and pharmacological consequences, in vivo imaging revealed an identical phenotype in male and female offspring of poly-1C-injected lactating dams, namely, reduced hippocampal and striatal volumes. This is consistent with the clinical picture where hippocampal and striatal volume reductions are seen in both schizophrenia (Kempton et al., 2001; McClure et al., 2013; Marsman et al., 2013) and depression (Drevets, 2001; Kempton et al., 2011; Olabi et al., 2011) as well as with animal studies showing that perturbations of both regions (Drevets, 2001; Kempton et al., 2011; Olabi et al., 2011) as well as with animal studies showing that perturbations of both regions can produce behavioral abnormalities seen here in the two sexes (Clarke et al., 2008; Der-Avakian and Markou, 2012; McDonald et al., 2002), but raises a question regarding structure-function relationship. It is possible that sex-specific structural changes exist in regions that were not assessed here (e.g., amygdala), in the microstructure of the regions assessed (Bakhshi and Chance, 2015), or in their developmental trajectories, as shown by us for gestational maternal immune activation (Piontkewitz et al., 2011a). Alternatively, it is possible that a similar neural pathology leads to sex-specific behavioral abnormalities due to sex differences in the neural circuits/substrates of these behaviors (de Vries and Sodersten, 2009).

Of interest in this context is the fact that structural deficits are sex-independent whereas neurotransmitter deficits are sex-specific. One interpretation of this difference is that volumetric reductions are a manifestation of a behaviorally nonspecific susceptibility conferred by the early risk factor that can predispose to multiple behavioral pathologies, whereas the distinct (sex-specific) nature of the behavioral pathologies is determined by other processes, such as different neurotransmitter dysfunctions. Importantly, sex is likely to be one of the major factors contributing to such differentiation because sex hormones can modulate all brain neurotransmitters (Barth et al., 2015; Gillies et al., 2017). For example, the well-established capacity of estrogen to regulate/modulate serotonin pathways, has been hypothesized to contribute to the heightened vulnerability to depression in women compared to men (Lokuge et al., 2011; Gillies and McArthur, 2010). We would further speculate that developmentally, structural reductions precede the emergence of neurotransmitter dysfunctions, the latter emerging together with behavioral abnormalities during adolescence, which is a highly sensitive period for the sex-specific effects of sex hormones on brain and behavioral development (Schulz and Sisk, 2016).

The mechanisms by which lactational poly-1C exposure “programs” suckling’s brain development in general, and in a sex-dependent manner in particular, remain to be investigated. Here we tested two potential mediating factors, maternal behavior and maternal immune response. Because poly-1C injection produces sickness behavior (Cunningham et al., 2007; Gibney et al., 2013), changes in maternal behavior could play a role in the effects observed here. However, we did not observe any effects of poly-1C on maternal behavior, although we did observe changes related to day of observation, in accordance with previous studies (Champagne et al., 2003), indicating that our observations were sensitive to changes in maternal behavior. It should be noted that studies reporting changes in maternal behavior typically use some type of interference (e.g. scattering the pups or introduction of new pups) to elicit such changes (Kosten and Nielsen, 2014; Moore and Morelli, 1979; Pan et al., 2014) whereas here animals were totally undisturbed during the observations, a condition that reduces the frequency and thus the detection of changes in maternal behaviors (Moore and Morelli, 1979). The only study we found that assessed the effects of immunostimulation in lactation (Aubert et al., 1997) is consistent with our findings as it showed that LPS in lactating mice impaired the quality of nest-building, but pup-retrieval, feeding and care including contact with the sucklings, remained unchanged.

Our focus on maternal immune response was based on findings that induction of maternal cytokines in the pregnant dam alters cytokine levels in the placenta and the fetal brain, which may interfere with normal brain development (Arrode-Bruses and Bruses, 2012; Bilbo and Schwarz, 2012; Garay et al., 2013; Gilmore and Jarskog, 1997; Kneeland and Fatemi, 2013; Meyer, 2013; Meyer et al., 2006; Urakubo et al., 2001). We hypothesized that a similar process would occur following lactational immune activation, namely, maternal production of cytokines would alter cytokine levels in the milk and the neonatal brain. We found that maternal poly-1C injection elevated cytokine (IL-1β and IL-6) and corticosterone levels in the lactating dams’ plasma, as well as in their milk. Furthermore, induction of maternal cytokines was followed by altered cytokine expression in the neonatal brain, hours after MIA, as was found in the fetal brain following gestational poly-1C (Arrode-Bruses and Bruses, 2012; Meyer et al., 2006). Specifically, lactational exposure to poly-1C led to elevated INF-γ and IL-6 levels in males 6 and 24 h after maternal poly-1C injection, and decreased TNF-α levels in both sexes 24 h after poly-1C injection.

There is evidence that elevation of cytokines occurs in milk following maternal infection or vaccination (Bannerman et al., 2003; Lanari et al., 2012; Lepage and Van de Perre, 2012; Wockel et al., 2008). Furthermore, it has been shown in mice that until PND8, maternally derived cytokines can cross the intestinal barrier into the neonates’ blood circulation and become functionally active in the neonates’ tissues (Aspinall et al., 2011). However, to the best of our knowledge, this is the first demonstration that maternal immune activation during lactation leads to increased cytokine levels in the milk and in the suckling’s brains. These results support the continuity between maternal-fetal and maternal-neonatal immune-neural interfaces, as well as the key role of altered mater-
nal and fetal/neonatal cytokines in mediating the link between maternal inflammation during pregnancy and lactation and abnormal brain development in the offspring.

Notably, the time of poly-I:C administration in the present study coincides with a postnatal period of largest sex differences in brain microglia colonization (Schwarz et al., 2012), which could explain sex differences in brain cytokine alterations found here, as well as in steroid levels (Konkle and McCarthy, 2011), and neurogenesis (Bowers et al., 2010). All of these differences could play a role in the sex-specific programming by lactational poly-I:C. However, our findings that direct neonate poly-I:C exposure did not lead to sex-specific behavioral phenotypes, and that the sex-specific effects of lactational exposure itself were restricted to some aspects of development, imply that the sex-specific behavioral phenotypes are not a result of sex-specific characteristics of the brain per se, but rather of their interaction with specific effects exerted by the early environmental insult as well as with other internal and external variables throughout development.

Finally, it is worth noting that the behavioral phenotype that emerged in the offspring exposed to lactational poly-I:C is totally different from the phenotype we described following gestational poly-I:C on GD 15, the latter including disrupted LI, faster reversal and enhanced AIA, all abnormalities modeling positive symptoms of schizophrenia, in both sexes (Piontkewitz et al., 2011a; Zuckerman et al., 2003; Zuckerman and Weiner, 2005). These findings extend those in mice showing that the nature of the behavioral consequences of maternal immune challenge in gestation emerging in the offspring depends on the challenge timing (early or late gestation; Bitanhirwe et al., 2010; Fortier et al., 2007; Meyer et al., 2006, 2008). Thus, the examination of the structural and functional consequences of poly-I:C-induced immune challenge at different developmental periods may reveal neuropathological mechanisms underlying the segregation of different symptom domains, either within the same disorder (Meyer and Feldon, 2012), or across different disorders (here).

4.1. Conclusion

Lactational immune activation provides a novel neurodevelopmental model in which a common risk factor produces sex-specific non-overlapping phenotypes of schizophrenia and depression recapitulating the known sex-bias in these disorders. We suggest that lactational poly-I:C exposure in female and male rats is a powerful experimental tool to induce and study the trajectories of neuropathological processes relevant to both disorders, as well as of neuropathological mechanisms underlying the segregation of depressive and negative/cognitive symptoms of schizophrenia, within the same model. In particular, the model enables to elucidate how neurobiological sex differences at various points in development interact with other biological and/or environmental factors to confer risk and resilience for the two disorders, and thus to identify novel treatment and/or early intervention targets, including trans-diagnostic prevention. Finally, the model exquisitely addresses the broadly acknowledged need to include sex as a variable in preclinical research.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.bbi.2017.01.015.

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