Università degli Studi di Cagliari

DOTTORATO DI RICERCA IN NEUROSCIENZE

Ciclo XXIX

EFFECTS OF PPAR-ALPHA ACTIVATION IN AN IMMUNE-MEDIATED NEURODEVELOPMENTAL MODEL OF SCHIZOPHRENIA

Settore/i scientifico disciplinari di afferenza

BIO/14 - Farmacologia

Presentata da: Marta De Felice

Coordinatore Dottorato Prof. Antonio Argiolas

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Esame finale anno accademico 2015 – 2016

Tesi discussa nella sessione d'esame marzo – aprile 2017

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1. INTRODUCTION

1.1. Schizophrenia

Schizophrenia is a complex neuropsychiatric illness, which strongly impacts the quality of life and social integration. The onset of the disorder usually occurs in early-adulthood and it is diagnosed evaluating the family history and clinical symptoms (Owen et al., 2016). In fact, no diagnostic tests currently exist and the overlapping of signs between schizophrenia and other diseases might make difficult to identify it. In the latest edition of Diagnostic and Statistical Manual of Mental Disorders (DSM-5), "*spectrum*" has been added to schizophrenia, including the range of those disorders with subtle differences in symptomatology. Six criteria have been proposed for a reliable diagnose, in which the heterogeneous array of symptoms (e.g. hallucinations, delusions, poverty of speech), the social withdrawal and the duration of pathological manifestations should be considered (DSM-5, 2013; Tandon et al., 2013). Moreover, cognitive impairments are often observed in schizophrenic patients (Green, 2016; Mier & Kirsch, 2016; Schulz & Murray, 2016). The etiology and symptomatological outcome of the disease has been long associated with an imbalance in neurotransmitter systems (Patel et al., 2014). A dysfunction in the dopaminergic mesocorticolimbic pathway is the oldest and most accepted theory reported (Meltzer & Stahl, 1976) and, the pharmacological modulation of such a system was the first strategy used against psychotic episodes. In addition, a crucial role in schizophrenia of the neurotransmitters serotonin, glutamate, acetylcholine and γ-aminobutyric acid (GABA) has been reported, so that new drugs acting on non-dopaminergic targets have been proposed (Keshavan et al., 2016).

Moreover, schizophrenia is defined as a multifactorial syndrome, in which genetic background, gender and environmental settings can impact on the development of the disorder. As demonstrated in heritability studies, genes are necessary but not sufficient. In

fact, the illness runs in family and a higher susceptibility in twins (Rijsdijk et al., 2011) has been found, but the risk ratio genes-related is far to be 100%. Various environmental factors during the early life, childhood or later are also deemed relevant (McDonald & Murray, 2000; Dean & Murray, 2005). Particularly, risks operating prenatally (e.g. obstetric complications, viral infections, maternal stress) have been found to increase the incidence to schizophrenia and other psychiatric disorders at adulthood. Such a finding paved the way to the neurodevelopmental hypothesis (Fatemi & Folsom, 2009; Gupta & Kulhara, 2010), suggesting that the complications during the vulnerable period of the fetus development can negatively interfere with the normal brain maturation. Lastly, gender differences in the disorder have attracted a special interest (Mendrek & Mancini-Marie, 2015). A theory reported that the prevalence of the illness is similar in both males and females (Leung & Chue, 2000). On the other hand, a different susceptibility between sexes was found (van der Werf et al., 2014). In fact, several reviews and meta-analyses provide evidence of higher incidence in men to develop schizophrenia, with a male/female risk ratio of 1.4:1 (Aleman et al., 2003; McGrath et al., 2004; Picchioni & Murray, 2007). However, whether these differences result from a different severity in illness course, an age-related incidence peak or molecular factors (e.g. hormones), remains unknown.

1.1.1. Role of the dopamine system in schizophrenia

The role of dopamine system has been investigated in cognition, reward, motivation as well as emotional processes related to schizophrenia (Laviolette, 2007; Brisch et al., 2014). The mesocorticolimbic dopamine system originates in the ventral tegmental area (VTA, A10 brain region). The mesolimbic afferents project to the ventral striatum/nucleus accumbens (VStr/NAc), whereas the mesocortical one project to the prefrontal cortex (PFC), (**Figure 1**). According to the "*dopaminergic hypothesis of schizophrenia*",

dopamine imbalance has been associated with the disorder. Specifically, a hyperactivity of the mesolimbic and a hypoactivity of the mesocortical portions have been shown (Toda & Abi-Dargham, 2007).

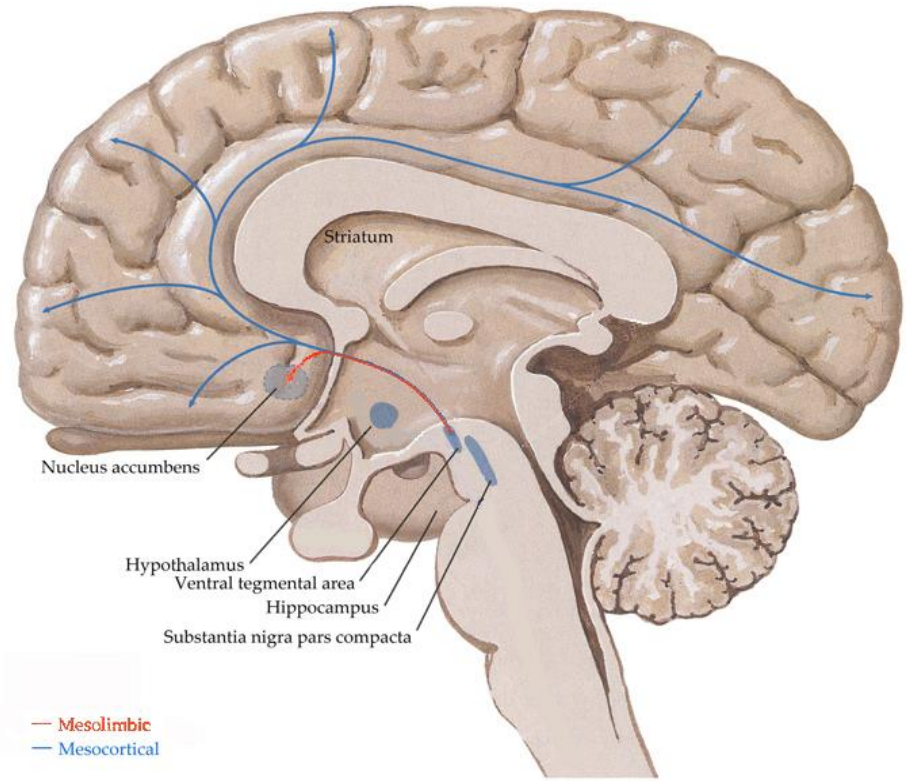

Figure 1. Schematic representation of mesocorticolimbic dopaminergic system. Figure adapted by (Felten & Shetty, 2010)

Increase of dopamine release in the VStr/NAc, in turn overstimulating dopamine D_2 receptors, is associated to positive symptoms. Accordingly, typical antipsychotic drugs alleviate pathological positive manifestations through D_2 receptors blockade (Kapur & Mamo, 2003). On the other hand, the psychostimulant amphetamine, which elevates extracellular dopamine, can induce psychotic-like signs in healthy subjects (Angrist et al., 1974), as well as exacerbates symptoms in schizophrenics (Laruelle et al., 1996). Neuroimaging in humans revealed different facets of the illness associated with disruption of the dopamine system. Positron emission tomography (PET) has been used to analyze

synthesis and storage of dopamine examining the uptake of radioactive analogues of L-DOPA, i.e. [^{18}F] or [^{11}C] DOPA (Hietala et al., 1995; Volkow et al., 1996; Lindstrom et al., 1999). In particular, DOPA uptake in the striatum has been found enhanced in schizophrenic patients and related with the severity of prodromal symptoms (Howes et al., 2007; Howes et al., 2009). Single photon emission computed tomography (SPECT) with the radioligand [^{123}I]IBZM in schizophrenic patients showed that *in vivo* D_2 receptor dopamine binding potential, induced by administration of amphetamine, is reduced, suggesting an enhanced dopamine release (Laruelle et al., 1996). A similar result was found using [^{11}C]raclopride with PET (Breier et al., 1997). In parallel with this finding, a higher baseline occupancy of D_2 receptors has been demonstrated. Specifically, the administration of the tyrosine hydroxylase (TH) inhibitor, alpha-methyl-para-tyrosine (α-MPT) elicited a higher availability of D_2 receptors in schizophrenic subjects (Abi-Dargham et al., 2000). Notably, a correlation between the enhancement in dopamine release and the occupancy of D_2 receptors in patients has been showed (Abi-Dargham et al., 2009). Whether or not alterations in striatal D_2 receptors density exist is still unclear (Brunelin et al., 2013). In particular, a significant elevation of D_2 receptors density has been demonstrated by a few studies (Weinberger & Laruelle, 2001). Finally, dopamine synapses have been analyzed measuring the striatal density of the dopamine transporter (DAT). The majority of the studies did not reveal differences in DAT density between schizophrenic individuals and controls, suggesting that the maladaptation of dopamine transmission is not directly correlated with the density of DAT (Fusar-Poli & Meyer-Lindenberg, 2013), (**Figure 2**).

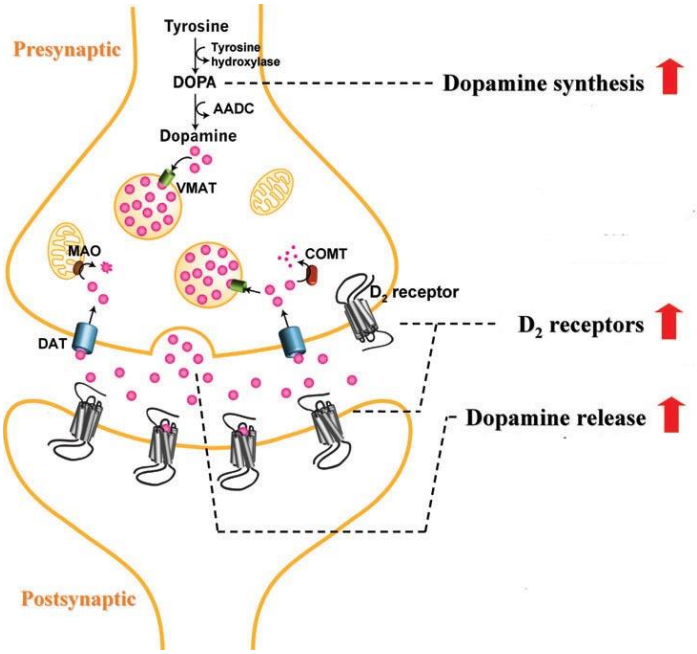

Figure 2. Dopamine system alterations in schizophrenia. AADC, aromatic acid decarboxylase; AMPT, *α*-methyl-*para*-tyrosine; COMT, catechol-*O*-methyltransferase; DAT, dopamine transporter; DOPA, 3,4-dihydroxyphenylalanine; MAO, monoamine oxidase; VMAT, vesicular monoamine transporter. Figure adapted by (Miyake et al., 2011).

1.1.2. Maternal immune activation (MIA) as a risk factor for neurodevelopmental disorders

Epidemiological evidence has provided an association between *in utero* insults and increased risk for schizophrenia and related disorders (Brown & Derkits, 2010; Brown, 2012; Canetta & Brown, 2012; Khandaker et al., 2013). Indeed, the first and the second trimester of pregnancy have been identified as the most critical periods in the fetal brain development (Brown & Susser, 2002; Brown et al., 2004a; Atladottir et al., 2010). Since 1919, Karl Menninger (Menninger, 1919) showed an enhanced rate of *"dementia precox"* among subjects prenatally exposed to the pandemic influenza in 1918. Moreover, a relationship between schizophrenia in hospitalized patients and epidemic influenza was found in Finland (Mednick et al., 1988; Mednick et al., 1994), Denmark (Barr et al., 1990;

Takei et al., 1996), England and Wales (O'Callaghan et al., 1991), as well as France (Limosin et al., 2003). On the other hand, several studies did not replicate these data (Kendell & Kemp, 1989; Erlenmeyer-Kimling et al., 1994; Selten & Slaets, 1994; Susser et al., 1994; Grech et al., 1997; Morgan et al., 1997; Selten et al., 1998; Selten et al., 1999; Westergaard et al., 1999; Mino et al., 2000). The discrepancies could result from technical limitations in classifying exposed and not-exposed fetuses to maternal infection. Nonetheless, serological analysis of archived maternal serum samples confirmed the link between MIA and psychosis in the offspring. Elevated levels of the proinflammatory chemokine interleukin-8 (IL-8) and cytokine tumor necrosis-factor α (TNFα) were found in mothers who gave birth to individuals developing schizophrenia (Buka et al., 2001b; Brown et al., 2004b). Further, the disease was found to be associated with prenatal exposure to other infection agents, such as *Toxoplasma gondii* (Brown et al., 2005; Mortensen et al., 2007), herpes simplex virus (Buka et al., 2001a; Buka et al., 2008), rubella (Brown et al., 2000; Brown et al., 2001), poliovirus (Suvisaari et al., 1999) and genital/reproductive microorganisms (Babulas et al., 2006).

MIA has been also considered as a risk factor for the development of autism spectrum disorders (Ciaranello & Ciaranello, 1995; Libbey et al., 2005; Patterson, 2009). Post-mortem brain analysis of autistic patients revealed an inflammatory-like state, as highlighted by increased cytokines levels. Elevated levels of proinflammatory cytokines and chemokines (IL-1β, IL-6, IL-8 and IL-12p40) has been also detected in plasma and cerebral spinal fluid (Patterson, 2011). Finally, enhanced cytokines and chemokines levels (i.e. IL-4, IL-5, IFNγ, and MPC-1) were found in maternal serum or amniotic fluid of autistic child mothers (Goines et al., 2011; Abdallah et al., 2012).

1.1.3. Polyriboinosinic polyribocytidilic acid (Poly I:C) as a MIA animal model for neurodevelopmental disorders

Exposure of rodent fetuses to maternal administration of Poly I:C, a proinflammatory cytokine inductor, is commonly used to obtain an animal model of neurodevelopmental disruption induced by maternal infection (Boksa, 2010; Meyer, 2014; Reisinger et al., 2015), which has been featured for construct, face and predictive validity. Poly I:C is a synthetic analogue of a double-stranded RNA showed to be involved in the innate immune response, which binds to transmembrane protein toll-like receptor 3 (TLR-3). Activation of the TLR-3 pathway induces a viral-like acute inflammatory state, leading to the synthesis of some proinflammatory cytokines, such as IL-1, IL-6, TNFα and IFNs (Boksa, 2010; Bronson et al., 2011; Meyer, 2014; Reisinger et al., 2015). In Poly I:C-administered dams, a sickness syndrome characterized by a body temperature variation and a food intake reduction, resulting in weight loss, has been found in approximately 24 hours (Bronson et al., 2011). Concurrently, a transplacental passage of maternally-derived cytokines can compromise the brain development in fetuses, inducing long-lasting alterations. The time slot for the exposure to maternal infection is considered to be relevant in the pathophysiology of neurodevelopmental disorders. Specifically, the deficits have been found more severe when induced in the early/middle prenatal period in rodents (Meyer et al., 2007; Meyer & Feldon, 2010), comparable with the first and the second trimester in human gestation. Morphological and molecular changes in the brain have been reported in rodents, including decreased hippocampal neurogenesis and increased lateral ventricular volume as well as reduced PFC expression of reelin and parvalbumin (Meyer et al., 2008b). In addition, a role of genes mainly involved in the disorder, such as disrupted in schizophrenia 1 (*Disc1*) (Abazyan et al., 2010; Ibi et al., 2010; Lipina et al., 2013),

neuregulin 1 (*Nrg1*) (O'Leary et al., 2014) and tuberous sclerosis 2 (*Tsc2*) (Ehninger et al., 2012; Ehninger, 2014) have been also found. Poly I:C MIA model reproduces long-term behavioral and cognitive aberrations in offspring, emerging in late adolescence or early adulthood, as usually observed in psychiatric conditions. Similarly, impairments in latent inhibition (Zuckerman *et al.*, 2003; Zuckerman & Weiner, 2005), sensorimotor gating (Wolff & Bilkey, 2008), social interaction (Smith et al., 2007) as well as novel object recognition (Ozawa et al., 2006) have been observed. Hypersensitivity to locomotor effects elicited by amphetamine (Zuckerman *et al.*, 2003; Meyer *et al.*, 2005), methamphetamine (Ozawa et al., 2006) and MK-801 (Zuckerman & Weiner, 2005) has been also reported. Moreover, alterations in acetylcholine (Wu et al., 2015), GABA (Richetto et al., 2015), serotonin (Ohkawara et al., 2015) and dopamine (Meyer et al., 2008a; Vuillermot et al., 2010) systems were found. Abnormalities in dopaminergic signaling have been specifically investigated for their central role in the etiology and treatment of schizophrenia. Early alterations of the ontogeny of dopamine neurons have been related to pathological maladaptations (Eyles et al., 2012). Mutations in the nuclear transcription factors NURR1 and Pitx3, involved in the development and maturation of dopamine neurons, have been largely investigated in the Poly I:C model (Meyer et al., 2008a; Vuillermot et al., 2010; Eyles et al., 2012). Several studies show an increased number of TH immunoreactive cells in the VTA and TH-positive terminals in the striatum (Meyer et al., 2008a; Vuillermot et al., 2010). In addition, an increase in evoked striatal dopamine release ex vivo (Zuckerman *et al.*, 2003) as well as in the levels of dopamine and DOPAC in the lateral globus pallidus and PFC (Winter et al., 2009) or only DOPAC in striatum (Ozawa et al., 2006) have also been reported. However, whether and how the dopamine transmission is affected by Poly I:C-induced MIA has not been investigated yet.

Furthermore, pharmacological approaches to prevent the impact of MIA in vulnerable subjects are currently missing. Given that our laboratory pay specific attention to the role of Peroxisome Proliferator-Activated Receptors (PPARs) in neuronal physiology and neuropathology (Pistis & Melis, 2010), in the present thesis we focus on a novel strategy involving such a receptor family.

1.2 Peroxisome Proliferator-Activated Receptors (PPARs)

The Peroxisome Proliferator-Activated Receptor (PPAR) is a family of nuclear receptor transcription factors, regulating gene expression through several mechanisms. Three isoforms, PPARα, PPARβ (also known as PPARβ/δ) and PPARγ, have been identified. Various and remarkable differences have been found in PPARs distribution and functionality. Specifically, PPARα is expressed in tissues involved in fatty acid metabolism, such as liver, intestine, heart, kidney and muscle, playing a crucial role in glucose homeostasis and inflammatory processes (Pyper et al., 2010). PPAR-β/δ, whose activation increases lipid catabolism, is prominently expressed in muscle, heart and adipose tissues. PPARγ is expressed in adipose tissue, having an effect on metabolic modulation (Lee & Kim, 2015). PPARs have also been shown to be ubiquitously expressed in the brain, and they are considered to play a role in the modulation of genes related to both neurotransmission and neurodegenerative pathologies (Moreno et al., 2004). PPARs share a general structural homology, showing a NH_2-terminal region, a ligand-independent transactivation domain (AF-1), a DNA-binding domain, a ligand binding and dimerization and a ligand-dependent activation (AF-2) domains, followed by a COOH-terminus (Ferre, 2004). According with the canonical genomic action, once activated by their ligand, PPARs undergo conformational changes promoting the association with coactivator and dissociation from corepressor proteins. Subsequently, PPARs form heterodimers with the

retinoid X receptors (RXR) and bind the peroxisome proliferator response element (PPRE) in the target genes ((Pistis & Melis, 2010) and references therein), (**Figure 3**).

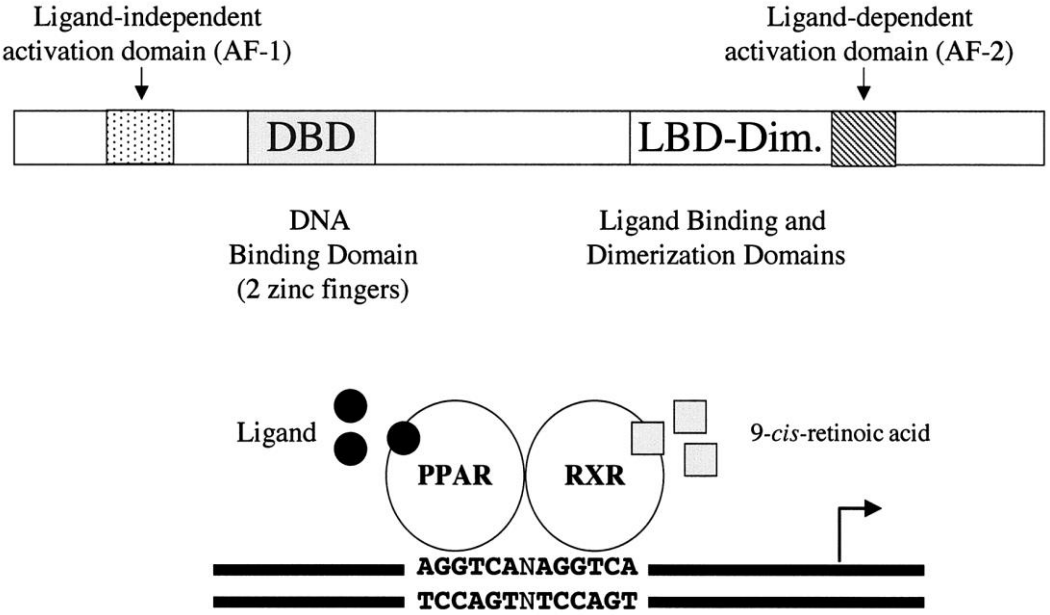

Figure 3. Structure of PPARs and "classic" genomic mechanism. On the top, PPARs have four main domains: ligand-independent transactivation domain (AF-1), a DNA-binding domain, a ligand binding and dimerization and a ligand-dependent activation (AF-2) domains. On the bottom, PPARs form heterodimers with the retinoid X receptors (RXR) and bind the peroxisome proliferator response element (PPRE) in the target genes (Ferre, 2004).

In parallel, other genomic and non-genomic effects has been demonstrated (Gelman et al., 1999; Escher & Wahli, 2000; Barbier et al., 2004; Gardner et al., 2005; Pistis & Melis, 2010). Non-genomic mechanisms mediated by PPARs are responsible for short time scale cellular modifications (Melis et al., 2008; Ropero et al., 2009). Activation of PPARα induces protein kinase-mediated phosphorylation of neuronal targets, such as $\alpha_4\beta_2$ nicotinic acetylcholine receptors (nAChRs) on VTA dopamine neurons, as previously reported in our laboratory (Melis et al., 2010). Moreover, activation of α7-nAChRs has been found to increase both the tyrosine phosphorylation of the β2 subunit of nAChRs on VTA dopamine

cells and the levels of two PPARα endogenous ligands in a Ca^{2+}-dependent manner (Melis et al., 2013b), (**Figure 4**).

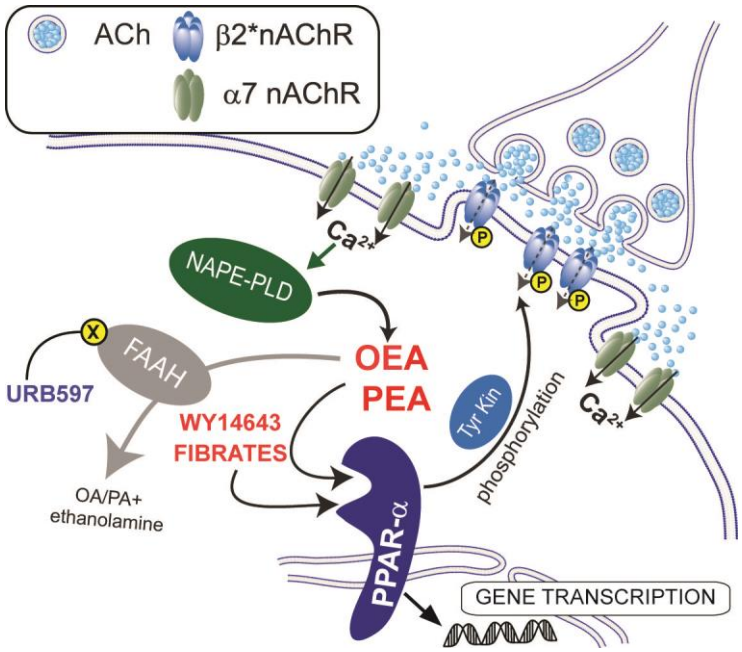

Figure 4. Schematic diagram illustrating non-genomic mechanisms mediated by PPARα. α7-nAChRs are located extrasynaptically on the somatodendritic region of dopamine neurons (Jones & Wonnacott, 2004). α7-nAChR-mediated increase in intracellular Ca^{2+} stimulates N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD), leading to the production of OEA and PEA These molecules, in turn, activate PPARα that exerts negative modulation of β2*-nAChRs operated by a tyrosine kinase-mediated phosphorylation (Tyr Kin). The effects of endogenous PPARα ligands are mimicked by the synthetic agonists WY14643 or fibrates. Increases in levels of N-acylethanolmines can also be triggered by blockade of FAAH by URB597. FAAH is the major inactivating enzyme for OEA and PEA and converts these molecules in ethanolamine and oleic acid (OA) and palmitic acid (PA), respectively. Figure adapted by (Melis et al., 2013b).

Endocannabinoid-related N-acylethanolamines (NAEs), such as the oleoylethanolamide (OEA) and the palmitoylethanolamide (PEA), are endogenous PPARα ligands. The biosynthesis of OEA and PEA is a two-step process induced in response to cellular activation, i.e. *on demand*, following intracellular Ca^{2+} increase or NMDA receptor activation. The formation of N-acylated ethanolamine phospholipids (NAPE), precursors of NAEs, is catalysed by N-acyltransferase, a ubiquitous Ca^{2+}-activated enzyme (Hansen et

al., 1997; Mackie & Stella, 2006). NAPE, in turn, is converted to NAEs by N-acyl-phosphatidylethanolamine-selective phospholipase D (NAPE-PLD). The hydrolysis of NAEs is mainly catalyzed by fatty acid amide hydrolase (FAAH) (Cravatt et al., 1996), although FAAH-2 (Wei et al., 2006) and NAE-hydrolyzing acid amidase (NAAA) (Tsuboi et al., 2005) are also involved. The modulation of PPARα has been largely investigated in different neural processes (O'Sullivan, 2016). For example, memory consolidation has been found to be facilitated by administration of OEA (Campolongo et al., 2009). Consistently, memory acquisition was enhanced by the synthetic PPARα agonist WY14643 as well as by the FAAH pharmacological inhibitor URB597 (Mazzola et al., 2009). URB597-mediated PPARα modulation has been also found to be relevant in sleep-wake cycle (Murillo-Rodriguez et al., 2007) and in nicotine addiction (Melis et al., 2008; Luchicchi et al., 2010; Melis & Pistis, 2014; Justinova et al., 2015).

1.2.1. Neuroprotective and anti-inflammatory effects of PPARα as a therapeutic strategy

Growing evidence suggests that gene expression modulated by PPARα has a therapeutic effect on inflammatory components of neurodegenerative disorders (Bordet et al., 2006; Rolland et al., 2013; Fidaleo et al., 2014; Moreno & Ceru, 2015). Indeed, regulation of inflammatory pathways, such as those associated with cyclo-oxygenase 2 (COX-2), adhesion proteins and cytokines (IL-6 and TNF-α), and antioxidant enzymes, e.g. superoxide dismutase and glutathione peroxidase, has been related with PPARα activity (Bordet et al., 2006). PPARα has been also found to control the activation of macrophage and microglial cells, which contribute to neuronal death through degenerative and inflammatory processes (Bordet et al., 2006). In preclinical studies, several endogenous and synthetic PPARα ligands have been tested as therapeutic tool against

neurodegenerative illness, such as Parkinson's (Chaturvedi & Beal, 2008; Barbiero et al., 2014a) and Alzheimer's (D'Agostino et al., 2012; Scuderi et al., 2014; Zhang et al., 2014) diseases as well as for psychiatric disorders, such as schizophrenia (Rolland et al., 2012) and epilepsy (Citraro et al., 2013; Puligheddu et al., 2013). In particular, fenofibrate, a PPARα agonist approved for human therapies, has been tested in schizophrenia-related PPI deficits and behavioral changes in rodents (Rolland et al., 2012; Grover et al., 2013). Impairments in PPI were reduced by administration of a diet containing fenofibrate 0.2% in adulthood, in a neurodevelopmental rodent model of schizophrenia induced by neonatal kainic acid injection (Rolland et al., 2012). Furthermore, symptoms of oral dyskinesia mimicked in rats by chronic administration of haloperidol, were dose-dependently inhibited by fenofibrate treatment (Grover et al., 2013). Whether or not PPARα activation might prevent neural aberrations induced by proinflammatory cytokines during fetal development, remains to be explored.

2. AIMS OF THE STUDY

Based on this background, the first aim of the present thesis was to investigate whether and how the Poly I:C MIA model might impact on the neurodevelopment in offspring. We took advantage of prepulse inhibition of startle reflex to evaluate behavioral alterations and *in vivo* electrophysiological recordings to examine the mesolimbic dopamine neuron activity in male and female littermates. The second aim was to test neuroprotective properties of prenatal chronic administration of fenofibrate, a PPARα agonist, against Poly I:C-induced behavioral and electrophysiological alterations

3. MATERIALS AND METHODS

All experiments were approved by the University of Cagliari Committee on Animal Use and Care and performed in strict accordance with the EEC Council Directive of 24 November 1986 (86/609). Animals were housed in groups of 3 to 6 in standard conditions of temperature and humidity under a 12 h light/dark cycle (with lights on at 7:00 A.M.) with food and water available *ad libitum*. We made all efforts to minimize animal discomfort and to reduce the number of animals used.

3.1. Prenatal treatment

Female Sprague Dawley rats (Harlan, Italy) were mated at the age of 3 months. The first day after the copulation was defined as gestational day (GD) 1.

3.1.1. Induction of MIA

At GD 15, dams were anesthetized with isoflurane 2%, and a single dose of Poly I:C (4.0 mg/kg, i.v.) or an equivalent volume of saline were administered in the lateral vein of the tail (**Figure 5**). Pregnant rats were weighed for the first 3 days after the administration of either Poly I:C or saline to evaluate weight loss.

3.1.2. Fenofibrate diet

From GD 8 to GD 18, Poly I:C- and saline-treated dams received a diet enriched with the PPARα agonist fenofibrate (0.2% w/w) or a control diet *ad libitum* (**Figure 5**).

We established four experimental groups: (I) control diet + vehicle (CTRL), (II) control diet + Poly I:C (Poly I:C), (III) fenofibrate diet + vehicle (FBR), and (IV) fenofibrate diet + Poly I:C (Poly I:C + FBR). After weaning, offspring were housed with littermates in groups of 3-5 individuals, and maintained undisturbed until experiments at adulthood.

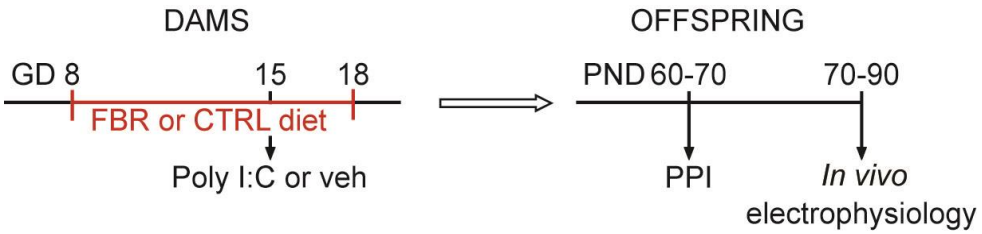

Figure 5. Schematic experimental protocol. Dams were fed with standard or 0.2% fenofibrate-enriched diet from gestational day (GD) 8 to GD 18. At GD 15, a single i.v. injection of Poly I:C (4 mg/kg) or vehicle (sterile pyrogen-free saline) was administered. Offspring was tested in prepulse inhibition (PPI) between postnatal day (PND) 60 and 70. In vivo electrophysiological recordings were performed beetween 70 and 90 PND.

3.2. Prepulse inhibition (PPI) of startle reflex

At 60-70 PND, male and female adult littermates were tested for prepulse inhibition (PPI) of startle reflex (**Figure 5**). Startle and PPI were performed as previously described by (Frau et al., 2014). The apparatus used for detection of startle reflexes (Med Associates, St Albans, VT) consisted of four standard cages placed in sound-attenuated chambers with fan ventilation. Each cage consisted of a Plexiglas cylinder of 9 cm diameter mounted on a piezoelectric accelerometric platform connected to an analogue-digital converter. Two separate speakers conveyed background noise and acoustic bursts, each one properly placed to produce a variation of sound within 1 dB across the startle cage. Both speakers and startle cages were connected to a main PC, which detected and analyzed all chamber variables with specific software. Before each testing session, acoustic stimuli and mechanical responses were calibrated via specific devices supplied by Med Associates. On

the testing day, each rat was placed in the cage for a 5 minute acclimatization period consisting of 70 dB white noise background, which continued for the remainder of the session. Each session consisted of 3 consecutive sequences of trials (blocks). During the first and third block, rats were presented with only 5 pulse-alone trials of 115 dB. In the second block was delivered a pseudorandom sequence of 50 trials, including 12 pulse-alone trials; 30 trials of pulse preceded by 74, 78, or 86 dB prepulses (10 for each level of prepulse loudness); and 8 no-stimulus trials, where only the background noise was delivered. Inter-trial intervals were selected randomly between 10 and 15 seconds, while the inter-stimulus intervals were set at 100 milliseconds. Startle response was based on the first positive wave that meets the minimum wave criteria and determined as mean startle amplitude of the pulse-alone trials relative to second block. Startle habituation across the 2 halves of the second block was evaluated as percent inter-block ratio using the following formula: (mean startle amplitude for first half of the second block/mean startle amplitude for second half of the second block) × 100. Latency to startle was based on the first peak value across pulse-alone trials of the second block. "Arbitrary units" were calculated by the Med Associates apparatus software by proportionally converting the analog voltage signal recorded by the startle sensor (ranging from -10 to +10 V) to a digital unit, within a range of values between –2048 and +2048. The % PPI was calculated only on the values relative to the second block using the following formula: ([mean startle amplitude pulse alone trials - mean startle amplitude prepulse + pulse trials]/mean startle amplitude for pulse alone trials) × 100.

3.3. In vivo single unit extracellular electrophysiology in the VTA

In vivo electrophysiological recordings were performed in male and female offspring at 70-90 PND (**Figure 5**). Rats were anaesthetized with urethane (1.3 g/kg, i.p.) and placed in

the stereotaxic apparatus (Kopf, Tujunga, CA, USA) with their body temperature maintained at 37±1°C by a heating pad. For the placement of a recording electrode, the scalp was retracted, and one burr hole was drilled above the parabrachial pigmented nucleus (PBP) of the posterior VTA (AP, 6.0 mm posterior from bregma, L, 0.4-0.6 mm lateral from midline) according to the Atlas of Rat Brain (Paxinos & Watson, 2007). We selected this subregion as it contains the larger density of dopamine cells as compared to the more medial portions of the posterior VTA. Extracellular single-unit activity of dopamine neurons located in the VTA (V, 7.0-8.0 mm from the cortical surface) was recorded with glass micropipettes filled with 2% Pontamine sky blue (PSB) dissolved in 0.5 M sodium acetate (impedance 2.5–5 MΩ). Putative VTA DA neurons were selected when all criteria for identification were fulfilled: firing rate <10 Hz and duration of action potential >2.5 ms as measured from start to end. The electrical activity for each neuron was recorded for 2-3 minutes. Different electrophysiological parameters were evaluated: the number of spontaneously active dopamine cells per electrode track (i.e., population activity), the basal firing rate and the bursting activity. Bursts were defined as the occurrence of two spikes at interspike interval <80 ms, and terminated when the interspike interval exceeded 160 ms (Grace & Bunney, 1983) (**Figure 6**). Single-unit activity was filtered (bandpass 0.1–10000 Hz) and individual action potentials were isolated and amplified (Neurolog System, Digitimer, Hertfordshire, UK), displayed on a digital storage oscilloscope (TDS 3012, Tektronics, Marlow, UK). Experiments were sampled on line and off line with Spike2 software (Cambridge Electronic Design, Cambridge, UK) by a computer connected to CED 1401 interface (Cambridge Electronic Design, Cambridge, UK). At the end of recording sessions, DC current (15 mA for 15 min) was passed through the recording micropipette in order to eject PSB for marking the recording site. Brains were then rapidly removed and were frozen in isopentane cooled to -40 °C. The position of

the electrodes was microscopically identified on serial 60 µm sections stained with Neutral Red.

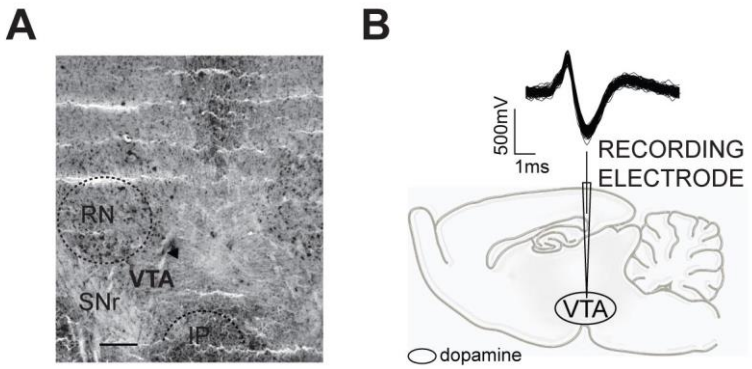

Figure 6. Electrophysiological recordings in the posterior VTA. (**A**) Representative histological section showing the recording site in the posterior VTA (the triangle indicates the PSB dye). Abbreviations: RN, red nucleus; IP, interpeduncular nucleus, SN, substantia nigra pars reticulata. Scale bar, 0.5 mm. (**B**) Schematic diagram illustrating the typical broad spike waveform of a dopamine neuron and the in vivo recording site in the rat brain

3.4. Estrous cycle staging

Vaginal smears were collected in female adult rats before experimental sessions. Samples were stained with Giemsa, and the cell morphology was microscopically examined to determine the estrous cycle stage (Marcondes et al., 2002). Four main phases were identified (**Figure 7**): estrous mainly displayed anucleated cornified cells (A), metestrous consisted of leukocytes, cornified and nucleated epithelial cells, proportionally (B), diestrous mainly showed leukocytes (C), and proestrous displayed a predominance of nucleated epithelial cells (D).

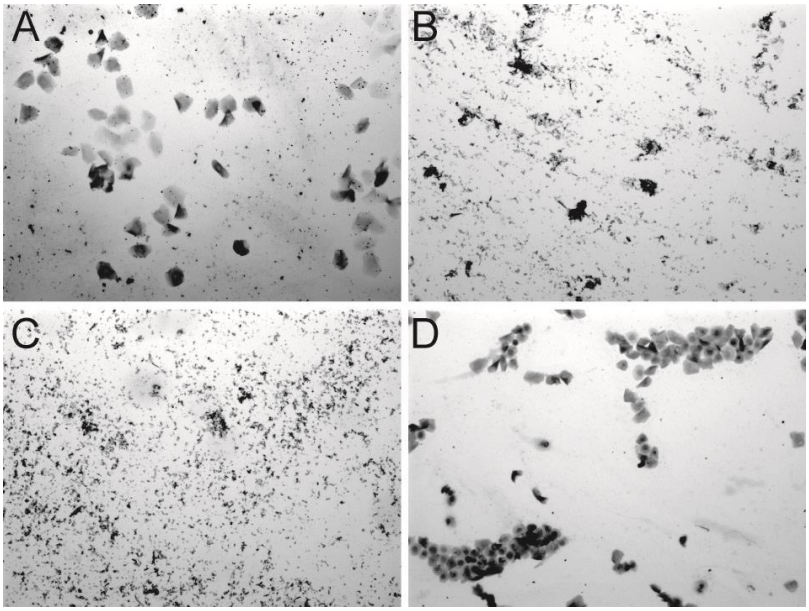

Figure 7. Representative photomicrographs of Giemsa's stained vaginal smears from female rats. (**A**) estrous, (**B**) metestrous, (**C**) diestrous and (**D**) proestrous.

3.5. Drugs

Poly I:C was purchased from InvivoGen, San Diego, CA (USA), and was dissolved in an endotoxin free saline solution. Fenofibrate was purchased from Sigma Aldrich (Italy), and used at concentration of 0.2% (w/w) to enrich a standard rodent diet (Harlan Teklad Global 2016, Italy).

3.6. Statistical analysis

Averaged data from different experiments are given as mean ± SEM. Statistical significance was assessed using one- or two-way ANOVA or Student's *t*-test, where appropriate. *Post-hoc* multiple comparisons were made using either the Bonferroni's test or the Newman-Keuls' test. All data were analyzed using GraphPad Prism (San Diego, CA, USA). The significance level was established at $P<0.05$.

4. RESULTS

4.1. Impact of Poly I:C treatment on dams weight

As previously reported (Zuckerman et al., 2003; Zuckerman & Weiner, 2005; Wolff & Bilkey, 2010), we found a weight loss in pregnant rats 24 hours after Poly I:C-injection. Poly I:C-treated dams lost weight irrespective of the diet consumed during pregnancy, whereas vehicle-injected dams increased their weight as expected (Poly I:C: -4.14 ± 3.13 g n=7, Poly I:C + FBR: -10.33 ± 1.02 g n=6, CTRL: 8.20 ± 2.87 g n=5 and FBR: 5.75 ± 3.50 g n=4, $F_{(3,21)}$=9.52; $F_{(3,21)}$=9.52, P<0.001, one-way ANOVA; **Figure 8**). These data suggest an efficacy of Poly I:C injection, which may be related to the inflammatory state.

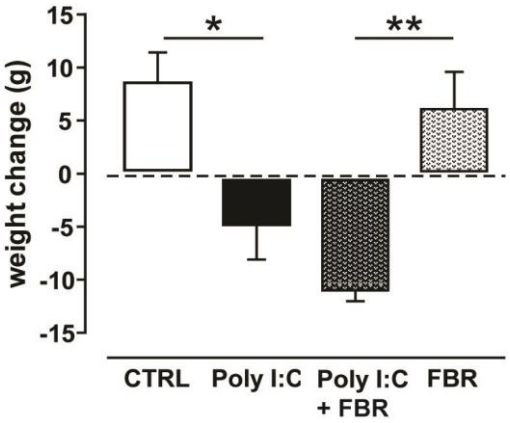

Figure 8. Impact of Polyriboinosinic-polyribocytidilic acid (Poly I:C) on dams' weight. Poly I:C treatment affects dams weight 24 hours after the injection. Data are expressed as mean ± SEM. *P<0.05, **P<0.01.

4.2. Effect of Poly I:C prenatal treatment on male rats

4.2.1. PPI was disrupted in offspring of Poly I:C-treated dams

To validate our model, we carried out the prepulse inhibition of startle reflex in controls and Poly I:C adult male rats. The acoustic startle response was measured at PND 60-70. Poly I:C treatment did not affect overall startle reflex values. Accordingly, no significant effects for mean startle amplitude (Poly I:C: 1129 ± 78.74 AU n=23 vs CTRL: 1049 ± 92.48 AU n=15; $t_{(36)}$=0.64, P>0.05, Student's *t*-test; **Figure 9A**), latency to peak (Poly I:C: 64.77 ± 1.00 ms n=23 vs CTRL: 65.63 ± 1.47 n=15; $t_{(36)}$=0.50, P>0.05, Student's *t*-test; **Figure 9B**) and startle habituation (Poly I:C: 165.7 ± 9.20 % n=23 vs CTRL: 161.2 ± 6.80 % n=15; $t_{(36)}$=0.35, P>0.05; Student's *t*-test; **Figure 9C**) were found between control and Poly I:C groups. PPI analyses revealed that maternal infection with Poly I:C significantly reduced this parameter (Poly I:C: 53.17 ± 3.97 % n=23 vs CTRL: 64.73 ± 2.92 n=15; $t_{(36)}$=2.12, P<0.05; Student's *t*-test; **Figure 9D**), suggesting a behavioral disruption related to maternal immune system activation.

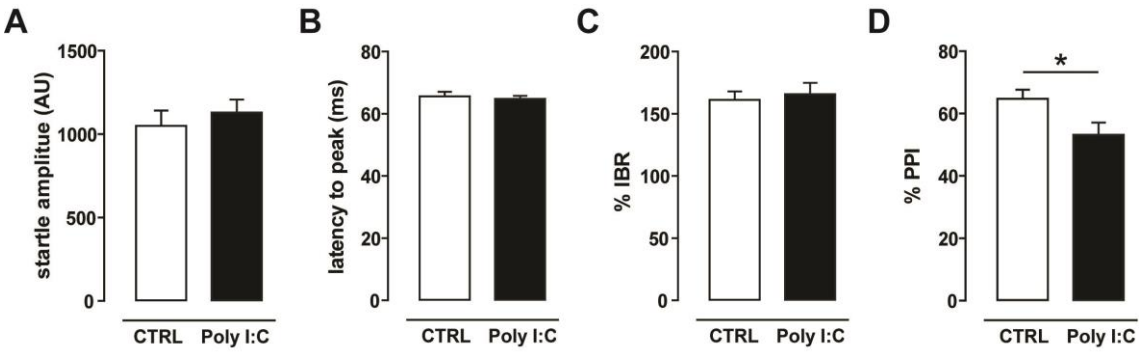

Figure 9. Behavioral abnormalities in Poly I:C male offspring. Startle amplitude (**A**), latency to peak (**B**) and startle habituation (**C**) were not altered by prenatal exposure to Poly I:C. However, PPI was significantly impaired by Poly I:C treatment (**D**). Values are expressed as mean ± SEM. *P<0.05. Prepulses are indicated by the intensity corresponding to decibels above background noise. AU, arbitrary units; % IBR, percent inter-block ratio.

4.2.2. The VTA dopamine cell activity was altered in Poly I:C prenatally-exposed rats

We examined whether the VTA dopamine cell spontaneous activity was affected in the Poly I:C model of psychiatric diseases. At PND 70-90, we performed a sampling of putative dopamine neurons in the PBP nucleus of the VTA. The number of spontaneously active dopamine cells was significantly reduced in Poly I:C rats as measured by the cells per track index (Poly I:C: 0.75 ± 0.11 n=19 vs CTRL: 1.27 ± 0.13 n=14; $t_{(31)}$=3.12, P<0.01, Student's *t*-test; **Figure 10A).** Dopamine neurons recorded from controls discharged at 3.40 ± 0.15 Hz with 16.38 ± 1.67 % (n=148) of spikes in bursts. On the other hand, Poly I:C rats showed a lower average frequency of 2.73 ± 0.18 Hz (n=109; $t_{(255)}$ =2.87, P<0.01, Student's *t*-test; **Figure 10B**) and a reduced percentage of burst firing of 12.21 ± 1.79 % (n=109; $t_{(255)=}$1.68, P<0.05, Student's *t*-test; **Figure 10C**) than controls. We further analyzed bursting parameters, such as mean spikes per burst and mean burst duration. In Poly I:C rats, burst episodes were shorter (Poly I:C: 100.9 ± 7.77 ms n=84 vs CTRL: 128.8 ± 8.64 ms n=126; $t_{(208)}$=2.26, P<0.05, Student's *t*-test; **Figure 10D**) and included less spikes on average (Poly I:C: 2.52 ± 0.08 n=84 vs CTRL: 2.76 ± 0.09 n=126; $t_{(208)}$=1.90, P<0.05, Student's *t*-test; **Figure 10E**).

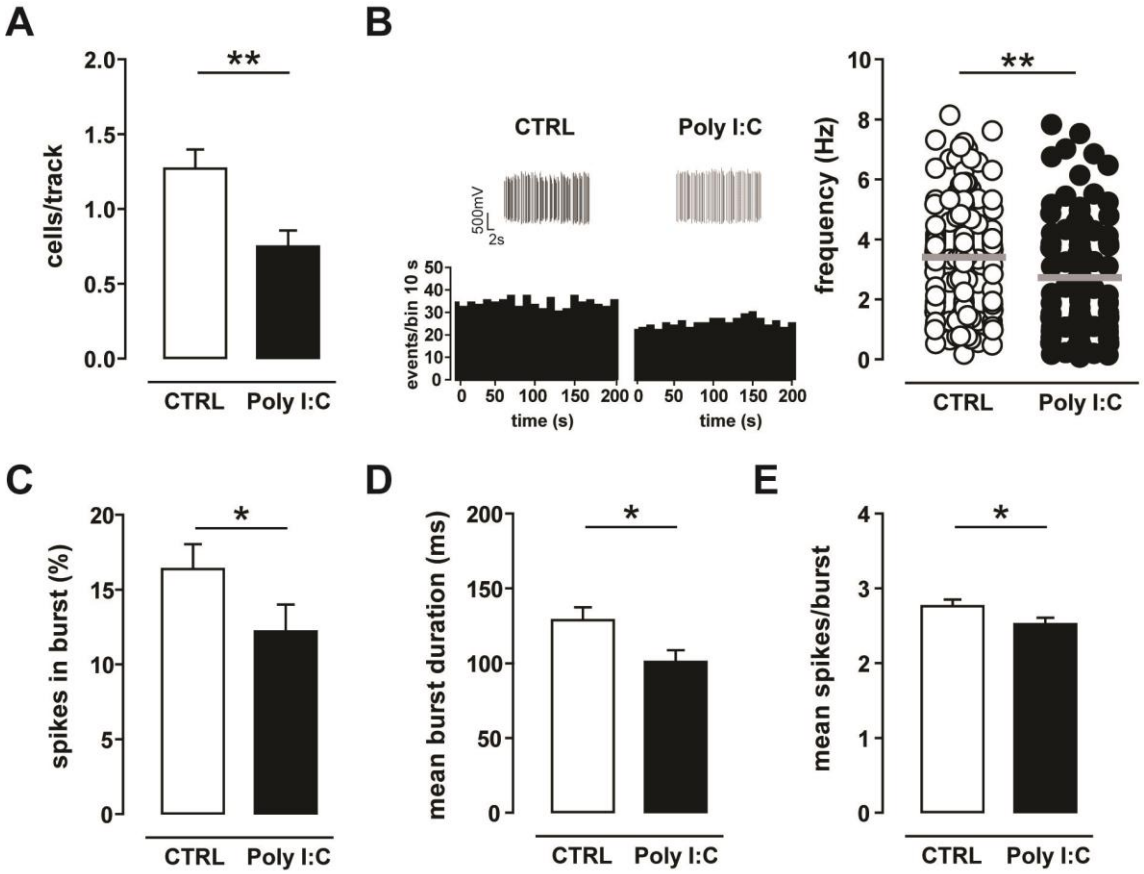

Figure 10. **Neuronal activity of VTA dopamine cells recorded from adult male rats were disrupted by prenatal Poly I:C treatment.** (**A**) The number of spontaneously active VTA dopamine neurons was reduced in offspring of Poly I:C-treated dams. (**B**) On the left, the panel shows traces (on the top) and rate histograms (on the bottom) illustrating representative extracellular recordings of a putative dopamine neuron from both control and Poly I:C rats. On the right, the scatter plot illustrates the firing rate of each dopamine neuron recorded from controls and Poly I:C. The frequency on average was significantly decreased in Poly I:C male rats. Analysis of bursting activity reveals that spikes in burst expressed as percentage (**C**), mean burst duration (**D**) and mean spikes per burst (**E**) were reduced in Poly I:C as compared to controls. Values are expressed as mean ± SEM. *P<0.05, **P<0.01.

4.3. Effect of Poly I:C prenatal treatment on female offspring

4.3.1. PPI was not affected by Poly I:C prenatal exposure

In parallel to males, female littermates underwent PPI experiments at PND 60-70. To minimize the impact of estrous cycle on PPI, rats were selectively tested during proestrous. However, a further analysis did not reveal hormones-related differences in startle values

and in sensorimotor gating during all stages of estrous cycle (P>0.05 for both parameters, two-way ANOVA, data not shown). Thus, we examined data independently of the cycle phase. Poly I:C prenatal treatment did not alter mean startle amplitude (Poly I:C: 700.5 ± 46.54 AU n=16 vs CTRL: 700.3 ± 66.14 AU n=17; $t_{(31)}$=0.003, P>0.05, Student's *t*-test; **Figure 11A**), latency to peak (Poly I:C: 69.96 ± 1.70 ms n=16 vs CTRL: 71.17 ± 2.04 ms n=17; $t_{(31)}$=0.45, P>0.05, Student's *t*-test; **Figure 11B**) and startle habituation (Poly I:C: 110.6 ± 12.75 % n=16 vs CTRL: 138.4 ± 19.02 % n=17; $t_{(31)}$=1.20, P>0.05, Student's *t*-test; **Figure 11C**). Moreover, sensorimotor gating was not affected in rats prenatally-treated with Poly I:C (Poly I:C: 46.68 ± 4.49 % n=16 vs CTRL: 47.75 ± 3.68 % n=17; $t_{(31)}$=0.18, P>0.05, Student's *t*-test; **Figure 11D**).

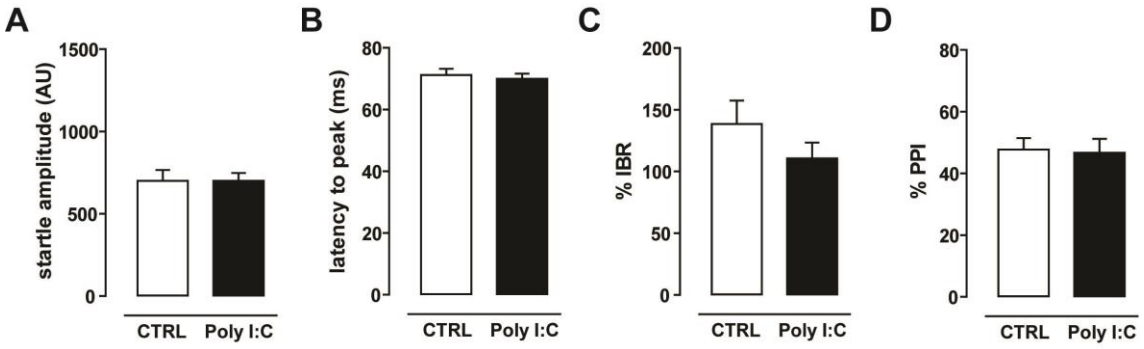

Figure 11. **Effects of prenatal exposure to Poly I:C on startle reflex and prepulse inhibition in female offspring.** Nor startle indices: startle amplitude (**A**), latency to peak (**B**) and startle habituation (**C**), neither PPI were altered by Poly I:C treatment (**D**). Values are expressed as mean ± SEM. Prepulses are indicated by the intensity corresponding to decibels above background noise. AU, arbitrary units; % IBR, percent inter-block ratio.

4.3.2. Electrical activity of VTA dopamine neurons did not differ between Poly I:C and control groups

We characterized dopamine neuron electrical activity in the PBP nucleus of the posterior VTA in the Poly I:C MIA model. The four stages of the estrous cycle were considered to evaluate the modulation of hormones on mesolimbic dopamine activity. Since

electrophysiological parameters did not statistically differ between diestrus I (aka metestrus) and diestrus II, we pooled the data and identified them as diestrus (P>0.05 for all parameters, Student's *t*-test, data not shown). Similarly to PPI results, dopamine neuron spontaneous activity from controls and Poly I:C rats was not impacted by hormonal levels (P>0.05 for all parameters, two-way ANOVA, data not shown). We combined our electrophysiological results and analyzed them independently from the estrous cycle. The number of spontaneously active dopamine cells was not altered by prenatal treatment with Poly I:C (1.22 ± 0.14, n=21) as compared to vehicle-treated offspring (1.57 ± 0.23, n=12, $t_{(31)}$=1.35, P>0.05, Student's *t*-test; **Figure 12A**). Moreover, Poly I:C and control rats showed similar average firing rate (Poly I:C: 2.83 ± 0.11 Hz n=155 vs CTRL: 3.00 ± 0.13 n=118; $t_{(271)}$=0.95, P>0.05, Student's *t*-test; **Figure 12B**) and percentage of spikes in burst (Poly I:C: 12.86 ± 1.35 % n=155 vs CTRL: 15.05 ± 1.76 % n=118; $t_{(271)}$=1.01, P>0.05, Student's *t*-test; **Figure 12C**). Analysis of burst parameters did not reveal differences (mean burst duration: Poly I:C:112.5 ± 5.24 ms n=131 vs CTRL:106.2 ± 5.92 ms n=97; $t_{(226)}$=0.79, P>0.05, Student's *t*-test; **Figure 12D**; mean spikes per burst: Poly I:C: 2.63 ± 0.06 n=131 vs CTRL: 2.58 ± 0.07 n=97; $t_{(226)}$=0.50, P>0.05, Student's *t*-test; **Figure 12E**).

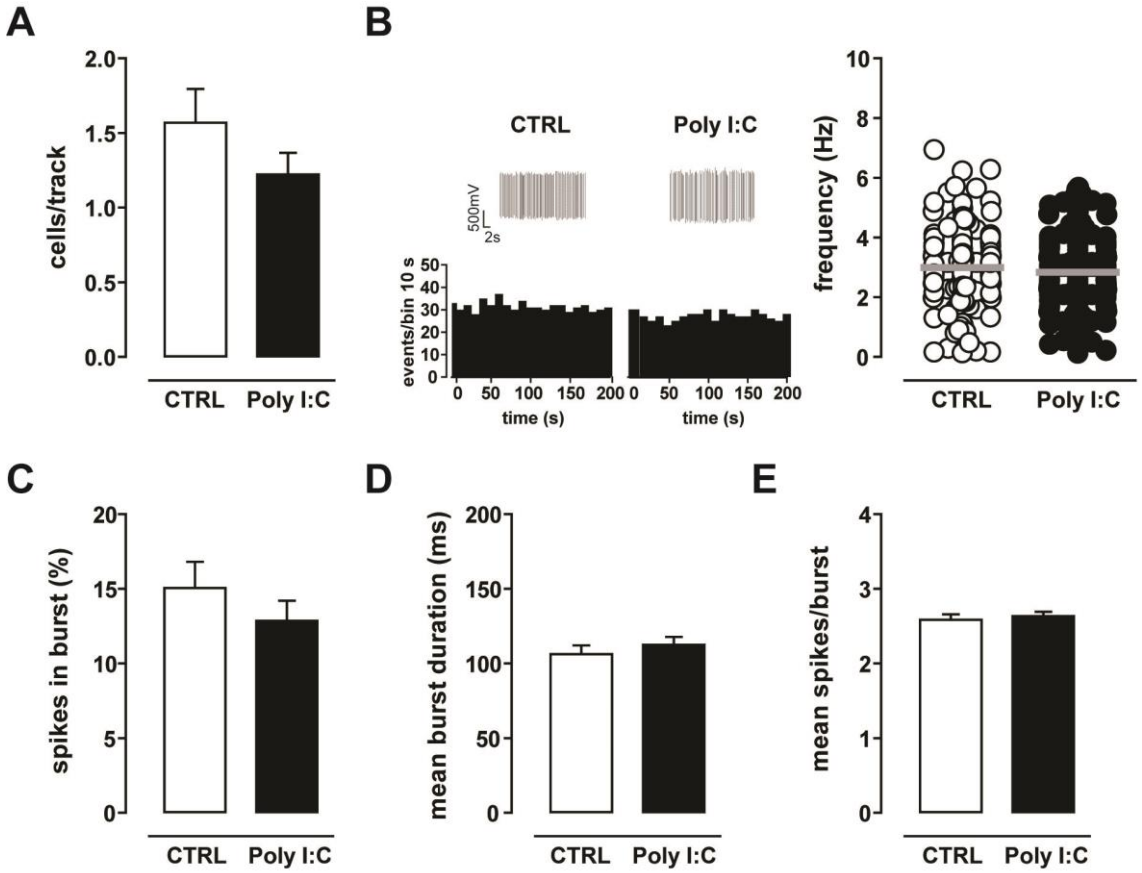

Figure 12. **Impact of Poly I:C treatment on VTA dopamine electrical activity in female offspring.** (**A**) The number of spontaneously active VTA dopamine neurons did not differ between controls and Poly I:C prenatally treated (**B**) On the left, the panel shows representative traces (on the top) and rate histograms (on the bottom) of putative dopamine neurons extracellularly recorded from both groups. On the right, the scatter plot illustrates that the mean firing rate was similar in controls and Poly I:C. Percentage of spikes in burst (**C**), burst duration (**D**) and mean spikes per burst (**E**) were not affected by prenatal exposure to Poly I:C. Values are expressed as mean ± SEM.

4.4. Impact of chronic fenofibrate during pregnancy on Poly I:C neurodevelopmental disruptions in male offspring.

4.4.1. PPI disruptions were not prevented by fenofibrate in male offspring of Poly I:C-treated dams

To investigate whether or not a chronic fenofibrate-enriched diet during pregnancy has neuroprotective and anti-inflammatory effects on behavioral aberrations, we carried out PPI in male offspring at adulthood (PND 60-70). A fenofibrate-enriched diet was

administered to dams from GD 8 to GD 18. On average, fenofibrate intake was quantified in 119.23 mg/kg per day.

Preliminary results showed that prenatal exposure to fenofibrate did not affect the mean startle amplitude (Poly I:C + FBR: 1020 ± 164.4 AU n=6 vs Poly I:C: 1196 ± 174.7 AU n=9, $F_{(3,31)}$=0.69; P>0.05, one-way ANOVA; **Figure 13A**), the latency to peak (Poly I:C + FBR: 68.11 ± 2.39 ms n=6 vs Poly I:C: 63.86 ± 1.64 ms n=9, $F_{(3,31)}$=1.98; P>0.05, one-way ANOVA; **Figure 13B**) and the startle habituation (Poly I:C + FBR: 113.5 ± 10.69 % n=6 vs Poly I:C: 119.3 ± 11.82 % n=9. $F_{(3,31)}$=0.89; P>0.05, one-way ANOVA; **Figure 13C**). Moreover, PPI disruptions were not restored by chronic fenofibrate administration (Poly I:C + FBR: 53.67 ± 6.01 % n=6; Poly I:C: 52.26 ± 4.90 % n=9, $F_{(3,31)}$=3.1; P<0.05, one-way ANOVA; **Figure 13D**).

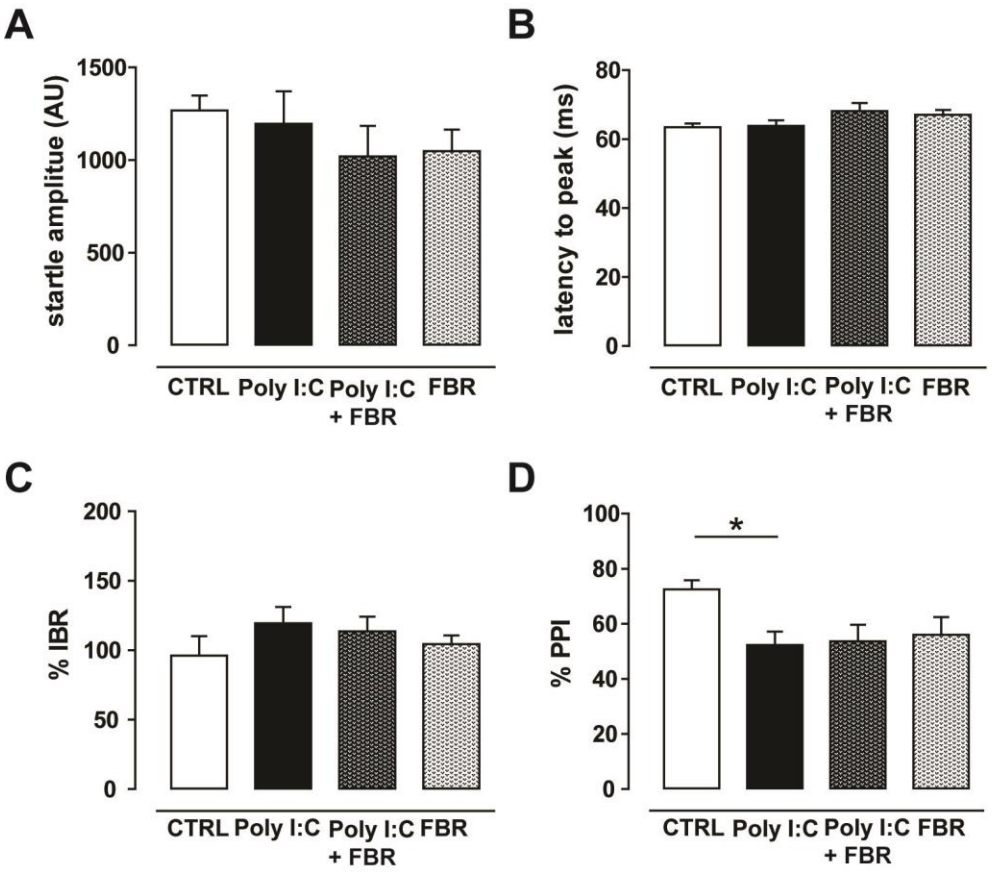

Figure 13. Impact of fenofibrate during pregnancy on startle reflex and prepulse inhibition in male offspring prenatally exposed to Poly I:C. Startle indices were not affected by chronic fenofibrate administration: startle amplitude (**A**), latency to peak (**B**) and startle habituation (**C**). In addition, the fenofibrate-enriched diet did not prevent the PPI impairments induced by Poly I:C (**D**). Values are expressed as mean ± SEM. *P<0.05. Prepulses are indicated by the intensity corresponding to decibels above background noise. AU, arbitrary units; % IBR, percent inter-block ratio.

4.4.2. Alterations of VTA dopamine neuron activity in male offspring were prevented by prenatal fenofibrate

We investigated whether chronic fenofibrate during pregnancy prevented Poly I:C-induced alterations on VTA dopamine cell activity in the offspring. In Poly I:C + FBR rats, we found a normalization of the number of spontaneously active dopamine cells (Poly I:C + FBR: 1.76 ± 0.23 n=10 vs Poly I:C: 0.73 ± 0.09 n=15; $F_{(3,46)}$=8.08, P<0.001, one-way ANOVA; **Figure 14A**) as well as in their firing rate (Poly I:C + FBR: 3.25 ± 0.15 Hz n=116 vs Poly I:C: 2.45 ± 0.17 Hz n=101; $F_{(3,433)}$=5.29, P<0.01, one-way ANOVA; **Figure 14B**). Analysis of burst episodes revealed that percentage of spikes in burst did not differ between groups (Poly I:C + FBR: 14.14 ± 1.80 % n=116 vs Poly I:C: 10.95 ± 1.65 % n=101; P>0.05, one-way ANOVA, **Figure 14C**). However, the fenofibrate-enriched diet prevented the reduction in mean burst duration (Poly I:C + FBR: 119.0 ± 7.40 ms n=96 vs Poly I:C: 91.81 ± 6.68 ms n=75; $F_{(3,360)}$=4.32, P<0.01, one-way ANOVA; **Figure 14D**) and in mean spikes per burst (Poly I:C + FBR: 2.73 ± 0.08 n=96 vs Poly I:C: 2.43 ± 0.07 n=75; $F_{(3,360)}$=3.13, P<0.05, one-way ANOVA; **Figure 14E**). Importantly, we found that fenofibrate administration was ineffective *per se* (P>0.05 for all parameters, one-way ANOVA).

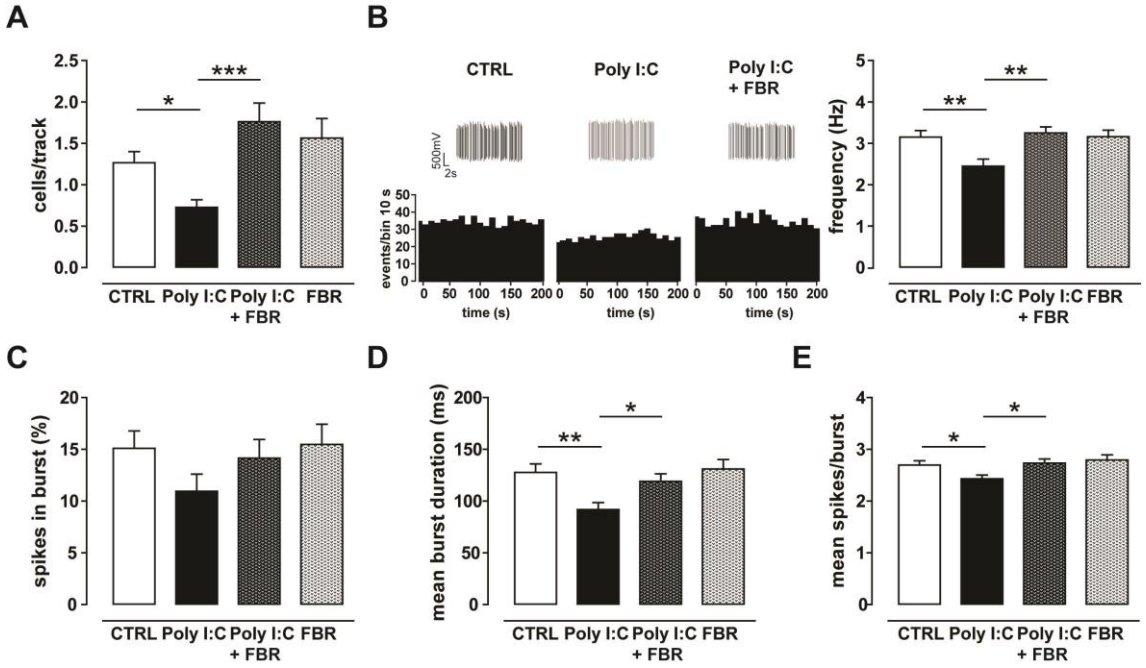

Figure 14. Effects of chronic fenofibrate during pregnancy on VTA dopamine cell activity alterations induced by Poly I:C prenatal treatment in male offspring. Fenofibrate chronic consumption prevented the Poly I:C-induced decrease in the number of spontaneously active VTA dopamine neurons (**A**) and in the firing rate (**B, on the left**). On the right (**B**), representative traces (top) and rate histograms (bottom) of putative dopamine neuron recordings from each group. (**C**) Spikes in burst expressed as percentage were not significantly different after prenatal exposure to Poly I:C and chronic fenofibrate diet. However, mean burst duration (**D**) and the mean spike/burst (**E**) aberrations were prevented by fenofibrate. Values are expressed as mean ± SEM. *P<0.05, **P<0.01, ***P<0.001.

5. DISCUSSION

The major findings of the present thesis are that in utero exposure to Poly I:C induces sex-specific detrimental effects and, the pharmacological modulation of PPARα during pregnancy partially prevents such deficits. As a result, prenatal Poly I:C treatment in the critical period for brain development, impairs sensory motor gating and electrophysiological features of VTA dopamine neurons in male but not female offspring. Moreover, the chronic activation of PPARα with fenofibrate normalized the number of spontaneously active VTA dopamine cells and their firing rate, as well as their bursting activity. The consequences of these results are relevant since point out that an imbalance in dopamine transmission is involved in schizophrenia and, the gender has a protective role on these alterations. Notably, our pharmacological strategy is the first evidence of a preventive role of a PPARα agonist in a MIA model of psychosis.

Dopaminergic abnormalities in Poly I:C-exposed male offspring have been previously demonstrated (Eyles et al., 2012; Hadar et al., 2015). In particular, the number of tyrosine hydroxylase (TH) immunoreactive cells in the VTA and TH-positive terminals in the striatum (Meyer et al., 2008a; Winter et al., 2009; Vuillermot et al., 2010) or dopamine output in striatal slices (Zuckerman et al., 2003) in the lateral globus pallidus and prefrontal cortex (Winter et al., 2009) were increased. Conversely, the electrophysiological data reported here show a reduced number of spontaneously active VTA dopamine cells and a decrease in their firing rate. Our results apparently diverge from the previous findings. However, by performing microdialysis experiments in terminal regions, we found higher baseline levels of dopamine in NAc in prenatally Poly I:C-treated male rats (see Appendix for details) when compared with controls. The discrepancy between the reduced

number and frequency of spontaneously VTA dopamine neurons and the enhanced dopamine output in NAc is apparently difficult to explain, but it is demonstrated that the firing activity and neurotransmitter release is not always correlated (Manta et al., 2013). In fact, dopamine release can be efficiently controlled at terminal regions without increased impulse activity of dopamine cells (Fawaz et al., 2009). Experiments in our laboratory excluded that this imbalance is the result of a dysregulation in D_2 dopamine receptor sensitivity and in DAT efficiency, since extracellular dopamine in NAc was similarly decreased by quinpirole and increased by cocaine, respectively, in both Poly I:C prenatally-exposed and controls (see Appendix for details). One additional hypothesis that can support our results is that the increased accumbal dopamine might trigger an inhibitory synaptic feedback loop that reduces or suppresses the firing rate of dopamine cells (Paladini et al., 2003; Watabe-Uchida et al., 2012). Dopamine release and firing rate as well as pattern of VTA dopamine neurons have also been found to be influenced by synchronized neural network activity in the neocortex. Such a functional coupling allows a bidirectional control of dopamine neurons by the PFC (Gao et al., 2007). Abnormal neural synchrony has been previous reported in schizophrenic subject and in animal models of the disorder (Uhlhaas & Singer, 2010; Dickerson & Bilkey, 2013; Gonzalez-Burgos et al., 2015). Thus, the disruption of dopamine neuron function could be due to an altered cortical state resulting from Poly I:C exposure. In fact, MIA has been shown to impair oscillatory activity in the hippocampus and the neocortex of offspring at adulthood, as similarly observed in human patients (Dickerson et al., 2010; Ducharme et al., 2012; Dickerson & Bilkey, 2013).

One can argue that our results contrast with previous findings in the mitotoxin methyl azoxymethanol acetate (MAM) model of schizophrenia (Lodge & Grace, 2007; Du & Grace, 2013). In these studies, an increase in the number of spontaneously active VTA

dopamine neurons, resulting in hyperdopaminergia, was reported. However, the considerable differences in the neuropathological mechanisms between the Poly I:C and the MAM models might explain the discrepancies. Exposure to the DNA chelating agent MAM, at GD 15 or earlier, has been found to induce gross neurodevelopmental abnormalities in macro- and microstructure, particularly in cortical regions, and microcephaly (Singh, 1980; Jongen-Relo et al., 2004). Instead, a later MAM administration during pregnancy (GD 17) elicited hyperdopaminergia with less severe abnormalities (Lodge & Grace, 2007; Du & Grace, 2013). On the other hand, MIA models recapitulate the effects of a maternal viral infection by activating microglia and cytokines production. These effects induce severe functional changes but are believed to induce minor anatomical abnormalities in the brain of offspring (Meyer, 2014).

As we expected, the electrophysiological alterations were paralleled by behavioral disruptions, as revealed by PPI deficits or disruptions in the social interaction and object recognition tests (see also Appendix). PPI is one of the most studied markers in schizophrenia and it is considered to have face, predictive and construct validity for this disease and for other psychiatric disorders (Swerdlow et al., 1994; Swerdlow & Geyer, 1998). Functionally, PPI provides an operational index of sensorimotor gating, a filtering mechanism that allows the brain to "gate out" and to process relevant stimuli from the amount of environmental sensory inputs (Braff & Geyer, 1990; Braff et al., 1995). Dysfunctions in sensorimotor gating are commonly observed in schizophrenic patients. In fact, the same forebrain regions involved in schizophrenia have been also found to regulate PPI (Takahashi et al., 2011), and PPI disruptions have been efficiently reversed by the benchmark antipsychotics (Braff et al., 2001).

Consistent with previous studies (Wolff & Bilkey, 2008; 2010), we found significant PPI impairments in adult rats prenatally exposed to Poly I:C. Nevertheless, PPI alterations have

not been constantly reported in MIA model, and some studies reported no PPI disruptions by Poly I:C exposure (Fortier et al., 2007; Van den Eynde et al., 2014).

The reason underling the discrepancies remains unknown, but it might arise from subtle differences in the timing of prenatal infections, the rat strain used or the settings of the PPI protocol. Unlike PPI parameters, no differences were found in the startle reflex, an unconscious defensive reaction to an intense acoustic stimulus, used to examine habituation process in schizophrenic subjects (Braff et al., 1995). Specifically, the average of startle reflex as well as the startle habituation and the latency were similar between groups. A possible explanation is that the startle parameters cannot be specifically assessed with our protocol. However, since PPI is extrapolated by startle values and PPI deficits occurred without concomitant alterations in the startle parameters, the reductions of PPI observed in Poly I:C male offspring likely reflect an effective impairment of sensorimotor gating.

In this thesis, it was also demonstrated a sex specificity in behavioral and electrophysiological alterations induced by prenatal Poly I:C exposure. Specifically, neither deficit in PPI nor changes in electrophysiological features of dopamine neurons were found in Poly I:C female littermates when compared to controls, indirectly suggesting a gender-related protection from MIA-induced aberrations. At best of our knowledge, only one study examined behavioral and cognitive disruptions in female rats of MAM-treated dams at GD 17. In this study, PPI impairments have been demonstrated (Hazane et al., 2009). Again, this discrepancy might be related to the differences between the two models. Moreover, neither the prenatal treatment nor the sex have been found to impact on PPI of animals exposed to MAM at GD 12, 13, 14 and 15, suggesting that behavioral deficits are less manifest following an earlier treatment (Jongen-Relo et al., 2004).

Studies investigating the effects of MIA in female offspring are still rare. Our results are consistent with the sex imbalance observed in neurodevelopmental disruption models of schizophrenia. In fact, a lower vulnerability of females to MIA-related dysfunctions has been demonstrated using behavioral tests validated in the psychopathology of the disease (Bitanihirwe et al., 2010; Romero et al., 2010; Zhang et al., 2012; Wischhof et al., 2015a; Wischhof et al., 2015b). An increase in latent inhibition effects (Bitanihirwe et al., 2010) and alterations in memory tasks (Zhang et al., 2012) were not observed in female offspring from Poly I:C-treated dams. Furthermore, following prenatal LPS exposure, deficits in the novel object recognition task were less marked in females (Wischhof et al., 2015b) and impairments in PPI and changes in startle reflex parameters have not been found (Wischhof et al., 2015b) or found to be less severe than in males (Romero et al., 2010). On the other hand, some studies reported that PPI disruptions were similar in both sexes (Howland et al., 2012) or were evident later in life in females than males (Borrell et al., 2002). Subtle differences in protocol settings or in the rat strains used might be involved in these inconsistent findings. Using our protocol, we can exclude a hormonal component on this effect. In fact, no difference related to the estrous cycle has been found in both Poly I:C- and vehicle-exposed offspring, in agreement with studies reporting that circulating hormones did not impact on sensory motor gating and startle reflex parameters (Plappert et al., 2005; Adams et al., 2008). Nevertheless, a disruption in PPI, but not in startle reflex, was previously demonstrated during proestrous, when estrogen level peaks (Koch, 1998). This result could underlie different experimental conditions that might allow the estrous cycle variations to affect PPI (Adams et al., 2008). Moreover, this finding is in contrast with the protective effect of estrogen on PPI impairments previously reported (Gogos et al., 2010).

In parallel to behavioral manifestation, morphological and molecular gender differences have been also demonstrated. A lower reduction of myelinated fibers and parvalbumin expression in the PFC, related with GABAergic abnormalities, were found in female offspring from LPS-treated dams (Wischhof et al., 2015b). Moreover, protein expression levels of prefrontal $5HT_{2A}$ receptors and their functionality were not changed (Wischhof et al., 2015a). These findings are apparently not related to serum cytokine profile. In fact, neither IL-1β nor IL-6 levels differ between male and female offspring exposed during pregnancy to LPS (Romero et al., 2010).

Consistently with this sex-related imbalance in schizophrenia, we also found that prenatal Poly I:C exposure did not disrupt dopamine transmission in female offspring. A study of Perez and colleagues (Perez et al., 2014) supports our result, as no differences in the firing of VTA dopamine neurons were found in female offspring in the schizophrenia MAM model. Contrary to our data, they demonstrated an increase in the number of spontaneously active dopamine cells across the estrous cycle. Given that the studies investigating the dopamine transmission in females and the evaluation of estrous cycle are limited, these discrepancies remain to be investigated. Notably, in humans, the activating transcription factor 4 (ATF4) and the phosphodiesterase 4B (PDE4B), two proteins coding Disc1-associated genes, were found to have protective effects against schizophrenia in women (Pickard et al., 2007; Qu et al., 2008). This result suggests that, beyond the hormones, also a genetic component confers a sex-specific protection to the disease.

In addition, MIA-induced abnormalities have been already demonstrated in juvenile animals. A decrease in the mean spontaneous firing rate of VTA dopamine cells recorded at PND 12-20 from male rats of Poly I:C-treated dams were found in our laboratory, performing ex vivo electrophysiology (unpublished data). Consistently, functional abnormalities in cultured hippocampal neurons from male offspring prenatally exposed to

Poly I:C have been demonstrated at neonatal/early postnatal ages, showing a lower spontaneous spike firing rate and a stronger inhibitory drive (Patrich et al., 2016).

An indirect relationship between hippocampus and VTA has been previously demonstrated (Floresco et al., 2001; Lodge & Grace, 2005). Specifically, the ventral subiculum (vSub) regulates the activity of VTA dopamine neurons through two inhibitory areas, NAc and ventral pallidum (VP). Given that the ventral subiculum (vSub) receives mainly projections from CA1 (Witter et al., 2014), a hypofunctionality of both areas, CA1 and vSub, could reduce the activity of the NAc, inhibiting the VTA dopamine cells via VP GABAergic neurons. Since behavioral changes do not appear before adolescence (Ozawa et al., 2006), it is likely that such alterations remain at subclinical level. Nonetheless, if female rats from Poly I:C-treated dams are protected since early life stages, it might confirm that the hormonal oscillations across the estrous cycle do not impact on our results, and it might be due to other components during brain maturation, such as genetic or epigenetic features.

Altogether, these findings point out that the Poly I:C MIA model can be considered as an appropriate animal model for neurodevelopmental disorders. The PPI disruption and the electrophysiological alterations found in male offspring at adulthood fulfill a multidimensional set of validity criteria predictive of psychiatric conditions in human.

In addition, the considerable differences detected between male and female littermates reflect the sex-specificity observed in schizophrenic patients. Adult female rats have been found to be protected against the detrimental effects of Poly I:C and this finding is not due to the impact of circulating hormones changes across the estrous cycle.

One important challenge is to investigate on potential therapeutic targets that might attenuate the disruptions induced in MIA models. To the best of our knowledge, few studies (Smith et al., 2007; Galvao et al., 2015; Kirsten et al., 2015) have verified the hypothesis that MIA-induced dysfunctions might be prevented with specific treatments

during pregnancy. In fact postnatal acute antipsychotic drug administration in adult or immature MIA offspring was demonstrated to block some of the behavioral deficits in influenza infected rodents and MIA models (Shi et al., 2003; Zuckerman et al., 2003; Ozawa et al., 2006; Meyer et al., 2008c; Piontkewitz et al., 2012; Farrelly et al., 2015). In addition, treatments with D-serine (Fujita et al., 2016), 7,8-dihydroxyflavone (Han et al., 2016), minocycline (Giovanoli et al., 2016) prevented the onset of cognitive deficits in adult offspring after MIA.

In this *scenario,* we demonstrated that the PPARα activation during pregnancy prevents the electrophysiological alterations in VTA dopamine neurons induced by MIA in male offspring. Our result is consistent with the anti-inflammatory properties of NAEs, endogenous PPARs ligands (Sepe et al., 1998; LoVerme et al., 2005; LoVerme et al., 2006). In addition, the administration of fenofibrate has been found to protect against the impairments associated with neurodegenerative disorders, such as Parkinson's (Kreisler et al., 2007; Barbiero et al., 2014a) and Alzheimer's disease, as well as for neurological (i.e. epilepsy) (Citraro et al., 2013; Puligheddu et al., 2013) or psychiatric disorders (i.e. schizophrenia) (Rolland et al., 2012). Evidence suggests that fenofibrate might minimize the oxidative stress implicated in the neuropathogenesis of these diseases (Kreisler et al., 2007; Rolland et al., 2012; Barbiero et al., 2014a). Specifically, the expression and activation of the antioxidant enzymes, glutathione peroxidase and superoxide dismutase, were induced by PPARα stimulation (Bordet et al., 2006; Barbiero et al., 2014a; Barbiero et al., 2014b). Since MIA has been found to be associated to a transient oxidative stress in fetal brain (Lante et al., 2007), we cannot exclude that fenofibrate exerts its protective effect through this pathway. Notably, a dopamine system dysfunction has been demonstrated as a result of oxidative stress (Juarez Olguin et al., 2016), supporting the

hypothesis that the reactive oxygen species may induce defects in the fetal brain development and impact dopamine activity in offspring.

Similarly, in MIA models the stimulation of the TLR-3 pathway induced by Poly I:C modulates the activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and the activator protein 1 (AP-1) through the mitogen-activated protein kinases (MAPK) cascade (Jiang et al., 2003), leading to the synthesis of several proinflammatory cytokines, such as IL-1, IL-6, TNFα and IFNs (Boksa, 2010; Bronson et al., 2011; Meyer, 2014; Reisinger et al., 2015). The passage across the placenta of maternal cytokines into the fetal compartment elicits an inflammatory response in fetuses and might induce long-term alterations in the brain of offspring. The inhibition of this mechanism by fenofibrate could result in the protective effects we reported here. In fact, PPARα activation has been found to negatively regulate the signaling of NFκB and the AP-1 (Delerive et al., 1999; Xing et al., 2007; Ramanan et al., 2008; Barbiero et al., 2014b), the expression of TNFα (Hill et al., 1999) as well as the production of cytokines (Yang et al., 2008) and INFs (Cheng et al., 2010).

The precise mechanism through which PPARα stimulation exerts its protective action in our model is not completely understood. Nonetheless, it is well established that PPARα activation induces transcriptional effects (transactivation and transrepression). Upon ligand binding, the PPARα heterodimerizes with RXR receptor and binds to the DNA at the specific sites PPRE in the promoter of target genes. The final result is the modulation of genes transcription via transactivation, in particular those involved in lipid metabolism, feeding behavior and energy balance (Pistis & Melis, 2010).

A great deal of evidence has indicated that PPARα controls various types of inflammatory response via transrepression (Daynes & Jones, 2002). These functions are mediated largely through the abilities of PPARα to transrepress (Ricote & Glass, 2007) functions of many

activated transcription factors, such as NF-κB and AP-1, signal transducers and activators of transcription (STATs) and nuclear factor of activated T cells (NFAT) (Daynes & Jones, 2002; Wahli & Michalik, 2012).

In this *scenario*, it is presumable that fenofibrate administration during pregnancy prevents the inflammatory process in a genomic-dependent manner. Moreover, the activation of PPARα has been found to increase the brain synthesis of OEA and PEA (Melis et al., 2013a). These molecules display marked anti-inflammatory properties in several models of acute and chronic inflammation (Sepe et al., 1998; Pistis & Melis, 2010).

These NAEs, which in turn sustain PPARα activity, elicit a positive feedback and might decrease inflammation. Similarly, we found that OEA and PEA levels were increased by α7-nAChRs stimulation (Melis et al., 2013b). This result is consistent with the anti-inflammatory role of the α7-nAChRs in the fetal brain development (Wu et al., 2015).

Beside the genomic effects, a non-genomic mechanism occurring in a short time scale was also demonstrated in our laboratory that might concur to fenofibrate protective activity in our MIA model. We discovered that α7-nAChRs modulates the excitability of dopamine neurons through phosphorylation of the β2-nAChRs subunit (Melis et al., 2013b).

An imbalance in dopamine and cholinergic systems might play a role in a critical phase of brain development and, consistently, has been found associated to psychiatric disorders (Scarr et al., 2013).

Unlike electrophysiological result, we found that prenatal fenofibrate exposure did not attenuate PPI impairments. A previous study reported a "*disease-modifying therapeutic effect*" of fenofibrate on PPI alterations induced by kainic acid (Rolland et al., 2012). However, both kainic acid and fenofibrate were administered postnatally whereas our treatments were carried out to the pregnant dams. Consequently, we cannot exclude that the exposure to the drug inducing neurodevelopmental modifications in a different life time

(pre- versus post-natal) could have a different impact on sensorimotor gating. On the other hand, the discrepancy might reside in the different neurobiological substrates involved in response to the pro-oxidative drug kainic acid and the proinflammatory cytokines inductor Poly I:C.

Further experiments are certainly needed in order to explain the lack of efficacy of fenofibrate treatment on PPI, since the sample size could be too small to have a reliable statistical power in the test used. Indeed, more experiments have been already schedule and will be completed in the near future. Moreover, schizophrenia is a complex disorder, in which dopamine is one of the major but not the only neurotransmitter involved, thus we cannot exclude that the fenofibrate exposure could not completely revert all the effects induced by MIA, particularly those related to specific behavioral disruptions. Additional behavioral tests, such those addressing cognitive performance and social interaction, should be carried out to integrate our findings.

As far as we know, our study is the first to investigate how the fenofibrate administration during pregnancy impact *per se* and on the neurodevelopmental aberrations of dopaminergic activity and sensorimotor gating.

Notably, we also found that the transient weight loss of dams following Poly I:C treatment was not prevented by fenofibrate. Nevertheless, rats prenatally exposed to fenofibrate were protected by the damaging effects of the Poly I:C treatment on VTA dopamine activity. This result might be explained by considering that PPARα and RXRα are expressed by two areas of the rat placenta involved mainly in uterine wall invasion and in cytokines production as well as in the transport barrier of nutrients and waste between dam and fetuses (Wang et al., 2002). It can be, therefore, speculated that fenofibrate exerts its neuroprotective effects preventing the transplacental passage of proinflammatory cytokines from maternal to fetal compartment.

Overall, our results provide evidence of the neuroprotective and anti-inflammatory effects of fenofibrate on neurodevelopmental aberrations induced by prenatal exposure to Poly I:C. Such preclinical finding might be predictive for a therapeutic use of the PPARα agonist during pregnancy to reduce/prevent the neuroinflammation and the risk to develop schizophrenia and related disorders in humans.

Fenofibrate is not recommended in pregnancy by the Food and Drug Administration (FDA) and by the European Medicine Agency (EMA) for potential hazardous effect on the fetus, as animal studies have revealed evidence of embryofetal toxicity and fetal abnormalities, but only at very high doses that also produced maternal toxicity. Its use during pregnancy is allowed weighing the potential benefits and risks for mother and unborn child. Notably, no fenofibrate-induced noxious effects in the development of fetuses have been observed in clinical studies (Ofori et al., 2007; Whitten et al., 2011; Sunman et al., 2012). At doses equivalent to those normally prescribed in clinics, this PPARα agonist was not found to induce teratogenesis in experimental subjects (Blane, 1987; Ujhazy et al., 1989; Peraza et al., 2006), inducing a mild fetotoxicity only at very high dose (Ujhazy et al., 1989).

In conclusion, given that the use of fenofibrate has been approved for decades in humans for the treatment of lipid metabolism disorders, by FDA, translational studies might be facilitated, although studies about the potential side effects of PPARα on fetal neurodevelopment are definitely needed. Nevertheless, our findings are relevant to increase knowledge about neurodevelopmental disorders and, it might represent an early-life therapeutical intervention in vulnerable subjects.

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APPENDIX

OXFORD

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RESEARCH ARTICLE

Maternal Immune Activation Disrupts Dopamine System in the Offspring

Antonio Luchicchi, PhD; Salvatore Lecca, PhD; Miriam Melis, PhD; Marta De Felice, BSc; Francesca Cadeddu, PhD; Roberto Frau, PhD; Anna Lisa Muntoni, MD; Paola Fadda, PhD; Paola Devoto, PhD; Marco Pistis, MD

Division of Neuroscience and Clinical Pharmacology, Department of Biomedical Sciences, University of Cagliari, Monserrato, Italy (Drs Luchicchi, Lecca, Melis, Ms De Felice, Drs Cadeddu, Frau, Fadda, Devoto, and Pistis); Neuroscience Institute, National Research Council of Italy, Section of Cagliari, Italy (Drs Muntoni and Pistis).
Present address (A.L.): Department of Integrative Neurophysiology, Center for Neurogenomics and Cognitive Research, Neuroscience Campus Amsterdam, Vrije Universiteit, Amsterdam, Netherlands.
Present address (S.L.): Institut du Fer à Moulin, 75005, Paris, France; Inserm, UMR-S 839, 75005, Paris, France.

Correspondence: Marco Pistis, MD, University of Cagliari, Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, Cittadella Universitaria di Monserrato, 09042 Monserrato (CA), Italy (mpistis@unica.it).

Abstract

Background: In utero exposure to maternal viral infections is associated with a higher incidence of psychiatric disorders with a supposed neurodevelopmental origin, including schizophrenia. Hence, immune response factors exert a negative impact on brain maturation that predisposes the offspring to the emergence of pathological phenotypes later in life. Although ventral tegmental area dopamine neurons and their target regions play essential roles in the pathophysiology of psychoses, it remains to be fully elucidated how dopamine activity and functionality are disrupted in maternal immune activation models of schizophrenia.
Methods: Here, we used an immune-mediated neurodevelopmental disruption model based on prenatal administration of the polyriboinosinic-polyribocytidilic acid in rats, which mimics a viral infection and recapitulates behavioral abnormalities relevant to psychiatric disorders in the offspring. Extracellular dopamine levels were measured by brain microdialysis in both the nucleus accumbens shell and the medial prefrontal cortex, whereas dopamine neurons in ventral tegmental area were studied by in vivo electrophysiology.
Results: Polyriboinosinic-polyribocytidilic acid-treated animals, at adulthood, displayed deficits in sensorimotor gating, memory, and social interaction and increased baseline extracellular dopamine levels in the nucleus accumbens, but not in the prefrontal cortex. In polyriboinosinic-polyribocytidilic acid rats, dopamine neurons showed reduced spontaneously firing rate and population activity.
Conclusions: These results confirm that maternal immune activation severely impairs dopamine system and that the polyriboinosinic-polyribocytidilic acid model can be considered a proper animal model of a psychiatric condition that fulfills a multidimensional set of validity criteria predictive of a human pathology.

Keywords: Schizophrenia, dopamine, electrophysiology, microdialysis

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Introduction

In the last decades, an association between in utero insults and enhanced risks for psychosis during adulthood has been underscored by both clinical and preclinical observations (Boksa, 2010). In particular, among the vast number of environmental factors deemed responsible of prenatal insults, the exposure to maternal infections in utero, and specifically to maternal immune response products, has attracted special interest. In fact, epidemiological studies show increased risk for schizophrenia and related disorders following prenatal maternal exposure to infection or inflammation (Brown and Derkits, 2010). In line with this notion, several studies have demonstrated that by mimicking a viral or bacterial infection in pregnant dams at specific ages of pregnancy (mainly comparable with the second trimester of human pregnancy), the progeny displays a wide array of psychotic-like anatomical, behavioral, and neurochemical aberrations (Patterson, 2002; Meyer et al., 2005; Patterson, 2009; Boksa, 2010).

One of the most studied rodent models of neurodevelopmental psychosis induced by maternal infection is obtained by exposing fetuses to the administration to the mother of a proinflammatory cytokine inductor, a double stranded RNA named polyriboinosinic-polyribocytidilic acid [poly(I:C)]), during gestational day (GD) 14 to 16 (Zuckerman et al., 2003). Poly(I:C) mimics the acute phase of viral infection by inducing a robust inflammatory response leading to the synthesis of several proinflammatory cytokines, such as interleukin-1β, interleukin-6, and tumor necrosis factor-α (Zuckerman et al., 2003; Meyer et al., 2006). Interestingly, this particular type of prenatal immune activation elicits in rodent offspring a broad spectrum of behavioral impairments commonly described in schizophrenic patients, such as deficits in sensorimotor gating, latent inhibition, and social interaction (Zuckerman et al., 2003).

Furthermore, poly(I:C) rats show hypersensitivity to the effects of amphetamine and phencyclidine, compounds that are known to produce positive, negative, and cognitive schizophrenia-like symptoms in healthy humans (Meyer et al., 2006; Ozawa et al., 2006). In addition, alongside of disrupted behavior, studies have also reported alterations in many neurotransmitter systems such as dopamine (Meyer et al., 2008; Vuillermot et al., 2010), acetylcholine (Wu et al., 2015), and GABA (Richetto et al., 2015).

In particular, dopaminergic abnormalities are considered a final common pathway in the neuropathogenesis of schizophrenia and remain central in both treatment and etiology (see Eyles et al., 2012 and references therein). Consistently, previous studies on maternal immune activation have found abnormalities in dopamine system in the offspring, such as increased numbers of tyrosine hydroxylase (TH) immunoreactive cells in the VTA and TH-positive terminals in the striatum (Meyer et al., 2008; Winter et al., 2009; Vuillermot et al., 2010) or dopamine output in striatal slices (Zuckerman et al., 2003) in the lateral globus pallidus and prefrontal cortex (Winter et al., 2009).

Although evidence is mounting that dopamine transmission is indeed impaired in several neurodevelopmental models of psychosis, including the poly(I:C) model, no study to our knowledge has yet combined behavioral, neurochemical, and electrophysiological analysis to assess and characterize this disruption.

To this aim, in the present study, we used the poly(I:C) neurodevelopmental model in rats. Male adult offspring were behaviorally assessed for deficits in sensorimotor gating, short-term memory impairment, and social interaction. In these animals, mesolimbic dopamine neuron activity was examined by in vivo electrophysiology, and extracellular dopamine levels were measured by brain microdialysis.

Materials and Methods

Prenatal Treatment and Subjects

Female Sprague Dawley rats (Harlan, Italy) were mated at the age of 3 months, and the first day after the copulation was defined as GD 1. We set our protocol basically following that already published by Zuckerman et al. (2003). At GD 15, pregnant dams were given either a single dose of poly(I:C) (4.0 mg/kg, i.v.; InvivoGen, San Diego, CA) or an equivalent volume of endotoxin free saline solution in the lateral vein of the tail. To minimize the animal discomfort, we induced pregnant dams with isoflurane (2%) anesthesia, and then we kept the anesthesia during the whole injection with an infusion system. Pregnant dams were weighed for the first 3 days after the administration of either poly(I:C) or saline to evaluate weight loss as reported by previous investigations (Zuckerman et al., 2003). Consistent with previous studies (Wolff and Bilkey, 2010), poly(I:C)-treated dams lost 2.6 ± 2.5 g (n = 11) 24 hours after the injection, whereas saline-treated dams increased their weight by 8.3 ± 2.1 g (n = 7). This difference is statistically significant ($P < .01$). The offspring were randomly assigned according to postnatal age to different experimental protocols (see Figure 1 for a schematic description).

Adult (60–90 days) Sprague Dawley rats, offspring of poly(I:C)- or vehicle-treated dams, were bred in our facilities and were used for the experiments described below. We housed animals in groups of 3 to 6 in standard conditions of temperature and humidity under a 12-h-light/-dark cycle (with lights on at 7:00 AM) with food and water available ad libitum.

All experiments were approved by the University of Cagliari Committee on Animal Use and Care and performed in strict accordance with the EEC Council Directive of 24 November 1986 (86/609). We made all efforts to minimize animal discomfort and to reduce the number of animals used.

Figure 1. Schematic representation of the experimental protocol. Polyriboinosinic-polyribocytidilic acid [poly(I:C)] treatment during pregnancy consisted in a single i.v. injection of poly(I:C) (4 mg/kg) or vehicle (sterile pyrogen-free saline) at the 15th gestational day (GD). Behavioral experiments were performed between postnatal day (PND) 60 and 70, whereas in vivo electrophysiology and microdialysis between 70 and 90 PND.

Behavioral Experiments

Prepulse Inhibition (PPI) of Startle Reflex

Startle and PPI were performed as previously described by Frau et al. (2014) with slight modifications. The apparatus used for detection of startle reflexes (Med Associates, St Albans, VT) consisted of 4 standard cages placed in sound-attenuated chambers with fan ventilation. Each cage consisted of a Plexiglas cylinder of 9-cm diameter mounted on a piezoelectric accelerometric platform connected to an analogue-digital converter. Two separate speakers conveyed background noise and acoustic bursts, each one properly placed to produce a variation of sound within 1 dB across the startle cage. Both speakers and startle cages were connected to a main PC, which detected and analyzed all chamber variables with specific software. Before each testing session, acoustic stimuli and mechanical responses were calibrated via specific devices supplied by Med Associates.

On the testing day, each rat was placed in the cage for a 5-minute acclimatization period consisting of 70-dB white noise background, which continued for the remainder of the session. Each session consisted of 3 consecutive sequences of trials (blocks). During the first and third blocks, rats were presented with only 5 pulse-alone trials of 115 dB. In the second block was delivered a pseudorandom sequence of 50 trials, including 12 pulse-alone trials; 30 trials of pulse preceded by 74-, 78-, or 86-dB prepulses (10 for each level of prepulse loudness); and 8 no-stimulus trials, where only the background noise was delivered. Inter-trial intervals were selected randomly between 10 and 15 seconds, while the inter-stimulus intervals were set at 100 milliseconds.

Novel Object Recognition (NOR) Test

NOR test was performed in adult rats (60–70 postnatal days [PND]) according to Spano et al. (2010). Each rat, after a habituation trial (10 minutes) on the arena (60 × 60 cm), was placed into the arena and allowed to explore 2 identical objects for 10 minutes (familiarization phase or T1). The second trial (choice phase or T2) started after an interval of 1 hour and lasted 3 minutes, with 2 objects placed in the same positions: one novel and the other a third copy of the object used in T1. The objects to be discriminated were made of glass, plastic, or metal, devoid of any natural significance, and were carefully cleaned with H_2O_2 between each trial to avoid olfactory cues. Exploratory behavior was defined as the animal directing its nose toward the object at a distance ≤2 cm and/or touching it with the nose. Conversely, turning around, climbing over, or sitting on the object was not considered as exploratory behavior. The following parameters were examined: (1) total time spent exploring the objects during T1 and T2; (2) latency of first approaches, that is, time taken by animal to approach any of the 2 objects when it is placed into the experimental arena; and (3) frequency of approaches, that is, numbers of approaching the 2 objects. A recognition index was calculated for each animal using the formula N/(N+F) × 100 (N = time spent exploring the novel object; F = time spent exploring the familiar one).

Social Interaction (SI) Test

SI test was performed in adult rats (60–70 PND) according to Spano et al. (2010). The experimental animal was placed into the arena (60 × 60 cm) with a novel unfamiliar conspecific rat of the same sex and of similar weight. Each test was performed with a novel rat that was exposed to neither the same test nor to any pharmacological treatment. Behavior was recorded for 10 minutes, and time spent by the experimental rat in social interactions (sniffing, following or grooming the partner, boxing, and wrestling) was monitored. The box was cleaned with H_2O_2 after each experimental session.

In Vivo Electrophysiological Experiments

Extracellular single unit recordings from VTA dopamine neurons in anesthetized male Sprague Dawley rats were carried out as described previously (Melis et al., 2008, 2009). We anesthetized animals (70–90 PND) with urethane (1.3 g/kg, i.p.), cannulated their femoral vein for i.v. administration of supplemental doses of the anesthetic, and placed them in the stereotaxic apparatus (Kopf, Tujunga, CA) with their body temperature maintained at 37 ± 1°C by a heating pad. The scalp was retracted and one burr hole was drilled above the parabrachial nucleus of the VTA (6.0 mm posterior from bregma, 0.3–0.6 mm lateral from midline) for the placement of a recording electrode. We localized structures according to the stereotaxic atlas of Paxinos and Watson (2007). Single unit activity of neurons located in the VTA (V 7.0–8.0 mm from the cortical surface) was recorded extracellularly with glass micropipettes filled with 2% pontamine sky blue dissolved in 0.5 M sodium acetate (impedance 2–5 MΩ). Single unit activity was filtered (bandpass 500–5000 Hz), and individual spikes were isolated by means of a window discriminator (Neurolog Instruments, Digitimer), displayed on a digital storage oscilloscope (TDS 3012, Tektronics), and digitally recorded. We sampled experiments on line and off line with Spike2 software (Cambridge Electronic Design, Cambridge, UK) by a computer connected to CED 1401 interface (Cambridge Electronic Design). The electrophysiological properties of spontaneously active VTA dopamine neurons were sampled by making 9 stereotaxic descents separated from each other by 200 μm, the sequence which was kept constant from animal to animal. Single units were identified according to already published criteria (Grace and Bunney, 1983; Ungless et al., 2004). We selected VTA dopamine neurons when all criteria for identification were satisfied, including: firing rate ≤10 Hz, duration of action potential ≥2.5 ms, and inhibitory responses to hindpaw pinching. Each neuron was recorded for 2 to 3 minutes. Different electrophysiological parameters were evaluated, the first being the number of spontaneously active dopamine cells per electrode track (ie, population activity). The other measurements were: (1) basal firing rate, (2) CV (SD of interspike intervals divided by the mean interspike interval; a measure of firing regularity), and (3) bursting activity. The onset of a burst was identified by 2 consecutive spikes with an interspike interval <80 milliseconds, and the termination of a burst was defined as an interspike interval exceeding 160 milliseconds (Grace and Bunney, 1983). The degree and mode of bursting activity were measured by the percentage of spikes that occurred in bursts (percentage of spikes in burst), mean spikes per burst, and burst duration.

At the end of each recording section, negative DC (10 mA for 15 minutes) was passed through the recording electrode to eject Pontamine sky blue, which allowed the identification of the recorded cells. Brains were removed and fixed in 8% formalin solution. We identified the position of the electrodes microscopically on serial sections (60 μm) stained with Neutral red.

Microdialysis Experiments

Extracellular dopamine levels were evaluated by microdialysis in freely moving animals as previously described (Devoto et al., 2015). Rats (70–90 days, 300–400 g) were anesthetized with Equithesin (0.97 g pentobarbital, 2.1 g $MgSO_4$, 4.25 g chloral hydrate, 42.8 mL propylene glycol, 11.5 mL 90% ethanol, distilled

water up to 100 mL, 5 ml/kg, i.p.) and placed in a stereotaxic apparatus (Kopf). Animals were implanted with vertical microdialysis probes (membrane AN 69-HF, Hospal-Dasco, Bologna, Italy; cut-off 40 000 Dalton) in the shell of the nucleus accumbens (NAc; AP 1.8, L ± 0.7, V -8.5 mm from bregma, 2 mm active membrane length) or in the medial prefrontal cortex (mPFC; AP 3.0, L ± 0.6, V -6.5 mm from bregma, 4-mm active membrane length) according to the coordinates of the atlas by Paxinos and Watson (1997).

The day after probe implantation, an artificial cerebrospinal fluid (147 mM NaCl, 4 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, pH 6–6.5) was pumped through the dialysis probes at a constant rate of 1.1 µL/min via a CMA/100 microinjection pump (Carnegie Medicine, Stockholm, Sweden). Samples were collected every 20 minutes and dopamine and 3,4-Dihydroxyphenylacetic acid (DOPAC) simultaneously analyzed by HPLC with electrochemical detection. The HPLC systems were equipped with 3.0- x 150-mm C18 (3.5 µ) Symmetry columns (Waters, Milan, Italy) kept at 30°C by Series 1100 thermostats (Agilent Technologies, Waldbronn, Germany) and ESA Coulochem II detectors (Chelmsford, MA). The mobile phase consisted of 80 mM Na_2HPO_4, 0.27 mM EDTA, 0.58 mM sodium octyl sulfate, 10% methanol, 4% acetonitrile, pH 2.8 with H_3PO_4, delivered at 0.35 mL/min; the Coulochem analytical cell first electrode was set at +200 mV, the second at -300 mV. In these conditions, the detection limit (signal to noise ratio 3:1) was 0.3 pg of dopamine on column.

Basal extracellular values are expressed as pg/20 µL dialysate injected on column immediately after collection. Changes produced by drug treatments were calculated as percent of mean basal value obtained from 3 consecutive samples with a variance not exceeding 10%.

At the end of experiment, rats were killed by decapitation, and the brain was frozen and dissected with a cryostat for visual inspection of dialysis probe position.

Statistical Analysis

Startle response was based on the first positive wave that meets the minimum wave criteria and determined as mean startle amplitude of the pulse-alone trials relative to second block. Startle habituation across the 2 halves of the second block was evaluated as percent inter-block ratio using the following formula: (mean startle amplitude for first half of the second block/mean startle amplitude for second half of the second block) ×100. Latency to startle was based on the first peak value across pulse-alone trials of the second block. "Arbitrary units" were calculated by the Med Associates apparatus software by proportionally converting the analog voltage signal recorded by the startle sensor (ranging from -10 to +10V) to a digital unit, within a range of values between –2048 and +2048. The % PPI was calculated only on the values relative to the second block using the following formula: ([mean startle amplitude pulse alone trials-mean startle amplitude prepulse+pulse trials]/mean startle amplitude for pulse alone trials) × 100.

According to normality and homoscedasticity assumptions verified by Kolmogorov-Smirnov and Bartlett's tests, data were compared across groups by Student's *t* test (startle parameters) and 2-way ANOVA for repeated measures (PPI values). Significance threshold was set at 0.05.

For the NOR test, the time spent in exploring objects during T1 was calculated by summating the time spent exploring each identical object to produce a single score. For the SI test, the amount of time spent sniffing, following the partner, wrestling/boxing, or grooming were summated for each rat to produce a single score. The unpaired Student's *t* test was used to compare differences between experimental groups. In all tests statistical significance was set at $P < .05$.

For electrophysiological and microdialysis studies, averaged data from different experiments are presented as mean ± SEM. Statistical significance was assessed using 1- or 2-way ANOVA for repeated measures followed by either Dunnett's or *t* test where appropriate.

Results

The Offspring of poly(I:C)-Treated Dams Display Abnormal PPI, NOR, and SI

To validate our model, we first carried out behavioral experiments in poly(I:C) rats and controls at adulthood (PND 60–70). The acoustic startle response was measured at PND 60 to 70. As shown in Figure 2A-C, poly(I:C) treatment did not affect overall startle reflex values. Accordingly, no significant effects for mean startle amplitude ($t_{(36)}=0.64$, n = 15–23, $P=.52$, Student's *t* test) (Figure 2A), latency to peak ($t_{(36)}=0.49$, n = 15–23, $P=.62$, Student's *t* test) (Figure 2B), and startle habituation ($t_{(36)}=0.35$, n = 15–23, $P=.72$, Student's *t* test) (Figure 2C) between control and poly(I:C) groups were found. Subsequently, a 2-way ANOVA (with treatment as independent factor and prepulse levels as repeated measures) assessed that the maternal infection with poly(I:C) significantly reduced PPI (main effect of treatment: $F_{(1,108)}=14.92$, $P<.001$; main effect of prepulse level: $F_{(2,108)}=12.99$, $P<.001$; interaction treatment × prepulse level: $F_{(2,108)}=2.1$, $P=.12$, NS) (Figure 2D).

Next, we explored the presence of memory deficits and social withdrawal by means of the NOR test and SI test, respectively. During the familiarization phase (ie, T1), no difference was observed in the time of interaction with the objects between poly(I:C) and control rats (time of interaction with left and right objects: controls: 24.5 ± 6.0 and 25 ± 5.9 seconds; poly(I:C) 42.3 ± 16.3 and 44.8 ± 17.7; n = 6, $P>.05$, Student's *t* test). During the choice phase (ie, T2), the poly(I:C) offspring exhibited a significantly lower preference for the novel object than control animals, as indicated by the ratio between the time spent with the novel and the familiar object, respectively (controls: 70 ± 5.6% and poly(I:C) 42.4 ± 3.65%, $t_{(10)}=4.129$, $P<.01$, n = 6) (Figure 2E). Thus, the poly(I:C) offspring expressed abnormally low sensitivity to a novel object, which is indicative of poor short-term memory.

A separate group of animals, poly(I:C) and controls, underwent the SI test. Poly(I:C)-exposed animals displayed a reduced time of interaction with the conspecific rats compared with controls (129.7 ± 7.3 vs 231 ± 25.4 seconds, n = 7, $t_{(12)}=3.83$, $P<.01$, Student's *t* test) (Figure 2F), while no differences were found in the number of contact (data not shown).

Taken together, these results suggest that maternal immune activation induces several behavioral alterations in the offspring that have been associated with schizophrenia-like symptoms in humans and support previous literature on the validity of poly(I:C) administration during pregnancy as a model for schizophrenia and related psychoses.

The Offspring of poly(I:C)-Treated Mothers Display Elevated Levels of Dopamine in the Shell of the NAc but Not in the mPFC

As dopamine imbalances are a hallmark of schizophrenia, we assessed whether offspring of poly(I:C)-treated dams display changes in baseline dopamine levels measured in the shell

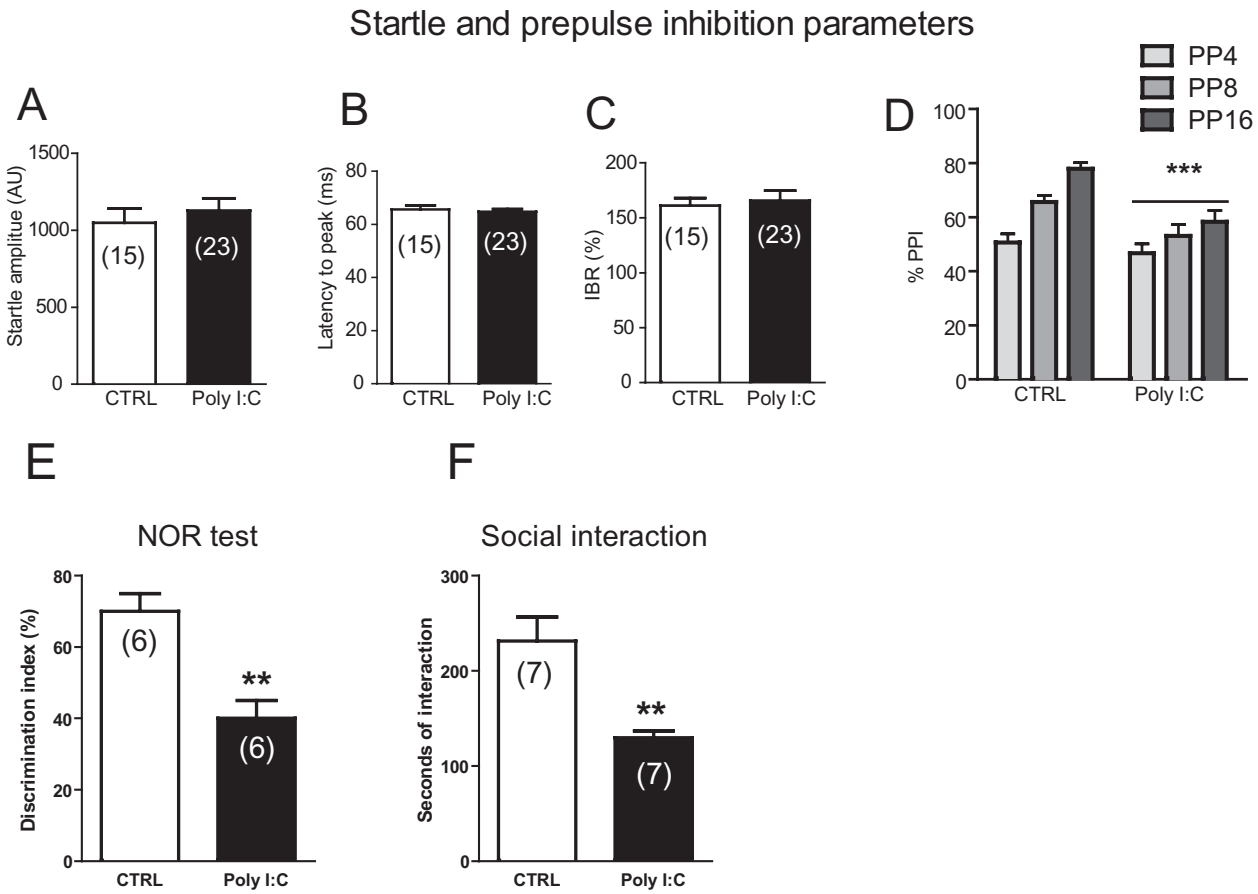

Figure 2. Behavioral abnormalities in polyriboinosinic-polyribocytidilic acid [poly(I:C)] offspring. Effects of poly(I:C) on startle reflex (A-C) and prepulse inhibition (PPI) (D) parameters. Graphs show that offspring from poly(I:C)-treated mothers exhibited impairments in PPI but not in startle indices. Values are expressed as mean ± SEM. ***$P < .001$ vs rats treated with vehicle (main effect of treatment). Prepulses are indicated by the intensity corresponding to decibels above background noise. AU, arbitrary units; %IBR, percent inter-block ratio; ms, milliseconds. For further details, see text. (E) Graphs show that offspring of poly(I:C)-treated mothers exhibited reduced discrimination index toward a novel object and (F) reduced social interactions. Data are expressed as means ± SEM. *$P < .01$ vs rats treated with vehicle (CTRL group).

of the NAc and in the mPFC by brain microdialysis in behaving animals. For experiments in the NAc, 14 control and 18 poly(I:C) rats were used. Basal values (mean ± SEM, expressed as pg/20 µL dialysate) are shown in Figure 3A. According to the hypothesis of enhanced dopamine transmission in poly(I:C) animals, extracellular dopamine levels were significantly higher in poly(I:C)- vs controls (+79%, $t_{(22.49)} = 2.536$, $P = .0186$, Student's *t* test with Welch's correction) (Figure 3A). No difference was found in DOPAC concentration (controls = 1627 ± 236, n = 9, Poly(I:C) = 2015 ± 274 pg/20 µL, n = 12, $t_{(19)} = 1.0.72$, $P = .297$, Student's *t* test with Welch's correction; data not shown).

To test the functionality of D2 dopamine receptors in controlling extracellular dopamine, we administered the selective D2 agonist quinpirole. Quinpirole (0.2 mg/kg, s.c.) decreased extracellular dopamine levels to about 40% of baseline level in both control and poly(I:C) rats (Figure 3C). Two-way ANOVA revealed a significant effect of time ($F_{(5, 45)} = 20.27$; $P < .0001$) but no effect of treatment ($F_{(1, 9)} = 0.08199$; $P = .7811$) or time x treatment interaction ($F_{(5, 45)} = 0.3689$; $P = .8672$). The functionality of dopamine transporter (DAT) was tested by local perfusion of cocaine (100 µM) into the NAc through the microdialysis probe. Cocaine perfusion increased extracellular dopamine levels to a maximum of about 1500% in both groups. Again, ANOVA analysis yielded a significant value for time ($F_{(5, 95)} = 29.57$; $P < .0001$) but no effect for treatment ($F_{(1, 19)} = 0.1234$; $P = .7292$) or time x treatment interaction ($F_{(5, 95)} = 0.2001$; $P = .9617$). However, following cocaine perfusion, extracellular dopamine levels expressed as absolute values were significantly higher in poly(I:C) rats compared with controls (controls: 60.2 ± 13.8 pg/sample, n = 9; poly(I:C) = 117.50 ± 23 pg/sample, n = 12; effect of poly(I:C) treatment: $F_{(1, 19)} = 12.98$, $P < .01$, 2-way ANOVA). Experiments in the mPFC did not show differences in baseline dopamine and DOPAC levels between poly(I:C) and controls. For these experiments, 11 controls and 12 poly(I:C) rats were used. Basal values are shown in Figure 3B. Dopamine levels did not differ in poly(I:C)- vs controls (Student's *t* test with Welch's correction, $t_{(21)} = 1.237$, $P = .22$) (Figure 3B). No difference was found in DOPAC concentration (controls = 115.4 ± 15.5, poly(I:C) = 123.7 ± 12.7 pg/20 µL, n, $t_{(21)} = 0.4174$, $P = .68$, Student's *t* test with Welch's correction; data not shown).

Effect of Prenatal poly(I:C) on Dopamine Cell Activity and Functionality in Vivo

The increase in extracellular dopamine in the shell of the NAc prompted us to study dopamine cell activity in the VTA. To address this question, a population sampling of dopamine cells was performed in the parabrachial nucleus of the VTA (Figure 4A). Although the VTA shows a degree of heterogeneity, the subregion where we recorded from contains the largest density of dopamine neurons projecting to the NAc compared with the more medial levels of the posterior VTA (Yamaguchi et al., 2011; Lammel et al.,

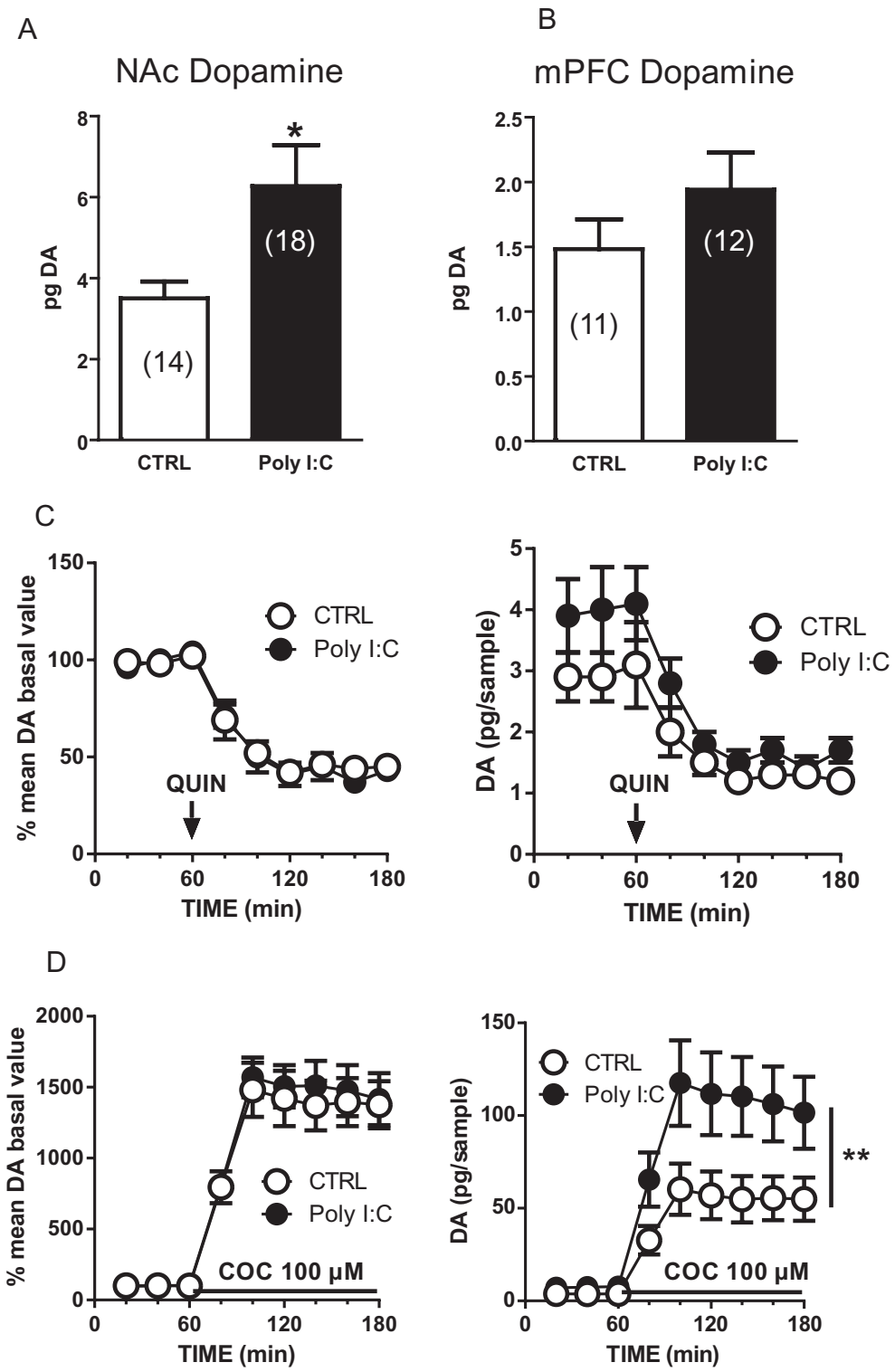

Figure 3. Enhanced extracellular dopamine (DA) levels in the nucleus accumbens (NAc) shell of polyriboinosinic-polyribocytidilic acid [poly(I:C)]-treated rats and effects of quinpirole and cocaine. Extracellular dopamine concentrations in the NAc shell (A) and medial prefrontal cortex (mPFC) (B) of control (CTRL) and poly(I:C) rats. (C) These graphs show the effect of systemically administered quinpirole (QUIN, 0.2 mg/kg, s.c., n = 5–6) on extracellular dopamine levels in the NAc shell expressed as percent of baseline (left) or in absolute values (pg/sample, right). (D) Locally perfused cocaine (COC, 100 µM, n = 9–12) enhances extracellular dopamine levels in the NAc shell. Data are expressed as percent of baseline (left) or in absolute values (pg/sample, right). The arrow represents the time of quinpirole administration, whereas the solid line represents the time of cocaine perfusion. Values are the mean ± SEM and are expressed as pg/20 µL dialysate. *$P < .05$, unpaired *t* test with Welch's correction.

2015). A total of 257 dopamine cells was recorded from urethane anesthetized adult rats, 109 from rats exposed to poly(I:C) (n = 19), and 148 from controls (n = 14). Baseline activity of each cell was recorded for 120 to 180 seconds (Figure 4B).

Poly(I:C) rats showed a reduced number of spontaneously active VTA dopamine neurons compared with controls as measured by the analysis of cells per track (1.27 ± 0.13 vs 0.75 ± 0.11 control vs poly(I:C); $t_{(31)} = 3.12$, $P < .01$; Student's *t* test) (Figure 4C).

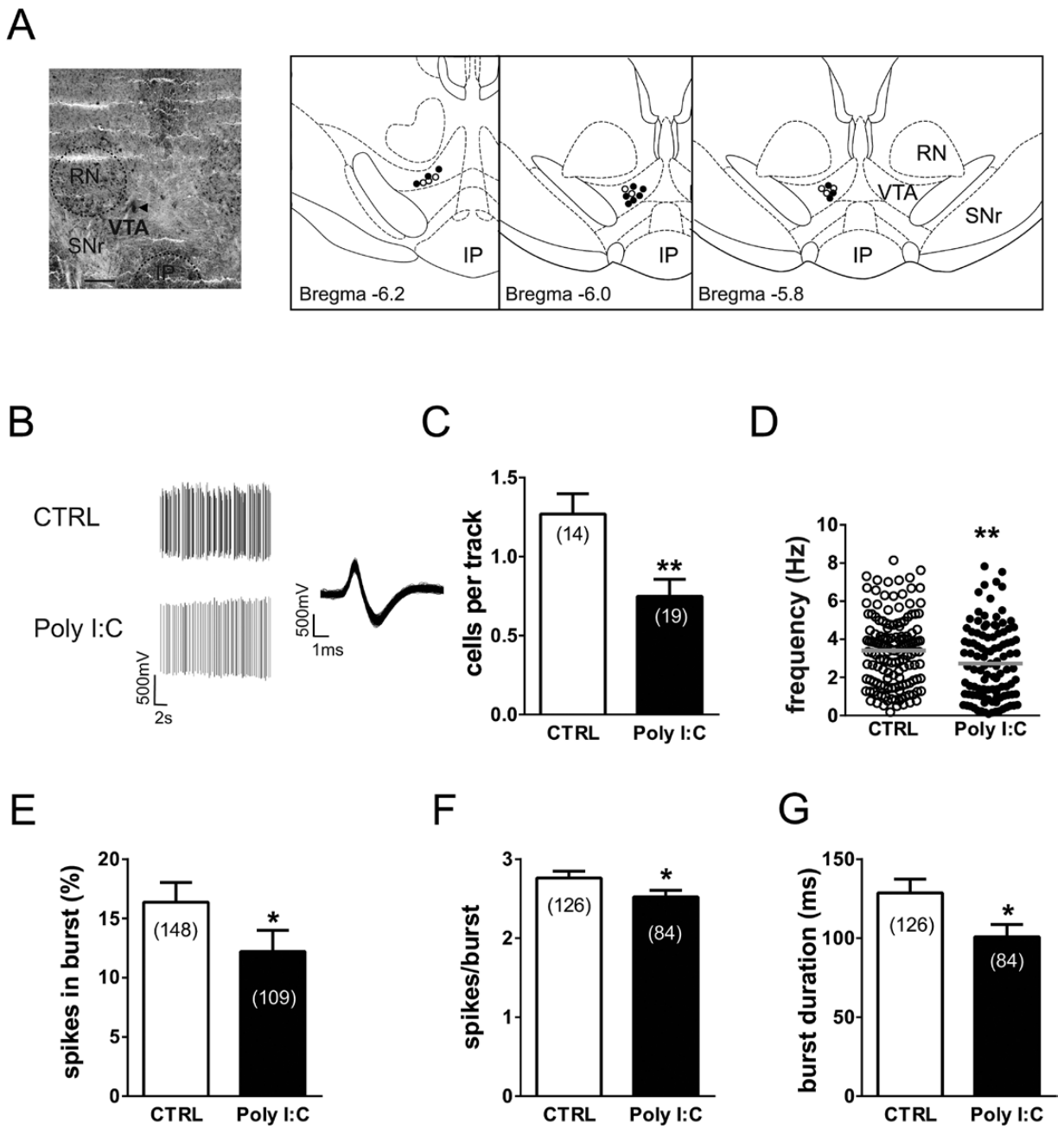

Figure 4. Prenatal polyriboinosinic-polyribocytidilic acid [poly(I:C)] treatment dysregulates dopamine neuron firing activity in adulthood. (A) Example of a recording location for a ventral tegmental area (VTA) dopamine neuron in a poly(I:C)-treated rat (the triangle indicates the pontamine sky blue dye). The diagrams at right show samples of localizations of recording sites in poly(I:C) rats (black dots) and controls (white dots) as verified by histological sections. IP, interpeduncular nucleus; RN, red nucleus; SNr, substantia nigra pars reticulata. Scale bar = 0.5 mm. (B) This panel shows traces illustrating representative extracellular recordings of a putative dopamine neuron in the VTA of anesthetized rats belonging to the control group (CTRL, above) and poly(I:C) group (below). Dopamine neurons recorded from poly(I:C) rats typically show slower firing activity and a reduced bursting compared with CTRL. The left trace shows the typical broad spike waveform of a dopamine neuron. (C) The bar graph shows the number of spontaneously active VTA dopamine neurons, which was different between the experimental groups. The scatter plot in (D) displays individual dopamine neuron firing rate in CTRL and poly(I:C) rats. The horizontal lines represent average values that are significantly different between the 2 groups. Graph histograms represent the percentage of spikes in bursts (E), the mean number of spikes per each burst (F), and the mean burst duration (G). Data are expressed as percentage or mean ± SEM. *$P < .05$, **$P < .01$.

In line with previous results in urethane-anesthetized rats (Kelland et al., 1990; Lecca et al., 2012), dopamine neurons recorded from control rats fired at 3.4 ± 0.15 Hz (n = 148) with a mean CV of interspike intervals of 64.1 ± 2.8% and presented 16.38 ± 1.67% of spikes in bursts. On the other hand, as depicted in Figure 4D, putative dopamine neurons (n = 109) recorded from poly(I:C) rats exhibited a lower discharge activity (2.73 ± 0.18 Hz; $t_{(255)} = 2.874$, $P < .01$; Student's *t* test) than controls.

We carried out a more detailed analysis of burst episodes, which included parameters as the mean spikes per burst and mean burst duration. Poly(I:C) showed significant differences in the percentage of spikes in burst compared with controls (16.38 ± 1.67% vs 12.21 ± 1.79%; controls vs poly(I:C); $t_{(255)} = 1.68$, $P < .05$; Student's *t* test) (Figure 4E). Additionally, values of mean spikes per burst and mean burst duration (n = 84) of VTA dopamine neurons were significantly altered compared with those

recorded from vehicle-injected littermates (n = 126). In poly(I:C)-treated rats, burst episodes were shorter (duration: 128.8 ± 8.64 milliseconds vs 100.9 ± 7.77 milliseconds; controls vs poly(I:C); $t_{(208)} = 2.26$, $P < .05$, Student's t test) (Figure 4G) and included fewer spikes on average (spikes per burst: 2.76 ± 0.09 vs 2.52 ± 0. 08; controls vs Poly(I:C); $t_{(208)} = 1.90$, $P < .05$, Student's t test) (Figure 4F).

Discussion

Our study, the first to combine electrophysiological and microdialysis experiments on the dopamine system in a maternal immune activation model of schizophrenia, reveals that poly(I:C) treatment during pregnancy induces a disruption in dopamine functions in the offspring. Specifically, we found increased basal levels of dopamine in the NAc but, surprisingly, a reduction in both the number and the firing rate of spontaneously active dopamine cells in the VTA. As expected, electrophysiological and neurochemical changes were paralleled by behavioral abnormalities, as revealed by deficits in PPI, SI, and NOR tests.

One of the hallmarks of positive symptoms of schizophrenia and related psychoses is an increased dopamine transmission in the mesolimbic system (Abi-Dargham et al., 2000). Accordingly, we found increased baseline levels of dopamine in the NAc, but not in the mPFC, in poly(I:C)-treated rats compared with controls. This finding is in agreement with previous studies showing that poly(I:C) animals exhibited hyperactivity of the dopamine system (Zuckerman et al., 2003; Hadar et al., 2015). In addition, it has been reported that poly(I:C) treatment increases the number of TH immunoreactive cells in the VTA and TH-positive terminals in the striatum (Meyer et al., 2008; Winter et al., 2009; Vuillermot et al., 2010). Other studies found increases in evoked striatal dopamine release ex vivo (Zuckerman et al., 2003) as well as augmented levels of dopamine and DOPAC in the lateral globus pallidus and PFC (Winter et al., 2009) or only DOPAC in the striatum (Ozawa et al., 2006). Conversely, our electrophysiological sampling of dopamine neurons in the VTA yielded a reduced number of spontaneously active cells together with a reduced firing activity. This finding is in contrast with previous studies that utilized the mitotoxin methyl azoxymethanol acetate (MAM) model of schizophrenia (Lodge and Grace, 2007; Du and Grace, 2013). The reason for this discrepancy might be due to the profound differences in the neuropathological mechanisms between the 2 animal models [MAM vs poly(I:C)]. Administration of MAM, a DNA chelating agent, to pregnant dams at GD 15 or earlier disrupts normal fetal brain development and induces gross neurodevelopmental abnormalities both in the macro- and microstructure, particularly in cortical regions, and microcephaly (Singh, 1980; Jongen-Relo et al., 2004). When MAM is administered at a later stage during pregnancy (GD 17), less severe abnormalities with hyperdopaminergia were reported (Lodge and Grace, 2007; Du and Grace, 2013). On the other hand, maternal immune activation models recapitulate the effects of a maternal viral infection by activating microglia and cytokines production. These effects induce severe functional changes but are believed to induce minor anatomical abnormalities in the brain of offspring (Meyer, 2014).

A decrease in spontaneously active dopamine neurons, and in their frequency of discharge, is apparently difficult to reconcile with an augmented dopamine output in terminal regions, but it confirms previous reports that firing rate and release might not always be correlated (Manta et al., 2013) and that dopamine release can be controlled efficiently at terminal regions without increased impulse activity of dopamine cells. We tested the hypothesis that a reduced dopamine cell activity is an adaptation to augmented synaptic dopamine, likely paralleled by a dysregulation in dopamine D2 autoreceptor sensitivity, as these receptors are key in determining the amount of released dopamine (Anzalone et al., 2012). However, our microdialysis experiments indicated that D2 receptor sensitivity did not change between poly(I:C) and controls, as quinpirole was equally effective in decreasing extracellular dopamine. We also tested the possibility that baseline increase in NAc dopamine was due to a deficient clearance of this neurotransmitter from the synaptic cleft. Hence, a study with lipopolysaccharide as a maternal immune activator showed reduced binding levels for the DAT in the NAc of the offspring of treated dams (Baharnoori et al., 2013). When cocaine was administered locally via the microdialysis fiber, dopamine levels were further markedly enhanced in poly(I:C) rats. This finding indicates that the effects of cocaine are not occluded and that dopamine clearance by DAT is similarly efficient in both groups of animals. However, this further increase in dopamine in spite of high baseline levels provides an explanation for the enhanced motor stimulating effect of amphetamine in poly(I:C) animals observed in other studies (Zuckerman et al., 2003; Ozawa et al., 2006).

Synchronized, neural network activity in the neocortex, termed cortical oscillations, has been known to impact the pattern of neuronal firing of dopamine neurons (Gao et al., 2007), and this functional coupling allows a bidirectional control of dopamine neurons by the mPFC. Cortical oscillations and neural synchrony are abnormal in patients with schizophrenia and in animal models of this disease (Uhlhaas and Singer, 2010; Dickerson and Bilkey, 2013; Gonzalez-Burgos et al., 2015). The disruption of dopamine neuron function could be due to a different cortical state resulting from poly(I:C) exposure. In fact, maternal immune activation has been shown to disrupt oscillatory activity in the hippocampus and the neocortex (Dickerson et al., 2010; Ducharme et al., 2012, 2014; Dickerson and Bilkey, 2013). Irrespective of the mechanism involved, firing rate of dopamine neurons might be reduced or suppressed through an inhibitory synaptic feedback loop arising from the NAc (Paladini et al., 2003; Watabe-Uchida et al., 2012).

Consistent with previous studies, adult poly(I:C) offspring exhibited significant PPI deficits. PPI provides an operational measure of sensorimotor gating, a neurophysiological mechanism that filters or "gates out" relevant stimuli from its surroundings, allowing the brain to focus attention on the most salient aspects of a stimulus (Braff and Geyer, 1990). The value of this experimental measure is based on its face, predictive, construct validity for schizophrenia and other psychiatric disorders (Wolff and Bilkey, 2008). In fact, PPI is regulated by the same forebrain regions involved in schizophrenia, patients with schizophrenia show PPI dysfunctions, and PPI deficits are efficiently reversed by the benchmark antipsychotics (Braff et al., 2001). Nevertheless, PPI alterations have not constantly been reported in MIA model, and even some recent studies reported no PPI disruptions by poly(I:C) administration (Fortier et al., 2007; Van den Eynde et al., 2014). While the reasons for these apparent discrepancies remain unknown, they may reflect subtle differences in the timing of prenatal infections, the rat strain used, and the setting of the PPI protocol.

Unlike PPI parameters, poly(I:C) exposure failed to induce significant changes in startle reflex. Indeed, although our protocol is not specifically designed for assessing startle parameters, poly(I:C) offspring did not display significant differences in the average of startle reflex as well as in the startle habituation and latency. Of note, since PPI is extrapolated by startle values and PPI deficits occurred without concomitant alterations in the startle parameters, the reductions of PPI observed in poly(I:C) offspring likely reflect an effective impairment of sensorimotor gating.

One question that arises from our findings and other studies is whether maternal immune activation models schizophrenia or

other neurodevelopmental disorders, such as autism spectrum disorders. In fact, several epidemiological studies have demonstrated an association between infection or inflammation during pregnancy and increased risk of autism (Atladottir et al., 2010). Prenatal infection and notably prenatal poly(I:C) and influenza virus administration can cause autism-relevant behaviors in the offspring and have been considered models of autism (Patterson, 2009; Schwartzer et al., 2013). This is not surprising when considering the similarities between schizophrenia and autism-related disorders, which might also share a neuroinflammatory pathogenesis during early fetal development (Meyer et al., 2011). Indeed, psychoses, ranging from autism to schizophrenia and bipolar disorders, might represent a continuum in both human pathology and animal models of these diseases.

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Statement of Interest

None.

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