Chronic lurasidone treatment normalizes GABAergic marker alterations in the dorsal hippocampus of mice exposed to prenatal immune activation

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ABSTRACT

Prenatal maternal infection represents a risk factor for the development of psychopathologic conditions later in life. Clinical evidence is also supported by animal models in which the vulnerability to develop a schizophrenic-like phenotype likely originates from inflammatory processes as early as in the womb. Prenatal immune challenge, for example, induces a variety of long-term behavioral alterations in mice, such as deficits in recognition and spatial working memory, perseverative behaviors and social impairments, which are relevant to different symptom clusters of schizophrenia.

Here, we investigated the modulation of GABAergic markers in the dorsal and ventral hippocampus of adult mice exposed to late prenatal immune challenge with the viral mimetic Poly(I:C) (*polyriboinosinic-polyribocytidilic-acid*) at gestational day 17, and we evaluated the ability of chronic treatment with the multi-receptor antipsychotic lurasidone to modulate the alterations produced by maternal infection. Poly(I:C) mice show a significant reduction of key GABAergic markers, such as GAD67 and parvalbumin, specifically in the dorsal hippocampus, which were normalized by chronic lurasidone administration. Moreover, chronic drug administration increases the expression of the pool of brain derived neurotrophic factor (BDNF) transcripts with the long 3'-UTR as well as the levels of mature BDNF protein in the synaptosomal compartment, selectively in dorsal hippocampus.

All in all, our findings demonstrate that lurasidone is effective in ameliorating molecular abnormalities observed in Poly(I:C) mice, providing further support to the neuroplastic properties of this multi-receptor antipsychotic drug.

INTRODUCTION

Schizophrenia is a psychiatric disorder with a neurodevelopmental origin, whose etiology relies on the interaction between genetic and environmental factors. Clinical and preclinical studies have demonstrated that exposure to infection or inflammation during gestation are important environmental risk factors for the susceptibility to neurodevelopmental disorders (Brown et al., 2001; Harvey and Boksa, 2012; Luoni et al., 2015c; Meyer, 2014). Indeed, it is believed that exaggerated levels of cytokines produced by the mother during gestation can interfere with brain development, thus increasing the risk of developing schizophrenia and related disorders later in life (Khandaker et al., 2013; Patterson, 2007). On this basis, several animal models have been established, which have the advantage of stringently controlling the type and timing during which the immune challenge is applied, while excluding confounding effects due to the genetic background. One of the models that has gained great recognition in the last decade is based on the use of polyriboinosinic:polyribocytidilic acid [Poly(I:C)], a synthetic analogue of a double-stranded RNA that produces a rapid inflammatory response, whose functional consequences in the adult offspring depend on the timing of injection to the pregnant dam (reviewed in Meyer et al., 2009). In particular, when the maternal immune response is induced on gestation day (GD) 17, adult offspring show profound behavioral alterations that recapitulate the negative and cognitive symptoms of schizophrenia, such as working memory deficits (Richetto et al., 2013; Richetto et al., 2014), as well as alterations of hippocampal-associated behaviour (Giovanoli et al., 2015). Late maternal immune activation is also associated with a number of molecular alterations, largely reproducing changes that have been observed in schizophrenic patients. As an example, we previously observed impairments in the cortical GABAergic transcriptome, including GAD65/67, VGAT and selected alpha-subunits of the $GABA_A$ receptor (Richetto et al., 2013; Richetto et al., 2014), which reproduce decreased GABAergic function, one of the most robust changes observed in schizophrenia (Hashimoto et al., 2008; Lewis, 2014; Lewis et al., 2011; Lisman et al., 2008).

Against this background, in the present study we aimed to investigate the impact of maternal immune activation late in gestation on the expression of GABAergic markers in the ventral (VH) and dorsal (DH) hippocampus. Of note, while the DH has a preferential role in mnemonic processes such as working memory and spatial learning, the VH has a preferential role in emotional behavior (Fanselow and Dong, 2010). Interestingly, it has been shown, with the help of many animal models, that altered functional interaction between the hippocampus and the prefrontal cortex contributes to cognitive impairments in schizophrenia (Godsil et al., 2013). As an example, rats exposed to maternal immune activation during the gestational period displayed decreased interactions between the hippocampus and the prefrontal cortex, and this deficit was reversed by the antipsychotic drug clozapine in a dose-dependent manner (Dickerson et al., 2012).

To this purpose we analyzed, in adult male offspring whose mothers were injected with Poly(I:C) at GD17, the modulation of key GABAergic markers, such as the GABA-synthetizing enzyme GAD67, the vesicular GABA transporter VGAT, and a marker of a subpopulation of GABAergic interneurons, namely parvalbumin, whose alterations may provide a molecular substrate for the cognitive impairments observed in this animal model (Canetta et al., 2016; Labouesse et al., 2015; Lewis et al., 2012; Lisman et al., 2008; Lodge et al., 2009; Tse et al., 2015). Furthermore, as an index of the specificity of the alterations for the excitatory versus the inhibitory synapses, we also investigated the modulation of neuroligin-1 and neuroligin-2, which

are postsynaptic membrane proteins that are respectively localized in excitatory glutamatergic and inhibitory GABAergic synapses (Kohl et al., 2015; Sudhof, 2008). In addition, we tested the ability of a pharmacological intervention to prevent or normalize at adulthood some of the alterations produced by the gestational manipulation. More specifically, we investigated the multi-receptor antipsychotic drug lurasidone, which is a high affinity antagonist of 5-HT_7 receptors and a partial agonist at 5-HT_{1A} receptors, besides having high affinity for D_2 and 5-HT_{2A} receptors. Interestingly, lurasidone's activity at 5-HT_7 and 5-HT_{1A} receptors may contribute to the antidepressant and pro-cognitive properties of the drug (Ishibashi et al., 2010; Tarazi and Riva, 2013).

EXPERIMENTAL PROCEDURES

Animals

C57BL/6 mice were used throughout the study. Female and male breeders were obtained from the in-house specific pathogen free colony of the Physiology and Behavior Laboratory (ETH) at the age of 12-14 weeks. Breeding began after 2 weeks of acclimatization to the new animal holding room, which was at temperature and humidity controlled (21±11°C, 55±5%) under a reversed light-dark cycle (lights off: 8:00 - 20:00 hours). All animals had *ad libitum* access to water and food (Kliba 3430, Klibamühlen, Kaiseraugst, Switzerland). All procedures described had been previously approved by the Cantonal Veterinarian's Office of Zurich and are in agreement with the principles of laboratory animal in the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication No. 86-23, revised 1985).

Maternal immune activation during pregnancy

For the purpose of the maternal immunological manipulation during pregnancy, 20 female mice were subjected to a timed mating procedure in which groups of 2-3 females were moved to a partitioned cage with one male, allowing olfactory but not physical contact between male and female animals. On the third day of partitioning, the females were brought together with one male and allowed to mate. Successful mating was verified the next morning by the presence of a vaginal plug and that day was referred as gestational day (GD) 0. Pregnant dams on GD17 received either a single injection of vehicle (sterile pyrogen-free 0.9% NaCl, Ctrl group) (*N*=8) or of the viral mimic polyriboinosinic-polyribocytidilic acid [Poly(I:C)] (potassium salt; Sigma-Aldrich) (*N*=10) at a dose of 5 mg/kg, that was dissolved in the vehicle to yield a final concentration of 1.0 mg/ml on the day of injection. Injections were made via the intravenous route at the mother's tail vein under mild physical constraint and the volume of injection was 5 ml/kg. The animals were returned to the home cage immediately post-injection.

Allocation of offspring

All the offspring were weaned and sexed at postnatal day (PND) 21. Littermates of the same sex were caged separately and maintained in groups of 2-4 animals per cage. For the purpose of this study, only male animals were included in all experiments because ours and previous research using the mouse prenatal Poly(I:C) administration model did not reveal sex-dependent effects on behavioural and cognitive functions, including social interaction and working memory (Bitanihirwe et al., 2010; Meyer et al., 2008; Richetto et al., 2013), for which the GABAergic system could be particularly relevant. The number of male offspring allocated to each experimental group, and the number of independent litters from which they stemmed, are described in Table S1.

Drug treatment

At PND100 mice pertaining to each experimental group (Ctrl and Poly(I:C)) were randomly divided into two groups and were chronically administered (until PND132) with vehicle (1% hydroxyethylcellulose solution, Veh) or lurasidone (LUR) at a final concentration of 0.33 mg/ml. All solutions were freshly prepared every two days and administered daily *per os* at a dose of 1 mg/kg, with a volume of 3 ml/kg. While LUR possesses a heterogeneous receptor profile, with high affinity for 5-HT_7, D_2, 5-HT_{2A} and 5-HT_{1A} receptors, this dose was chosen based on studies that show antidepressant and pro-cognitive effects

(Cates et al., 2013; Enomoto et al., 2008). Indeed, at this dose, LUR should exert its activity primarily through 5-HT_7 receptors with a reduced blockade for dopamine D_2 receptors.

Brain tissue collection

24 hours after the last drug administration, animals were sacrificed by decapitation in order to collect brain samples for the purpose of measuring gene and protein expression levels in candidate brain regions. In particular, the dorsal (DH; bregma −1.5 to −2.5 mm) and the ventral (VH; bregma −2.5 to −3.5 mm) hippocampi have been dissected. Both left and right brain hemispheres were collected and were used for either gene or protein expression analyses. Brain specimens were collected in 96-well microtiter plates kept on dry ice and allowed to freeze before storage at −80°C until further use.

RNA preparation and quantitative Real-Time PCR analysis

Total RNA was isolated using the Qiagen RNeasy Mini kit (Qiagen, Italia) according to the manufacturer's instructions, and quantified by spectrophotometric analysis.

An aliquot of each sample was then treated with DNase to avoid DNA contamination. RNA was analysed by TaqMan qRT-PCR instrument (CFX384 real-time system, Bio-Rad Laboratories) using the iScript one-step RT-PCR kit for probes (Bio-Rad Laboratories). The samples were run in 384-well formats in triplicates as multiplexed reactions with *36B4* as a normalizing internal control, since its expression was not affected by prenatal injection and further manipulations.

Thermal cycling was initiated with an incubation at 50°C for 10 min (RNA retrotranscription) and then at 95°C for 5 min (TaqMan polymerase activation). After this initial step, 39 cycles of PCR were performed. Each PCR cycle consisted of heating the samples at 95°C for 10 s to enable the melting process and then for 30 s at 60°C for the annealing and extension reaction. Relative target gene expression was calculated according to the $2^{(-\Delta\Delta Ct)}$ method. The probe and primer sequences used are summarized in Table S2.

Protein extraction and Western blot analysis

Brain samples were manually homogenized using a glass-glass potter in a pH 7.4 cold buffer (containing 0.32 M sucrose, 0.1 mM EGTA, 1 mM HEPES solution and 0.1 mM phenylmethylsulfonyl fluoride), in presence of a complete set of protease (Roche) and phosphatase (Sigma-Aldrich) inhibitors and then sonicated for 10 s at a maximum power of 10-15% (Bandelin Sonoplus). The homogenate was clarified (1000 *g*; 10 min), obtaining a pellet (P1) enriched in nuclear components, which was resuspended in a buffer (1 mM HEPES, 0.1 mM dithiothreitol, 0.1 mM EGTA) supplemented with protease and phosphatase inhibitors. The supernatant (S1) was then centrifuged (13000 *g*; 15 min) to obtain a clarified fraction of cytosolic proteins (S2). The pellet (P2), corresponding to the crude membrane fraction, was resuspended in the same buffer used for the P1. Total protein content was measured according to the Bradford Protein Assay procedure (Bio-Rad Laboratories), using bovine serum albumin as calibration standard.

Protein analyses were performed in the whole homogenate (GAD67, VGAT, PV, NLGN1, NLGN2, mBDNF) and in the P2 fraction (mBDNF). Equal amounts of protein (10µg for the homogenate, 7µg for the P2) were run under reducing conditions on Any Kd Criterion TGX precast gels (Bio-rad Laboratories) and then electrophoretically transferred onto polyvinylidene fluoride or nitrocellulose membranes. Unspecific binding sites were blocked with 10% non-fat dry milk, then the membranes were incubated overnight with

the primary antibodies and, the following day, for 1h at room temperature with a peroxidase-conjugated anti-rabbit or anti-mouse IgG (Table S3). Immunocomplexes were visualized by chemiluminescence using the ECL Star (Euroclone), LiteAblot Plus (Euroclone) or Clarity Western ECL substrate (Bio-Rad Laboratories). Results were standardized using β-actin as the control protein, which was detected by evaluating the band density at 43 kDa. Protein levels were calculated using the Chemidoc MP imaging system together with Image Lab software (Bio-Rad Laboratories). To ensure that autoradiographic bands will be in the linear range of intensity, different exposure times were used.

Statistical analysis

The effects of drug treatment (LUR) and prenatal infection [Poly(I:C)] on the mRNA or protein levels of our molecular targets were analysed by two-way ANalysis Of VAriance (ANOVA) followed by Fisher's Least Significant Difference (LSD) *post hoc* comparisons. The statistical outcomes obtained in the 2-way ANOVA are listed in Table S4. Significance for all tests was assumed for $p<0.05$. Data are presented as means ± standard error of the mean (S.E.M.). SPSS for Mac OS X (Release 22.0.0.0) was used to perform the statistical analyses.

RESULTS

Analysis of GABAergic markers

Since mice exposed to maternal immune activation with Poly(I:C) at GD17 show defects in working memory (Giovanoli et al., 2015; Richetto et al., 2013; Richetto et al., 2014), we decided to investigate if such manipulation may result in persistent alterations of key components of the GABAergic system within the dorsal and ventral hippocampus (DH and VH, respectively), in order to evaluate the effects of a pharmacological intervention in adulthood using the novel antipsychotic drug lurasidone (LUR).

We first analysed two pre-synaptic GABAergic markers, the primary GABA-synthesizing enzyme, GAD67 (glutamate acid decarboxylase 67, also namely GAD1), and VGAT, the vesicular GABA transporter, responsible for uptake and storage of GABA within pre-synaptic vesicles. In the DH, while *Gad67* mRNA levels were not significantly affected by Poly(I:C) or LUR treatment (Fig. 1A), we found that GAD67 protein levels showed a significant LUR X Poly(I:C) interaction (Table S4). Indeed, Poly(I:C)-treated mice showed a significant reduction of GAD67 protein levels (-20% vs. Ctrl/Veh, $p<0.05$), an effect that was normalized by chronic LUR treatment ($p<0.01$ Poly(I:C)/LUR vs. Poly(I:C)/Veh) (Fig. 1C). The effects of Poly(I:C) and LUR treatment on GAD67 appears to be specific for the DH, since within the VH we did not observe any significant effect on *Gad67* mRNA levels (Fig. 1B) or protein levels (Fig. 1D) (Table S4).

Regarding the analysis of VGAT modulation, as shown in Fig. 1E we found a significant main effect of LUR on *Vgat* mRNA levels in the DH, while we did not observe any effect of Poly(I:C) or any LUR X Poly(I:C) interaction (Table S4). In particular, LUR treatment produced an up-regulation of *Vgat* mRNA levels both in Ctrl as well as in Poly(I:C) mice, which however is greater in the latter group (Fig. 1E). This effect seemed to be paralleled by alterations at protein levels where again LUR determined a more noticeable effect when administered to Poly(I:C)-treated animals, although the statistical analysis did not support this trend (Fig. 1G). In addition, we did not observe any significant effect on VGAT in the VH, both at mRNA (Fig. 1F) and protein levels (Fig. 1H) (Table S4).

Since several neuroanatomical studies have found impaired parvalbumin (PV) expression in cortical and hippocampal regions of schizophrenic individuals (Lewis, 2014; Lewis et al., 2012), we next investigated PV expression in order to establish possible alterations as a consequence of maternal infection and LUR treatment. In the DH, as shown in Fig. 1I, we found a significant effect of LUR treatment on *Pv* mRNA levels, with no Poly(I:C) effect and no LUR X Poly(I:C) interaction (Table S4). In detail, chronic LUR caused a greater increase on *Pv* mRNA levels when administered to Poly(I:C)-treated mice, as compared to the effect that it exerted in Ctrl mice. In parallel, at protein levels, we detected a significant LUR X Poly(I:C) interaction (Table S4). Indeed, we found that PV protein levels were significantly reduced in Poly(I:C) mice treated with vehicle (-29% vs. Ctrl/Veh, $p<0.05$), and that this effect was completely normalized by chronic administration with LUR ($p<0.05$ Poly(I:C)/LUR vs. Poly(I:C)/Veh) (Fig. 1K). In line with the changes of GAD67, again the modulation was specific for the DH, since we did not detect any statistically significant change of PV mRNA expression levels in the VH (Fig. 1J) as well as of its protein levels (Fig. 1L) (Table S4).

We next focused our attention on neuroligins (NLGNs), which are postsynaptic proteins that link pre- and postsynaptic membranes by binding to presynaptic neurexins (Sudhof, 2008). The NLGN family members are encoded by different genes in mammals and are localized in different postsynaptic specializations. Specifically, NLGN1 and NLGN2 appear to be mainly localized in excitatory glutamatergic and inhibitory

GABAergic synapses, respectively, and may influence their synaptic properties (Kohl et al., 2015; Varoqueaux et al., 2004). In the DH, as shown in Fig. 2, neither Poly(I:C) treatment, nor chronic LUR administration had any effect on *Nlgn*1 mRNA levels (Fig. 2A). Similarly, we did not observe any significant effect on its protein levels (Fig. 2E) (Table S4). On the other hand, we found a significant effect of chronic LUR administration, but not of Poly(I:C) exposure, on the mRNA levels for *Nlgn2*, (Fig. 2B) (Table S4). Interestingly, when investigating NLGN2 protein levels, we found a significant interaction of LUR X Poly(I:C) treatment (Fig. 2F) (Table S4). In detail, while Poly(I:C) mice displayed a tendency toward a reduction of NLGN2 protein levels (-19% vs. Ctrl/Veh, $p>0.05$), chronic LUR administration was able to significantly up-regulate NLGN2 when given to Poly(I:C)-treated mice ($p<0.01$) (Fig.2F).

The analysis of NLGNs in the VH showed a different pattern of modulation. As shown in Fig. 2C, while we did not detect any significant change on *Nlgn1* mRNA levels or NLGN1 protein levels (Fig. 2G) (Table S4), we found a significant main effect of Poly(I:C) treatment as well as of LUR administration on *Nlgn2* mRNA levels, with no significant LUR X Poly(I:C) interaction (Fig. 2D) (Table S4). Indeed, LUR treatment was able to up-regulate *Nlgn2* mRNA levels, an effect that is larger when administrated to Poly(I:C)-treated mice. On the contrary, no significant effect was found on NLGN2 protein levels (Fig. 2H) (Table S4).

Analysis of Brain-Derived Neurotrophic Factor (BDNF)

Various findings demonstrate a role for BDNF in the maturation and function of GABA neurons (Hong et al., 2008; Marty et al., 2000). On this basis, and considering the important role of BDNF in drug-induced plasticity (Autry and Monteggia, 2012; Calabrese et al., 2009), we decided to investigate the potential contribution of BDNF in the changes observed following Poly(I:C) infection and LUR treatment.

Within the DH (Fig. 3A, C), while Poly(I:C) exposure did not alter total *Bdnf* mRNA levels, it produced a significant effect on *Bdnf* transcripts with the long 3'-UTR (Table S4). In parallel, also LUR treatment produced a significant modulation exclusively of long 3'-UTR *Bdnf* mRNA levels within this brain region (Table S4). Indeed, the expression of long 3'-UTR *Bdnf* mRNA appears to be primarily increased by LUR when given to Poly(I:C) mice. In the VH (Fig. 3B, D), neither Poly(I:C) treatment, nor LUR administration had any significant effect on total *Bdnf* mRNA levels (Fig. 3B), while we observed a significant main effect of LUR on the pool of *Bdnf* transcripts with the long 3'-UTR (Fig. 3D) (Table S4). Specifically, we found that LUR treatment produced a decrease of the pool of transcripts with the long 3'-UTR, which appeared to be more pronounced in Ctrl animals.

Given the fact that lurasidone modulates *Bdnf* mRNA expression levels primarily in the DH, we also examined the levels of the mature form of BDNF protein in this brain region. We found a significant effect of Poly(I:C) on mBDNF protein levels, both in the whole homogenate (Fig. 4A) and in the crude membrane fraction (P2) (Fig. 4B) (Table S4). Moreover, while chronic LUR treatment did not affect mBDNF expression in the whole homogenate (Fig. 4A), it produced a significant effect on mBDNF protein levels specifically in the P2 fraction (Fig. 4B) (Table S4). Indeed, mBDNF levels were increased by drug administration in Ctrl as well as in Poly(I:C)-treated mice (Fig. 4B), as compared to Veh-treated mice.

DISCUSSION

The present study confirms our previous findings demonstrating that late prenatal immune activation in mice induces long-term effects on the GABAergic system (Labouesse et al., 2015; Richetto et al., 2013; Richetto et al., 2014; Richetto et al., 2015), and expands our knowledge to the hippocampal sub-regions, specifically the ventral and the dorsal parts (VH and DH, respectively). In particular, we demonstrate regional-specific alterations in the dorsal part of the hippocampal formation that is primarily involved in regulating cognitive function. Furthermore, we found that chronic lurasidone treatment is able to ameliorate these alterations, likely through the modulation of the neurotrophin BDNF.

It is well established that mice exposed to Poly(I:C) infection at GD17 show deficits in spatial working memory and recognition memory, together with perseverative behaviors and social interaction deficits, which represent a hallmark of schizophrenia-related dysfunction (Bitanihirwe et al., 2010; Meyer et al., 2006a; Meyer et al., 2008; Meyer et al., 2006b; Richetto et al., 2013; Richetto and Riva, 2014). Deficits of the GABAergic system have been postulated to play a relevant role in cognitive impairments observed in schizophrenia (Heckers and Konradi, 2015; Lisman et al., 2008; Lodge et al., 2009; Murray et al., 2011; Tamminga, 2006). Among these, the most consistently reported cellular alteration observed in the brain of schizophrenic patients is the reduction in the expression of the calcium-binding protein, parvalbumin (PV), a marker of a sub-population of GABA-interneurons (Curley et al., 2013; Fung et al., 2010). Within the hippocampus and the PFC, the fast-spiking PV-expressing interneurons are vital for the generation of gamma oscillation, which are likely one of the best index of functional activity in these brain regions. Normal oscillatory function is essential for optimal information processing and intellectual function, and these depend on the integrity of GABAergic interneurons (Gill and Grace, 2014). Animal models of schizophrenia provide results that are consistent with the loss of PV interneurons observed in schizophrenia patients (Canetta et al., 2016). As an example, it has been shown with the methylazoxymethanol acetate (MAM) neurodevelopmental model of schizophrenia that a reduction of PV-positive neurons is associated with alterations in task-associated oscillatory activity during performance of a latent inhibition task (Lodge et al., 2009). Our results are consistent with a role for PV neurons, since we found a significant reduction of its protein levels, specifically in the DH. Interestingly, we observed that lurasidone administration was able to normalize this reduction. We detected a similar effect and a similar regional specificity in the modulation of GAD67 protein levels, one of the two enzymes required for the decarboxylation of glutamate to GABA, which is responsible for the majority of GABA synthesis in the Central Nervous System. The effects on PV and GAD67 are the mostly replicated and observed alterations in clinical studies examining the modifications occurring in brain structures of patients suffering from schizophrenia (Curley et al., 2011; Hashimoto et al., 2008)). Interestingly, we found that lurasidone was able to increase different GABAergic markers, both at mRNA and protein levels, primarily in the DH of Poly(I:C)-treated mice.

This specificity was further confirmed through the analysis of neuroligins (NLGNs), which are postsynaptic membrane proteins anchoring pre- and postsynaptic compartments by binding to presynaptic neurexins (Sudhof, 2008). As shown above, specifically in the DH, we reported a significant interaction between Poly(I:C) and lurasidone treatment showing that while NLGN2 protein levels are reduced in Poly(I:C)-treated animals, its levels are up-regulated by the pharmacological treatment, suggesting a primary effect on this sub-hippocampal region. These results provide further support to the notion that the prenatal

manipulation determines specific alterations on the organization and function of the GABAergic system, since while NLGN1 is primarily involved in regulating the organization of the glutamatergic system, NLGN2 is mainly localized at inhibitory GABAergic synapses (Sudhof, 2008). Indeed, NLGN2 binds to the postsynaptic scaffolding protein gephyrin, which is involved in $GABA_A$-receptor recruitment and may therefore regulate GABAergic synaptic properties (Poulopoulos et al., 2009). It has been previously demonstrated that chronic stress is also able to specifically reduce the protein levels of NLGN2, but not NLGN1, in the hippocampus of adult rats, an effect that may contribute to impaired sociability (van der Kooij et al., 2014). Moreover, consistent with a decreased inhibitory synaptic function, NLGN2 knockout mice showed heightened anxiety-related behavior, as compared to wild-type animals, possibly related to an altered function of the inhibitory transmission, rather than to a decreased number of inhibitory synapses (Blundell et al., 2009). Together, these alterations may set the stage for excitation/inhibition unbalances that, in turn, may contribute to the alterations affecting specific behavioral domains (working and spatial memory) of adult animals exposed to maternal immune activation, which rely on a proper DH functioning (Bitanihirwe et al., 2010; Giovanoli et al., 2015; Meyer et al., 2009; Meyer et al., 2008; Richetto et al., 2013; Richetto et al., 2015).

Strikingly, these results demonstrate that lurasidone treatment is quite effective in promoting GABAergic function in Poly(I:C)-injected mice, suggesting that this multi-receptor modulator may therefore be able to counteract the alterations of the GABA system that, as mentioned above, are a key feature of schizophrenic patients (Gill and Grace, 2014). It has already been reported that at least some of the behavioral and cognitive abnormalities induced by prenatal immune activation can be normalized by acute or chronic antipsychotic treatment. Zuckerman and coworkers demonstrated that prenatal infection induced alterations in latent inhibition that were normalized by an acute treatment with both haloperidol and clozapine (Zuckerman et al., 2003). In line with these initial findings, two other groups reported the beneficial effects of chronic clozapine treatment at adulthood in ameliorating Poly(I:C)-induced memory impairments (Meyer et al., 2010; Ozawa et al., 2006). In addition, recent findings demonstrated that both clozapine or risperidone during adolescence can prevent the manifestation of behavioral and structural brain abnormalities, characterizing schizophrenia, in adult offspring exposed to gestational Poly(I:C) (Piontkewitz et al., 2011; Piontkewitz et al., 2009). Of note, we have previously shown that lurasidone is able to normalize alterations of the GABA system in SERT knockout rats, a genetic model of depression (Luoni et al., 2013). Although the background is very different, since it involves a genetic predisposition to a psychopathologic phenotype, we could find some similarities with our experimental paradigm. Indeed, lurasidone treatment was able to ameliorate the impaired expression of GABAergic markers in SERT knockout rats, such as PV or $GABA_A$-y2 receptor subunit. These findings confirm that the GABAergic system is pharmacologically targeted by lurasidone administration in an attempt to ameliorate the psychopathologic phenotype, suggesting that the modulation of the GABAergic transcriptome may contribute to its therapeutic efficacy, particularly with respect to the pro-cognitive effects that characterize this antipsychotic drug (Enomoto et al., 2008; Ishiyama et al., 2007; Tarazi and Riva, 2013).

Interestingly, previous studies from our group have consistently demonstrated that one of the mechanisms through which chronic lurasidone treatment may provide beneficial effects on brain function is by enhancing the expression of neuroplastic molecules, such as BDNF, in key brain regions involved in psychiatric disorders (Calabrese et al., 2013; Fumagalli et al., 2012; Luoni et al., 2014a; Luoni et al.,

2015a; Luoni et al., 2015b; Luoni et al., 2014b; Tarazi and Riva, 2013). In particular, BDNF has been shown to exert an important role in regulating the development of GABA interneurons and in regulating GABAergic transmission (Bolton et al., 2000; Hashimoto et al., 2005; Marty et al., 2000; Sakata et al., 2009; Waterhouse et al., 2012; Yamada et al., 2002). Consistently with our previous study (Giovanoli et al., 2015), we found that also this cohort of adult Poly(I:C)-treated mice did not display significant alterations of BDNF mRNA and protein levels. Nevertheless, we found that a 5-week treatment with lurasidone regulated *Bdnf* mRNA levels in the DH, an effect that appears to be larger in Poly(I:C)-treated mice. Interestingly, in agreement with our previous results (Luoni et al., 2014a; Luoni et al., 2015a; Luoni et al., 2013; Luoni et al., 2015b), the effects of lurasidone are prominent for the pool of *Bdnf* transcripts with the long 3'UTR, which are characterized by dendritic targeting (An et al., 2008; Lau et al., 2010), suggesting the potential role for *Bdnf* in this compartment. This possibility is substantiated by the specific up-regulation of BDNF protein levels in the synaptic compartment of lurasidone-treated rats. This effect may be the result of the combinatorial effect of an increase of the pool of *Bdnf* transcripts with the long 3'-UTR, in association with a direct modulation of its translation, the latter likely explaining the apparent discrepancy observed between the modulation of *Bdnf* mRNA and protein levels after lurasidone administration in control mice.

The anatomical and cellular selectivity of lurasidone on the neurotrophin BDNF may underlie the specificity for the effects produced by the antipsychotic on the modulation of GABAergic markers. Interestingly, while several studies reported alterations of GAD67 specifically in PV-positive interneurons (Hashimoto et al., 2003), it has also been demonstrated that PV-containing neurons highly express the high affinity receptor for BDNF, TrkB (Waterhouse et al., 2012), thus explaining the potential for lurasidone to regulate this system through BDNF modulation.

Although we believe that the data presented in this paper bear important clinical implications, we recognize that they represent a starting point for future studies aimed at elucidating the underlying molecular mechanisms that mediate the beneficial effects of lurasidone through BDNF modulation, which may be achieved using specific tools to inhibit BDNF function, such as TrkB antagonists or BDNF mutant mice. Moreover, the investigation of other antipsychotic drugs with different mechanisms of action will help to establish similarities and differences based on drug classes as well as the contribution of the different receptor subtypes.

In conclusion, this study provides further evidence regarding the molecular mechanisms underlying the behavioral and cognitive alterations relevant for schizophrenia observed in a well-characterized animal model of prenatal immune activation. Moreover, these results demonstrate the ability of the antipsychotic drug lurasidone to restore Poly(I:C)-induced GABAergic alterations, possibly through the modulation of the neurotrophin BDNF. All in all, our results provide further support to the ability of lurasidone to promote neuroadaptive mechanisms in selected brain regions, which may contribute to ameliorate functions that are deteriorated in psychiatric disorders (Fumagalli et al., 2012; Luoni et al., 2014a; Luoni et al., 2013; Luoni et al., 2015b; Tarazi and Riva, 2013).

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FIGURE LEGENDS

Fig.1 Analysis of GABAergic markers at mRNA (A, B, E, F, I, J) and protein levels (C, D, G, H, K, L) in the dorsal (A, C, E, G, I, K) and ventral hippocampus (B, D, F, H, J, L) of control (Ctrl) and Poly(I:C) mice treated with vehicle (Veh) or lurasidone (LUR). In particular, the data of GAD67 (A, B, C, D), VGAT (E, F, G, H) and PV (I, J, K, L) are shown. The data, expressed as % change vs. Ctrl/Veh group, set at 0%, are the mean ± S.E.M. of at least 8 animals per group. *$p<0.05$ vs. Ctrl/Veh; $^{\$}p<0.05$, $^{\$\$}p<0.01$ vs. Poly(I:C)/Veh (2-way ANOVA followed by post-hoc test). For main effects of 2-way ANOVA please refer to Table S4.

Fig.2 Analysis of *Nlgn1* (A, C) and *Nlgn2* (B, D) mRNA levels and NLGN1 (E, G) and NLGN2 (F, H) protein levels in the dorsal (A, B, E, F) and ventral hippocampus (C, D, G, H) of control (Ctrl) and Poly(I:C) mice treated with vehicle (Veh) or lurasidone (LUR). The data, expressed as % change vs. Ctrl/Veh group, set at 0%, are the mean ± S.E.M. of at least 7 animals per group. $^{\$\$}p<0.01$ vs. Poly(I:C)/Veh (2-way ANOVA followed by post-hoc test). For main effects of 2-way ANOVA please refer to Table S4.

Fig.3 Analysis of total (A, B) and long 3'-UTR *Bdnf* (C, D) mRNA levels in the dorsal (A, C) and ventral hippocampus (B, D) of control (Ctrl) and Poly(I:C) mice treated with vehicle (Veh) or lurasidone (LUR). The data, expressed as % change vs. Ctrl/Veh group, set at 0%, are the mean ± S.E.M. of at least 10 animals per group. For main effects of 2-way ANOVA please refer to Table S4.

Fig.4 Analysis of mBDNF protein levels in the whole homogenate (A) and in the crude membrane fraction (B) of the dorsal hippocampus from control (Ctrl) and Poly(I:C) mice treated with vehicle (Veh) or lurasidone (LUR). The data, expressed as % change vs. Ctrl/Veh group, set at 0%, are the mean ± S.E.M. of at least 6 animals per group. For main effects of 2-way ANOVA please refer to Table S4.

FIGURE 1

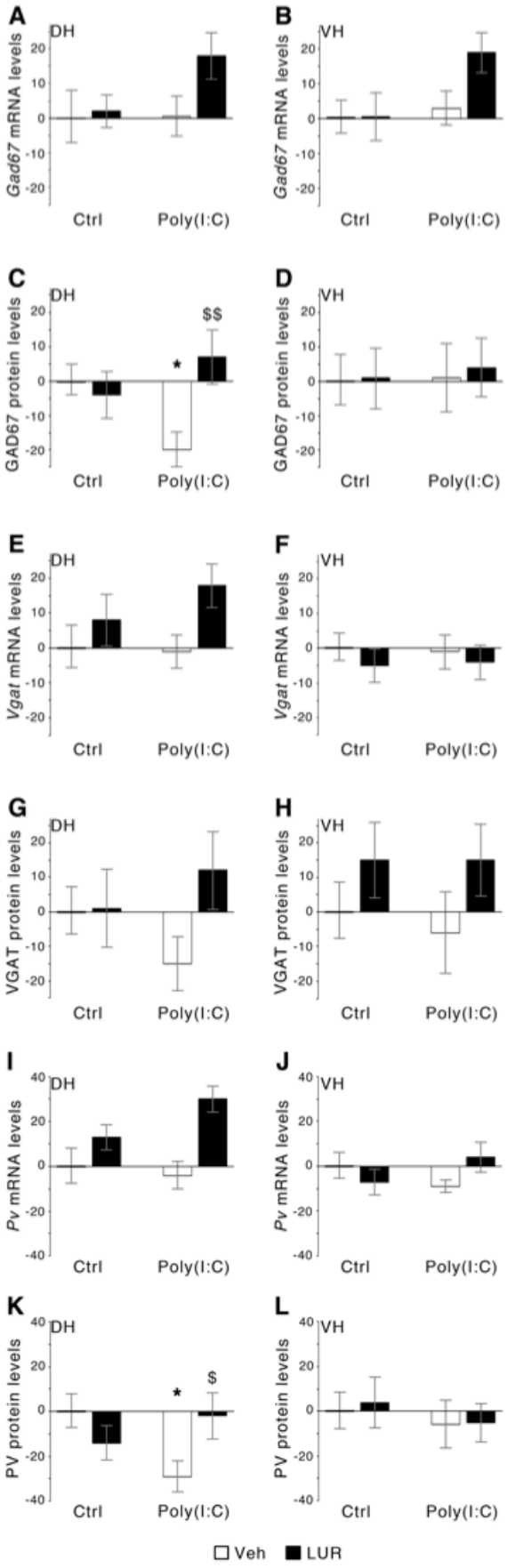
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FIGURE 2

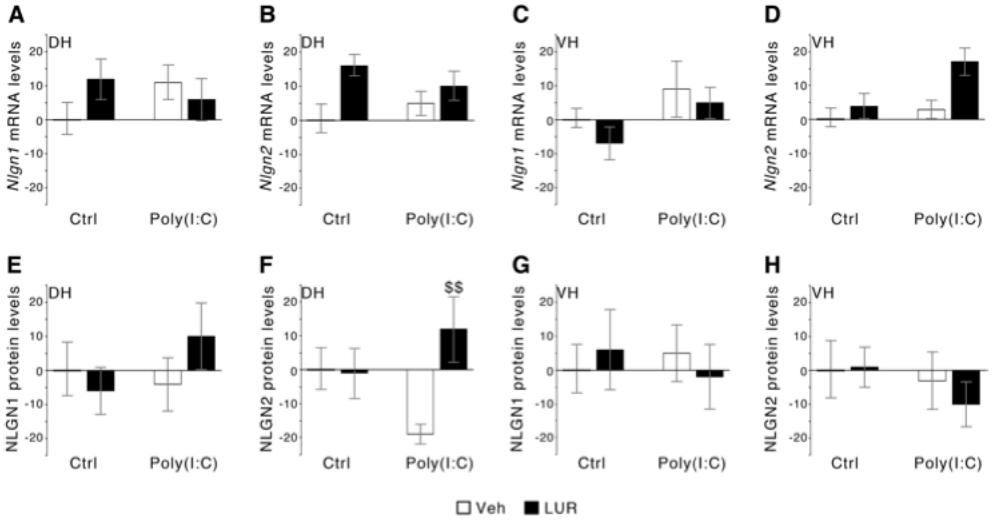
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FIGURE 3

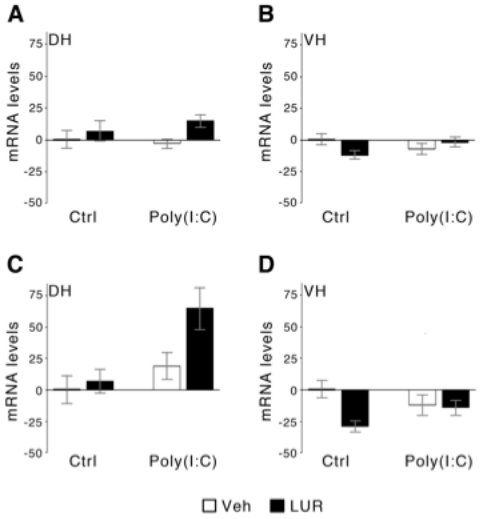
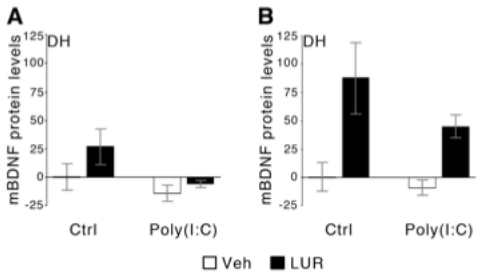


FIGURE 4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