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Reduced cortical somatostatin gene expression in a rat model of maternal immune activation

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ABSTRACT

Alterations in GABAergic interneurons and glutamic acid decarboxylase (GAD) are observed in the brains of people with schizophrenia. Studies also show increased density of interstitial white matter neurons (IWMN), including those containing GAD and somatostatin (SST) in the brain in schizophrenia. Maternal immune activation can be modelled in rodents to investigate the relationship between prenatal exposure to infections and increased risk of developing schizophrenia. We reported that maternal immune activation induced an increase in density of somatostatin-positive IWMN in the adult rat offspring. Here we hypothesised that maternal immune activation induced in pregnant rats by polyinosinic:polycytidylic acid would alter SST and GAD gene expression as well as increase the density of GAD-positive IWMNs in the adult offspring. SST gene expression was significantly reduced in the cingulate cortex of adult offspring exposed to late gestation maternal immune activation. There was no change in cortical GAD gene expression nor GAD-positive IWMN density in adults rats exposed to maternal immune activation at either early or late gestation. This suggests that our model of maternal immune activation induced by prenatal exposure of rats to polyinosinic:polycytidylic acid during late gestation is able to recapitulate changes in SST but not other GABAergic neuropathologies observed in schizophrenia.

1. Introduction

GABAergic interneurons are the main inhibitory neuron in the brain (Lewis et al., 2005). These interneurons can be identified by the expression of various markers such as glutamic acid decarboxylase (GAD) ,[2] calcium binding proteins; parvalbumin (Pv) and calretinin (Cr) and neuropeptides such as neuropeptide Y (NPY) and somatostatin (Lewis et al., 2012). These GABAergic interneuron markers have been implicated in schizophrenia including a reduction of GAD mRNA and protein (Akbarian et al., 1995; Duncan et al., 2010; Guidotti et al., 2000; Hashimoto et al., 2008a; Thompson et al., 2009) and reductions in gene expression for Pv (Fung et al., 2014; Hashimoto et al., 2008b, 2003), SST+ (Fung et al., 2014; Hashimoto et al., 2008a, 2008b; Morris et al., 2008) and NPY (Fung et al., 2014; Hashimoto et al., 2008a). These and other changes in interneurons are postulated to affect GABA-mediated inhibition, pyramidal neuron excitation and the

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[2] Abbreviations: calbindin gene (*Calb1*); calretinin (Cr); calretinin gene (*Calb2*); cingulate cortex (CC); glutamic acid decarboxylase (GAD); glutamate decarboxylase 1 gene (*Gad1*); glutamate decarboxylase 2 gene (*Gad2*); interstitial white matter neurons (IWMNs); neuronal nuclear antigen (NeuN); neuronal nitric oxide synthase (*Nos1*); neuropeptide Y (NPY); Neuropeptide Y gene (*Npy*); parvalbumin (Pv); parvalbumin gene (*Pvalb*); polyinosinic:polycytidylic acid (PolyI:C); quantitative RT-PCR (qPCR); reelin gene (*Reln*); somatostatin gene (*Sst*).

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generation of gamma oscillations in the cortex that leads to the development of cognitive deficits in schizophrenia implicating a role for interneuron pathologies in schizophrenia (Bartos et al., 2007; Behrens and Sejnowski, 2009; Salinas and Sejnowski, 2001).

While most neurons, including GABAergic interneurons were thought to exist only in grey matter tissues, cells within the white matter or interstitial white matter neurons (IWMNs) were first described by Meynart in 1867 (Meynert, 1867) and have since been shown to express GABAergic markers (Joshi et al., 2012). Cajal (Cajal, 1901a) and others (Judaš et al., 2010) (Clancy et al., 2001) have shown that the dendritic tree and axons from these IWMNs project into the overlying cortical layers suggesting they may be integrated into local neuronal networks of healthy brains. Furthermore, electrophysiological studies confirm that IWMNs are integrated into local circuits (Clancy et al., 2001; Torres-Reveron and Friedlander, 2007). In schizophrenia, these IWMNs are increased in density in the white matter adjacent to brain regions implicated in dysfunction in the disorder (Duchatel et al., 2019). Whilst, most IWMN studies have focused on general neuronal markers such as neuronal nuclear antigen (NeuN), there have been three studies to date examining the densities of known GABAergic markers in IWMNs including NPY (Ikeda et al., 2004), SST (Yang et al., 2011) and GAD (Joshi et al., 2012). The density of these IWMNs was increased in post mortem brain tissue of people with schizophrenia, with SST+ (Yang et al., 2011) and GAD+ IWMNs (Joshi et al., 2012) increased in the superficial white matter whereas NPY+ IWMNs were increased in only deep white matter (Ikeda et al., 2004). Given that IWMNs are integrated into cortical circuits, to test whether increased density of IWMNs, including those containing GAD, is sufficient to alter brain dysfunction requires studies in animal models.

It has long been suggested that schizophrenia has a neurodevelopmental origin. A key aspect of this hypothesis is that exposure to an environmental factor(s) early in development triggers a pathophysiological process that alters brain development, before the onset of symptoms of schizophrenia. Indeed, maternal infection during pregnancy is one of the known environmental factors that can significantly increase the risk of schizophrenia in the offspring (Brown, 2006). Rodent maternal immune activation models have been developed in order to understand how exposure of offspring to infection during pregnancy leads to an increased risk of schizophrenia (Duchatel et al., 2018b). Experimental evidence shows that maternal infection can change neuropathology and behaviour similar to that observed in schizophrenia (Meyer et al., 2005; Ozawa et al., 2006; Zuckerman et al., 2003; Zuckerman and Weiner, 2005). Indeed, maternal immune activation using polyinosinic:polycytidylic acid (PolyI:C) exhibits numerous neurochemical and brain morphological abnormalities similar to people with schizophrenia (Meyer and Feldon, 2010; Meyer et al., 2009; Piontkewitz et al., 2011), and importantly with the same maturation delay (Ozawa et al., 2006; Piontkewitz et al., 2011). Extensively reviewed elsewhere (Meyer and Feldon, 2012), maternal immune activation rodent models exhibit deficits in sensorimotor gating, selective attention, and working memory, as well as increased sensitivity to psychotomimetic drugs (Meehan et al., 2016; Meyer and Feldon, 2010, 2012; Meyer et al., 2009). Animals exposed to immune activation also incur brain structural abnormalities such as increased lateral ventricle volume (Piontkewitz et al., 2011) and decreased cortical brain volumes (Piontkewitz et al., 2009).

GABAergic interneurons form the main inhibitory network in the brain and it has been hypothesised that maternal infection may affect the development of this network by affecting expression of GABAergic markers leading to abnormal synaptic inputs by these interneurons (Behrens and Sejnowski, 2009). In the context of the developing brain, alterations in GABAergic markers could disrupt the normal development of inhibitory circuitry that is seen in schizophrenia. Indeed, IWMNs are a subgroup of GABAergic neurons and thus the possibility arises that in the case of maternal infection, these neurons may be increased in density in the white matter of the brain in this model and contribute to the development of schizophrenia-like changes to cortical functions.

We have previously identified that prenatal exposure to immune activation results in an increase in SST+ IWMNs (early and late gestation maternal immune activation)(Duchatel et al., 2016), as well as Iba1+ immunoreactivity (late gestation maternal immune activation) (Duchatel et al., 2018b) in the white matter of the corpus callosum and corresponding increases in cortical *C4* gene expression (late gestation maternal immune activation) (Duchatel et al., 2018a), suggesting that these pathologies are particularly susceptible to late gestation immune activation. In this study we hypothesised that maternal immune activation induced in pregnant rats by PolyI:C would alter gene expression for SST and GAD in the cortex as well as induce an increase in the density of GAD+ IWMNs underneath the cortex. This will determine if maternal immune activation via PolyI:C specifically affects the SST+ IWMN subpopulation and whether it also models the more widespread effects on GABAergic neurons observed in cases with schizophrenia.

2. Methods

2.1. Animals

The use and monitoring of animals in this project was performed in accordance with the National Health and Medical Research Council's Australian code of practice for the care and use of animals for scientific purpose, with approval from the University of Newcastle Animal Care and Ethics Committee, Newcastle, Australia (Approval numbers A-2009–108 and A-2013–319).

Briefly, pregnant Wistar rats were randomly allocated to a treatment group (PolyI:C or saline) and on the appropriate gestational day (GD10 or 19) dams were anaesthetised with isoflurane (induction 5%, maintenance 2.5–3% - Abbott Australasia Pty Ltd - Australia) and administered either 4.0 mg/kg of PolyI:C (Sigma-Aldrich – AUS) or phosphate-buffered saline (PBS) via lateral tail vein injection (at 1 mL/kg body weight). Litters were weaned at postnatal day (PND) 21, at which no more than 3 animals per sex from each litter were allocated for any analyses. Litters for qPCR analyses included GD10 Saline = 6, GD10 PolyI:C = 4, GD19 Saline = 4, GD19 PolyI:C = 6. Litters for IHC analyses included GD10 Saline = 4, GD10 PolyI:C = 4, GD19 Saline = 2, GD19 PolyI:C = 4. Confirmation of maternal immune activation was verified using IL-6 ELISA (see Duchatel et al. (2016)).

2.2. Quantitative real time PCR

Animals used for qPCR are from the same cohort described in Duchatel et al. (2018b). Expression of GABAergic mRNA markers in the cingulate cortex (CC) was determined by quantitative RT-PCR (qPCR), in accordance with Duchatel et al. (2018b). A total of 24 male and 24 female rats euthanised at postnatal day 70–84 were included with $n = 6$ per sex from each of the four experimental groups; GD10 Saline, GD10 PolyI:C, GD19 Saline, GD19 PolyI:C. CC samples were obtained using the methods previously described by Duchatel et al. (2018b) from which subsequent dissection of the CC (including parts of the infralimbic, prelimbic cortices; bregma +2.5 – +3.5) was made. Brain samples from each hemisphere were kept on dry ice before being stored at −80 °C. Only samples from one hemisphere were used for qPCR analysis, with the same number of left and right hemispheres across the 4 treatment groups and sex. Tissue samples were thawed and homogenized with TissueLyzer® (Qiagen – Australia; 4 min at 20 Hz) in a RNAse-free microtube containing 1 ml of QIAzol® Lysis Reagent (Qiagen) and a 5 mm diameter stainless steel bead (Qiagen). Total RNA was then extracted using the RNeasy® Mini Kit (Qiagen) and DNase I treated (Invitrogen – USA) as per manufacturer's instructions. RNA was quantified using the NanoDrop Pearl (Implen – USA) and stored at −80 °C. RNA was reverse transcribed using a Superscript III Reverse

Transcription kit (Life Technologies – USA), as per manufacturer's instructions. A reverse transcriptase enzyme – negative control reaction was also performed for each sample. The cDNA was diluted in nuclease free water and stored at −20 °C. qPCR primers for a number of genes expressed in GABAergic neurons (Supplementary Table 1) were designed using Primer Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi). For each animal, qPCR reactions were performed in triplicate for each gene using 1x SYBR Green Select Master Mix (Thermo Fisher Scientific), 200 nM of each forward and reverse primer, and 5 µl of cDNA sample, in a total volume of 12 µl per reaction. Amplification reactions were performed at 0.2 ng/µl cDNA concentration. qPCR amplification was performed using an Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems – USA), with an initial denaturation and activation step at 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. Melt curves were generated to confirm amplification of a single gene product.

The average cycle threshold (Ct) value was calculated using the 7500 SDS software v2.0.6 (Applied Biosystems) for each gene. The relative expression of each gene was determined using the delta-delta Ct method (Schmittgen and Livak, 2008) with the geometric mean of, *Actb, Gapdh* and *Tubb3* used as the internal reference genes. Changes in gene expression between maternal immune activation at GD10 and GD19 and control groups for each sex were assessed using three-way ANOVA (SPSS) with gestational day (GD10 or GD19), treatment (control or PolyI:C) and sex (male or female) as factors. If any interaction involving treatment and gestational day was significant, post-hoc Bonferroni correction for multiple comparisons of simple treatment effects was conducted using either t-tests or Mann-Whitney tests when there were concerns about normality or homogeneity of variance of the data. Any gene expression data points that were more than two standard deviations from the mean were removed as outliers. The data are presented as increase or decrease in fold change relative to controls with the standard error of the mean (SEM).

2.3. Immunofluorescence and immunohistochemistry

Animals used for immunofluorescence and immunohistochemistry are from the same cohort described in Duchatel et al. (2016). Brain sections used for immunohistochemistry are from the same cohort of rats used to investigate IWMN density in our previous study of SST+ IWMN density by Duchatel et al. (2016). This is a separate cohort to those used in the above-mentioned qPCR analysis. Briefly, 12 week old (PND 84), male and female offspring from dams exposed to PolyI:C or saline at GD10 or GD19 were perfuse-fixed, brains collected, sectioned at 30 µm then processed for DAB immunohistochemistry as described in Duchatel et al. (2016). This current study used primary antibodies directed against GABAergic neuron markers as per Supplementary Table 2. Sodium citrate antigen retrieval was utilised for GAD primary antibodies. Free floating sections were incubated in 1 M sodium citrate for 30minutes at 80 °C then allowed to cool in antigen retrieval solution before commencing immunohistochemistry procedure.

2.4. Neuronal counting, quantification and statistical analysis

Images of sections immunolabelled for GAD+ and NPY+ using DAB immunohistochemistry were captured using the Aperio™ Digital Pathology System (Leica Biosystems) at 20X magnification and then used to calculate the area of white matter sampled in each section as described in Duchatel et al. (2016). Four sections sampled 180 µm apart from region 1 (3.2 mm – 2.5 mm from Bregma) and 2 (2.3 mm – 0.7 mm from Bregma) were used to count GAD+ or NPY+ IWMNs in the entire corpus callosum. This delineation is based on the anatomically significant difference in the white matter of the corpus callosum between these regions, where the genu of the corpus callosum becomes visible and joins both hemispheres of the cortex. All immunopositive IWMNs within this area were counted, except those touching the grey/white matter boundary. The final GAD+ and NPY+ IWMN density was calculated as the mean of the sections counted for each region in each animal. The investigator performing the counting was blind to the treatment status (i.e. maternal immune activation or vehicle) throughout experimental, analysis and quantification steps.

GraphPad Prism 7 software was used to analyse cell density data and produce descriptive statistics, including the mean and standard deviation (presented as mean +/- SD). The true p-value is reported where $p < 0.05$ was considered statistically significant. Grouped data sets were tested for normality. One-way ANOVA with Bonferroni multiple comparisons were used to compare IWMN density between control and PolyI:C GD10 and GD19 rats for each region separately. As the IWMN densities between saline treated rats at GD10 and GD19 were not significantly different ($p = 0.80$), they were pooled into a single control group. This pooling resulted in a total of 23 rats (13 M, 10 F) in the control group, 8 in the GD10 group (3 M, 5 F) and 19 in the GD19 group (7 M, 12 F). Power calculations justifying sample sizes are reported in (Duchatel et al., 2016).

3. Results

3.1. Reduced cortical somatostatin gene expression after late gestation maternal immune activation

Previously we reported that IWMNs expressed NeuN and somatostatin and that somatostatin-positive IWMNs were significantly increased in density after maternal immune activation (Duchatel et al., 2016) which is in accordance with postmortem studies of the brain in schizophrenia (Yang et al., 2011). As stated above, there is reduced expression of the *SST* (Fung et al., 2014; Hashimoto et al., 2008a, 2008b; Morris et al., 2008) and *GAD* genes (Akbarian et al., 1995; Duncan et al., 2010; Guidotti et al., 2000; Hashimoto et al., 2008a; Thompson et al., 2009) in the cortex in schizophrenia. Here we investigated whether the expression of GABAergic neuron genes in the cortex was affected by maternal immune activation in our model. We chose glutamate decarboxylase 1 (*Gad1* – codes for isoform GAD67), glutamate decarboxylase 2 (*Gad2* – codes for isoform GAD65), somatostatin (*Sst*) parvalbumin (*Pvalb*) (Fung et al., 2014; Hashimoto et al., 2008b, 2003), calbindin (*Calb1*) (Fung et al., 2014), and reelin *(Reln*) (Guidotti et al., 2000) since these genes have altered cortical expression in schizophrenia including in the CC. Neuronal nitric oxide synthase (*Nos1*) has been linked to schizophrenia from postmortem and genetic studies (reviewed in Freudenberg et al., 2015). Neuropeptide Y (*Npy*) and calretinin (*Calb2*) were chosen since they are not changed in schizophrenia (Fung et al., 2014; Mirnics et al., 2000) and thus served to rule out global effects on gene expression.

Overall three-way ANOVA identified a significant effect of gestational day on *Sst* gene expression ($F(1,38) = 6.271$, $p = 0.017$; Supplementary Table 3), but with a significant treatment x gestational day effect ($F(1,38) = 6.252$, $p = 0.017$) indicating that the effect of treatment on *Sst* gene expression was different for GD10 and GD19. There were no interactions or main effects involving sex on the Sst gene. Post hoc Mann-Whitney tests identified a significant decrease in *Sst* gene expression in the CC of rats exposed to late gestation maternal immune activation at GD19 ($p = 0.032$ after Bonferroni correction) compared to GD19 controls (Fig. 1). There was no significant treatment effect on *Sst* gene expression for GD10 rats. We then re-examined the *Sst* gene expression to determine if there were any litter effects. A total of 27 litters (7 GD10 Control, 8 GD10 MIA, 4 GD19 Control, 8 GD19 MIA) were used for qPCR. The maximum number of animals used per litter in the qPCR experiment was 3. Where 3 animals were used in a litter, it was all of a single sex, so there were no instances where 3 males and 3 females were used from the same litter. To ensure that effects were not attributable to uneven litter distributions, we averaged the gene expression data within litters so that there is only one male and one female sample from each litter. This resulted in sample sizes of 7 GD10

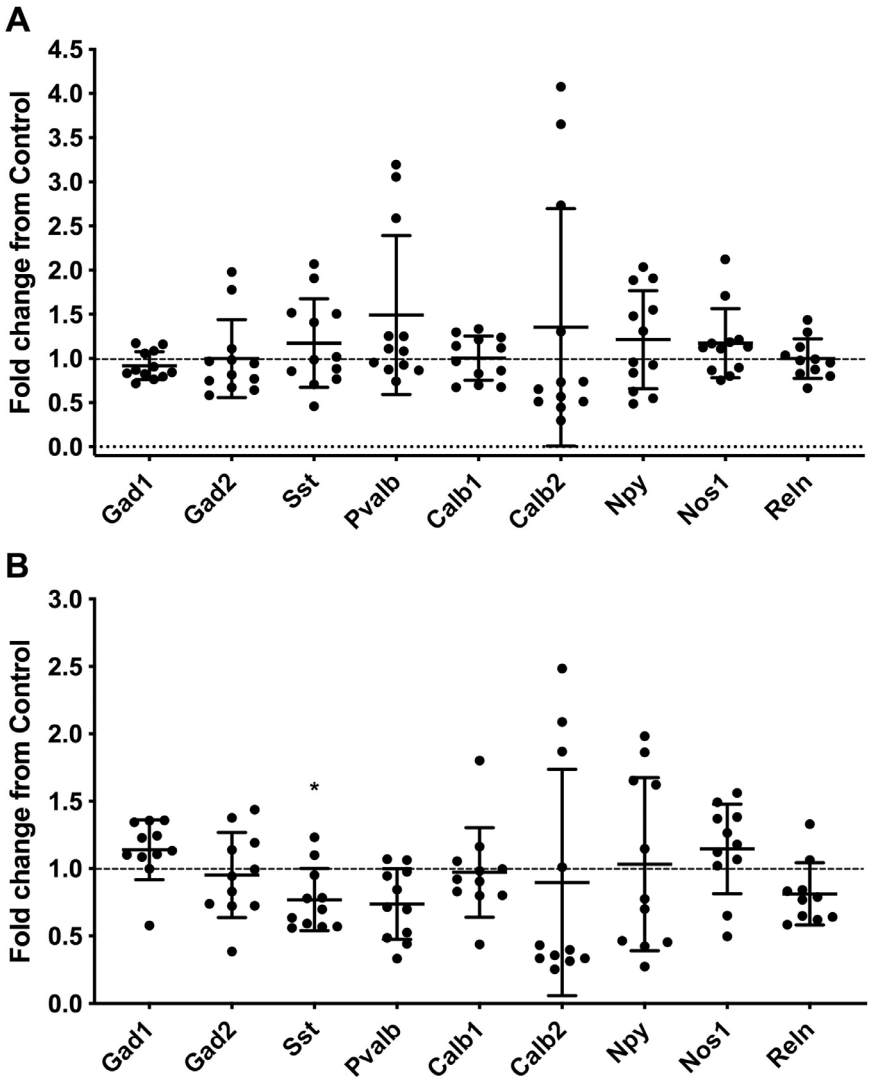

Fig. 1. Effects of maternal immune activation on GABAergic neuron-related gene expression. Relative gene expression analysis showing fold change in expression for glutamate decarboxylase 1 (*Gad1*), glutamate decarboxylase 2 (*Gad2)*, somatostatin (*Sst*), neuropeptide Y (*Npy*), parvalbumin (*Pvalb*), calbindin (*Calb1*), calretinin (*Calb2*), neuronal nitrox oxide synthase (*Nos1*) and reelin (*Reln*) in the cingulate cortex of offspring from dams exposed to PolyI:C at either GD10 (A) or GD19 (B) normalised and compared to controls where the expression was set at 1 (dashed line). *Sst* gene expression was significantly reduced in offspring of dams exposed to PolyI:C at GD19 compared to controls (* = $p < 0.05$). There was no significant changes in expression of the other GABAergic neuron-related genes. Each group consisted of 6 males and 6 females. Bars represent mean fold change from control +/- SEM.

Control (3 male, 4 female), 4 GD19 Control (2 male, 2 female), and 8 GD10 and GD19 MIA (both 4 male and 4 female). Even though this creates a low sample size that is not ideal, three-way ANOVA of the 'per litter' dataset identified a significant Treatment x GD effect ($F(1,23) = 8.86$, $p = 0.007$). The MIA < CON pairwise posthoc effect was also significant ($p = 0.016$ [Mann Whitney]). This shows that litter effects did not influence the reduction in *Sst* gene expression in rats exposed to maternal immune activation at GD19.

With respect to other markers of GABAerigc interneruons, we did not find any change in the expression of *Gad1, Gad2, Npy, Pvalb, Calb1, Calb2, Nos1*, or *Reln* genes in the CC of either GD10 or GD19 Poly:IC rats compared to their GD-specific control groups (Fig. 1, Supplementary Table 3). However, there was an overall effect of sex for the *Calb1* ($F(1,37) = 5.901$, $p = 0.02$) and *Reln* ($F(1,37) = 6.219$, $p = 0.017$) genes where females had significantly lower expression than males. For the *Calb2* gene, there was a gestational day x sex interaction ($F(1,40) = 5.587$, $p = 0.023$) where the females had lower expression, but only in the GD19 gestational timepoint. There were no other sex effects or interactions with sex on the expression of other genes.

3.2. Expression of GABAergic markers in rat IWMNs

To determine if maternal immune activation specifically induced changes in SST+ IWMNs, we investigated other IWMN subtypes in our maternal immune activation model. Firstly, we used immunofluorescence co-localisation experiments to determine what other GABAergic neuron markers were expressed by rat NeuN+ IWMNs. These studies showed that NeuN+ IWMNs express several GABAergic markers including GAD (Fig. 2A-C), NPY (Fig. 2D-F), Pv (Fig. 2G-I), Cr (Fig. 2J-L) and nNOS (Fig. 2M-O) in the corpus callosum of the white matter of control rats. Since all GAD+, NPY+, Pv+, Cr+ and nNOS+ IWMNs within the white matter also expressed NeuN, this suggested that these were all populations of mature neurons.

3.3. Effect of maternal immune activation on GAD+ and NPY+ IWMN density

We then examined whether maternal immune activation affected GAD+ IWMN density since Joshi et al. (2012) had reported increased GAD+ IWMN density in cases with schizophrenia. In our model, a two-way ANOVA showed there was no treatment effects at either GD10 or GD19 on the mean density of GAD+ IWMNs identified within the corpus callosum (Fig. 3), in either region 1 ($F(1,41) = 0.45$, $p = 0.956$; Fig 3F), or region 2 ($F(1,41) = 1.35$, $p = 0.269$; Fig 3G) compared to controls. There was also no overall effect of sex nor any treatment x sex interaction ($p > 0.05$).

Other than SST and GAD, NPY is the only other GABAergic IWMN marker examined in people with schizophrenia to date. Ikeda et al. (2004), observed an increase in NPY+ IWMN density in the deep white matter, but not superficial white matter in people with

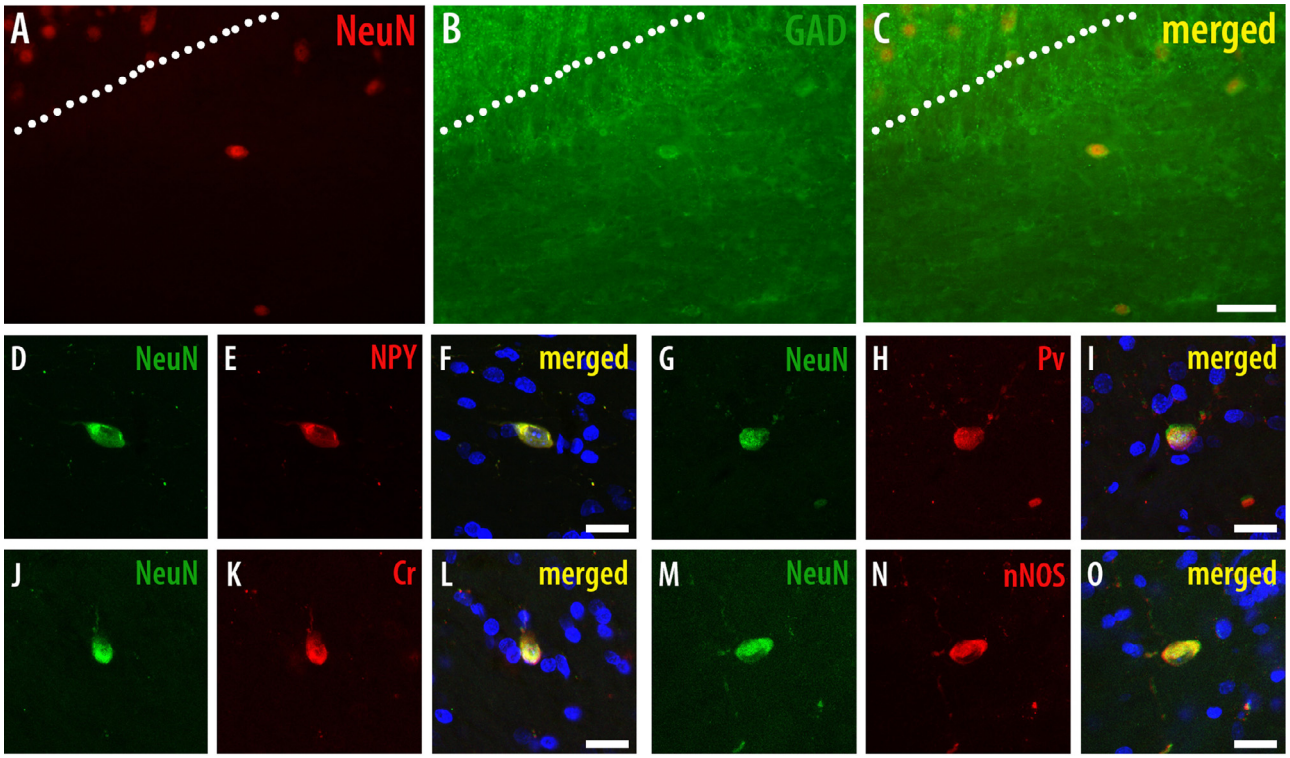

Fig. 2. NeuN+ IWMNs co-express GABAergic neuron markers. IWMNs identified by immunofluorescence in the corpus callosum of the adult rat brain by the expression of NeuN (A, D, G, J, M), were shown to co-express the GABAergic neuron markers glutamic acid decarboxylase (GAD65/67; A-C), neuropeptide Y (NPY; D-F), parvalbumin (Pv; G-I), calretinin (Cr; J-L) and neuronal nitric oxide synthase (nNOS; M-O). Merged overlays of NeuN, and GABAergic neuron subset (Yellow), with DAPI (blue) are shown in F,I,L and O. Scale bar 10 μm.

schizophrenia. In our model, a two-way ANOVA showed there was no treatment effects at either GD10 or GD19 on the mean density of NPY+ IWMNs identified within the corpus callosum (Fig. 4), in either region 1 ($F(1,21) = 3.10$, $p = 0.066$; Fig 4F), or region 2 ($F(1,21) = 2.25$, $p = 0.129$; Fig. 4G) compared to controls. There was also no overall effect of sex nor any treatment x sex interaction ($p > 0.05$).

4. Discussion

Gestational exposure to agents that cause maternal immune activation are used to model the effects of infections during pregnancy on the development of the offspring. The timing of gestational exposure to maternal immune activating pathogens such as PolyI:C has been shown to be a critical factor in the development of behavioural and cellular changes displayed by the offspring of mothers exposed to maternal infection paradigms (Boksa, 2010; Meyer and Feldon, 2012). maternal immune activation paradigms have primarily focused on early/middle vs. late gestation insults as these periods are homologous to end of the first trimester and middle to end of the second trimester in humans (Macedo et al., 2012). In this regard, we previously reported that prenatal exposure to PolyI:C in both early and late gestation induced an increase in the density of IWMNs expressing SST (Duchatel et al., 2016) in a similar manner to that observed in a postmortem brain tissue study of schizophrenia (Yang et al., 2011). In particular we showed that these changes were more widespread in the white matter after maternal immune activation during late gestation. Furthermore, we showed that the complement component C4 gene was overexpressed in the cortex in rats exposed to maternal immune activation during late gestation (Duchatel et al., 2018a). This suggested that our model of maternal immune activation was able to induce both changes in the cortex and white matter of the brain. Here we questioned how extensive the impact of maternal immune activation is on the brain by investigating whether this model was capable of inducing schizophrenia-like changes in other types of GABAergic interneurons. This would also determine whether the SST+ neurons are particularly susceptible to maternal immune activation or if other neurons are affected.

Other subtypes of GABAergic interneurons were chosen because a number of studies have shown changes in gene and protein expression for markers of GABAergic interneurons in cases with schizophrenia. In this regard, decreased expression of GAD at the gene and protein level in subjects with schizophrenia is probably the most robustly repeated finding in the post mortem tissue studies conducted on the disorder (Akbarian et al., 1995; Duncan et al., 2010; Guidotti et al., 2000; Hashimoto et al., 2008a; Thompson et al., 2009). But, particularly pertinent to our studies several reports have shown a reduction in *SST* gene expression in the cortex in schizophrenia (Fung et al., 2014; Hashimoto et al., 2008a, 2008b; Morris et al., 2008) and this appears to correlate to changes in SST+ IMWNs (Yang et al., 2011). Based on this we first investigated whether maternal immune activation could induce schizophrenia-like changes to gene expression for markers of GABAergic neurons in the CC. The CC was chosen since this brain region not only has altered structure and function in patients with schizophrenia (reviewed in Bersani et al. (2014)), as well as altered gene expression in cases with schizophrenia (Scarr et al., 2018) and in animal models of the disorder (Bosker et al., 2012). Whilst we observed no alterations in the expression of a range of markers of GABAergic neurons, including the rat homologues of *GAD*, we did observe a significant reduction in *Sst* gene expression in the cingulate cortex of rats exposed to late gestation maternal immune activation. These findings provide further evidence to support the notion that SST+ neurons are susceptible to maternal immune activation when this occurs at late gestation which is in accordance with our previous study (Duchatel et al., 2016). As such this model of late gestation maternal immune activation is able to recapitulate the changes to SST+ IWMNs and cortical *Sst* gene expression that have been observed in postmortem brains from cases with schizophrenia (Yang et al., 2011).

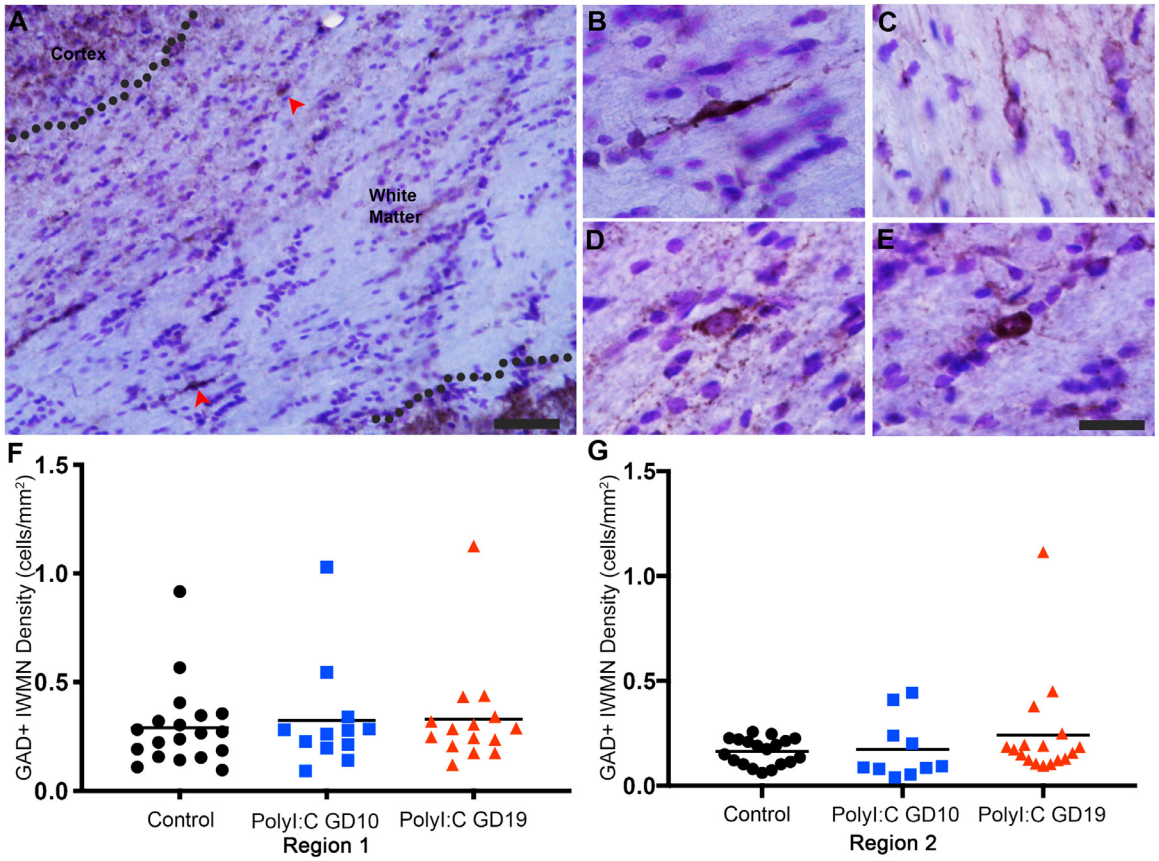

Fig. 3. Effects of maternal immune activation on glutamic acid decarboxylase positive (GAD+) interstitial white matter neurons (IWMNs) in the rat corpus callosum. Representative images of GAD+ neurons in the cortex and white matter of the corpus callosum in control rats (A). (B-E) Higher magnification of GAD+ IWMNs from A. Red arrowheads indicate representative GAD^+ IWMNs that were quantified. Density of GAD+ IWMNs assessed in the corpus callosum from offspring of animals exposed to maternal immune activation via polyinosinic:polycytidylic acid (PolyI:C) treatment at either gestational day 10 (GD10) (blue) or GD19 (red), and controls (black). Each data point represents the mean density (cells/mm^2) from one animal. Comparisons are presented for two rostrocaudally adjacent areas, region 1 (F) and region 2 (G). There was no difference in the density of GAD^+ IWMNs between control and maternal immune activation rats at either gestational time point. Scale bar: A – 30 μm, B-E – 10 μm.

So the question is whether there is a physiological consequence(s) caused by an increased density of SST+ IWMNs and a reduction in cortical *SST* gene expression? Yang et al. (2011) proposed that these two changes in the brains of schizophrenia cases may actually be linked. It is possible that an insult to the cortex leads to development of a deficit in GABAergic interneurons including a reduction in *SST* gene expression in cases with schizophrenia (Yang et al., 2011). The cause of this deficit or 'damage' to the cortex is uncertain; one possibility is neuroinflammation. In this regard, we previously showed that IBA1+ immunoreactivity is increased in these same GD19 PolyI:C rats (Duchatel et al., 2018b). Furthermore, Zhao et al. (2019) showed that maternal immune activation at GD18 induced long-term changes to microglia that was correlated with neurogenesis in the hippocampus of male rats. Therefore this deficit or 'damage' to the cortex in turn may trigger new neurons to be born, including those expressing SST, that migrate towards the cortex (Yang et al., 2011) in an effort to restore cortical function. In support of this notion Yang et al. (2011) showed that not all SST+ IWMNs expressed NeuN, a marker of mature neurons suggesting they have an immature phenotype. Furthermore, studies in primates show that IWMNs express doublecortin and polysialylated neural cell adhesion molecule which are markers of immature migrating neurons (Cai et al., 2009). Whilst studies on reelin, a protein known to be involved in directing neuronal migration (D'Arcangelo et al., 1995; Meyer and Goffinet, 1998; Meyer et al., 2000) may provide clues to altered migration in this context (reviewed in Duchatel et al. (2019) we observed no change in cingulate *Reln* gene expression in animals exposed to maternal immune activation. There are some limitations to our study that need to be acknowledged as these affect our interpretation of the data. We only measured changes in gene expression and not protein levels, so any link to function would need to be confirmed at the protein level to begin with. We also acknowledge that the immunohistochemistry for SST+ IWMNs was conducted on separate animals to the *Sst* gene expression analysis. Even with these limitations, late gestation maternal immune activation is an animal model that could be pursued to further investigate this association between reduced cortical *Sst* gene expression and increased SST+ IWMN density.

The lack of alterations in *GAD* gene expression in the cingulate cortex of maternal immune activation rats, was not what we expected. Two studies have reported the effects of maternal immune activation on GAD gene expression. Richetto et al. (2014) used PolyI:C at late gestation (GD17) and showed a reduction in *GAD* mRNA and protein expression in the offspring at adulthood (PND100). This study was conducted in mice in the medial prefrontal cortex (mPFC) which overlaps with the region used in this current study. It is possible that the mouse brain is more susceptible to the effects of maternal immune activation. However, Cassella et al. (2016) used PolyI:C injected at GD14 in rats and showed a reduction in *GAD* mRNA in the offspring at P60. Whilst it appears a similar region of the cortex was sampled to our study here, there were a number of differences that may account for the lack of concordance between the two studies. We induced maternal immune activation at early and late gestation in Wistar rats and used qPCR on brains collected between PND70-84, whereas Cassella et al. (2016) used a mid-gestation time point in Sprague

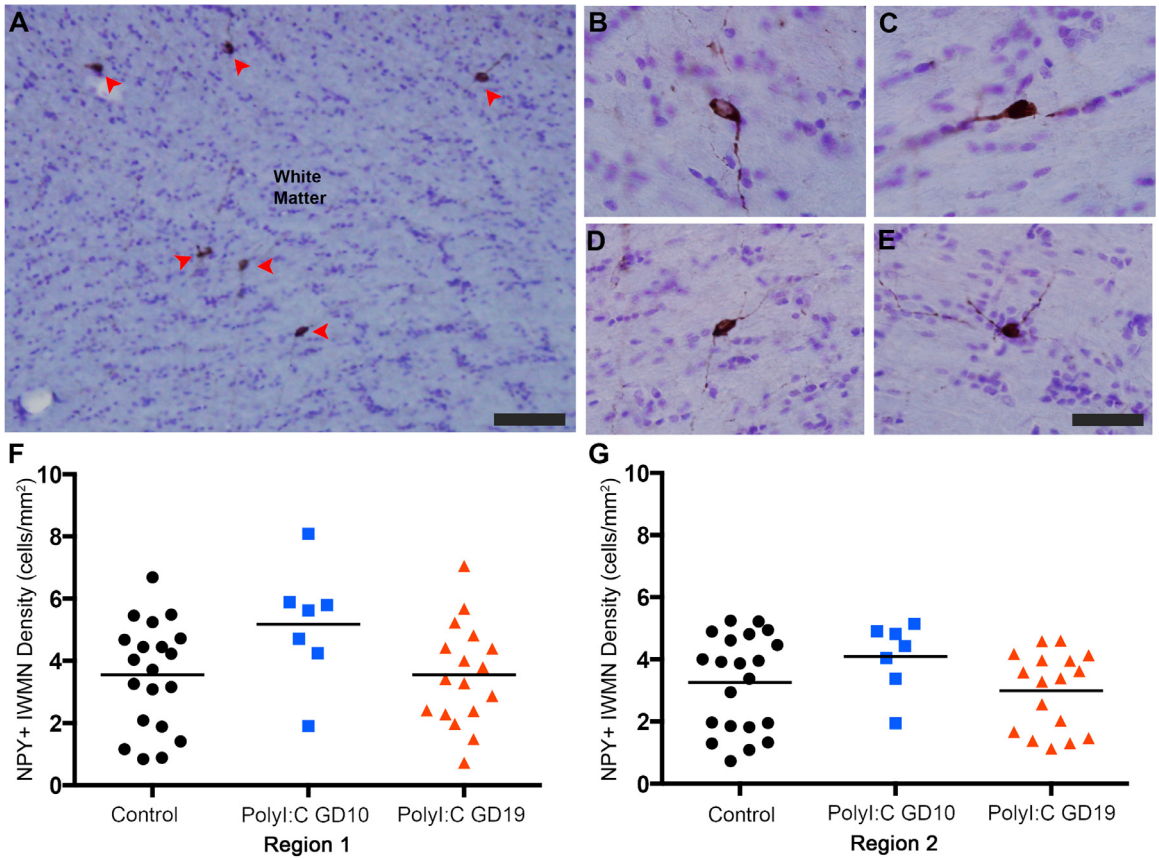

Fig. 4. Effects of maternal immune activation on neuropeptide Y positive (NPY+) interstitial white matter neurons (IWMNs) in the rat corpus callosum. Representative images of NPY+ neurons in the cortex and white matter of the corpus callosum in control rats (A). (B-E) Higher magnification of NPY+ IWMNs from (A). Red arrowheads indicate representative NPY+ IWMNs that were quantified. (F-G) Density of NPY+ IWMNs assessed in the corpus callosum from offspring of animals exposed to maternal immune activation via polyinosinic:polycytidylic acid (PolyI:C) treatment at either gestational day 10 (GD10) (blue) or GD19 (red), and controls (black). Each data point represents the mean density (cells/mm^2) from one animal. Comparisons are presented for two rostrocaudally adjacent areas, region 1 (F) and region 2 (G). The density of NPY+ IWMNs was similar in control and maternal immune activation rats. Scale Bar: A – 30 μm, B-E – 10 μm.

Dawley rats and used in situ hybridisation. Whether these differences in maternal immune activation timing, techniques or rat species can account for the lack of concordance of our study with the published literature requires further investigation.

We then moved on to study whether the density of other subtypes of IWMNs was affected by maternal immune activation. One of the limitations to this study was the sparse distribution across the corpus callosum of GAD+, Pv+, Cr+ and nNOS+ IWMNs. This limited the subsets that could be counted. As such, we were restricted to counting only the GAD+ and NPY+ IWMN subsets. Upon completion, we observed no effect of maternal immune activation at either GD10 or GD19 on the density of both the GAD+ and NPY+ IWMNs in the corpus callosum of the rat offspring. Since the tissue used here was from the same cohort of animals we reported on previously (Duchatel et al., 2016), this further suggests that SST+ IWMNs are particularly susceptible to prenatal exposure to immune activation in our model.

Given Joshi et al. (2012) had shown that the density of GAD+ IWMNs in the orbitofrontal cortex was increased by 42% in subjects with schizophrenia compared to controls, the lack of change to GAD+ IWMN density in our maternal immune activation model was not what we had expected to observe. With the assumption that all GABAergic interneurons express GAD and since Fig. 2 showed that there are multiple GABAergic populations in the rat corpus callosum, we were surprised to observe that the density of GAD+ IWMNs in the rat corpus callosum was so low. Indeed, GAD+ IWMN density (0.16–0.32 cells/mm^2) was significantly lower than that for the NPY+ IWMNs (3.25–5.17 cells/mm^2) and the SST+ IWMNs reported in our previous study (3.13–4.66 cells/mm^2) using tissue from the same cohort of animals (Duchatel et al., 2016). Indeed since the density of the total population of NeuN+ IWMNs in this cohort ranged from 16.27- 22.8 cells/mm^2 (Duchatel et al., 2016), this suggests that the majority of IWMNs in the rat corpus callosum do not express GAD. This is even more puzzling considering IWMN density in human control subjects was 31.91 cells/mm^2 for GAD+ IWMNs compared to 41.5 cells/mm^2 for the NeuN+ IWMNs (Joshi et al., 2012; Yang et al., 2011). Could it simply be the significant difference in size of the rat and human brain? Significant optimisation of the GAD antibody including antigen retrieval was required to gain successful labelling, therefore we ensured that each section where GAD+ IWMNs were counted also had labelling of GAD+ neurons in distinct layers of the overlying cortex. Interestingly, Yang et al. (2011) observed a number of SST+ IWMNs that did not express NeuN, but expressed doublecortin (Dcx) in the human white matter, suggesting that a proportion of IWMNs may be immature. Perhaps an explanation for the low density of GAD+ IWMNs is that GAD is not actively transcribed until the neurons mature when they reach the cortex in the rat brain.

Only one study to date has examined the density of NPY+ IWMNs in postmortem brains from people with schizophrenia. Ikeda et al. (2004) showed that the density of NPY+ IWMNs was increased (~8%) in the deep white matter but not changed in the superficial white matter of the brains of subjects with schizophrenia compared to controls. This is an interesting observation, considering all studies that examined NeuN, the most robust marker for IWMNs, observed increases in only the superficial white matter and not the deep white matter. However, the rodent white matter is significantly smaller in volume and the corpus callosum is directly bounded by cortical

regions and subcortical structures unlike the cortex investigated in the human studies of IWMNs. Furthermore, the IWMNs are non-uniformly distributed and at much lower density in rodent brain. Thus, we did not distinguish between superficial and deep white matter in this study, which may have limited our ability to identify this change in NPY+ IWMNs in our rat model of maternal immune activation.

In conclusion, we have shown that neurons containing SST are particularly susceptible to maternal immune activation, especially when this intervention occurs during late gestation. This is strikingly similar to what is observed in the brains of people with schizophrenia. However, even though we confirmed maternal immune activation was induced by PolyI:C in the dams in our model via IL-6 ELISA and behavioural effects were observed (Meehan et al., 2016), it appears that this intervention alone was not sufficient to recapitulate some of the other schizophrenia-like changes observed in GABAergic interneurons by previous studies of maternal immune activation and the postmortem literature in schizophrenia. Moving forward, this maternal immune activation model does provide us with the capability to study the physiological consequences of an increased density of SST+ IWMNs and changes to cortical SST gene expression. However, it may not be suitable to assess the impact of the broader GABAergic interneuron deficits observed in schizophrenia. Indeed, schizophrenia is known to be multifactorial likely involving several environmental insults and genetic changes. It is possible that a second environmental hit in conjunction with maternal immune activation might be needed to induce these broader GABAergic interneuron deficits in our model.

Declaration of Competing Interest

The authors have no competing interests.

Acknowledgments

RD, PJ, PM, DH, PT designed the study. RD conducted all experiments. RD, LH and PM completed the statistical analyses. DHA, CM, LH provided animals to be used in these experiments. MB, DS provided assistance with qPCR analysis. RD, PT drafted the manuscript with input from each of the other authors.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2019.112621.

References

Akbarian, S., Kim, J.J., Potkin, S.G., Hagman, J.O., Tafazzoli, A., Bunney Jr., W.E., Jones, E.G., 1995. Gene expression for glutamic acid decarboxylase is reduced without loss of neurons in prefrontal cortex of schizophrenics. Arch. Gen. Psychiatry 52 (4), 258–266.

Bartos, M., Vida, I., Jonas, P., 2007. Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks. Nat. Rev. Neurosci. 8 (1), 45–56.

Behrens, M.M., Sejnowski, T.J., 2009. Does schizophrenia arise from oxidative dysregulation of parvalbumin-interneurons in the developing cortex? Neuropharmacology 57 (3), 193–200.

Bersani, F.S., Minichino, A., Fojanesi, M., Gallo, M., Maglio, G., Valeriani, G., Biondi, M., Fitzgerald, P.B., 2014. Cingulate cortex in schizophrenia: its relation with negative symptoms and psychotic onset. A review study. Eur. Rev. Med. Pharmacol. Sci. 18 (22), 3354–3367.

Boksa, P., 2010. Effects of prenatal infection on brain development and behavior: a review of findings from animal models. Brain Behav. Immun. 24 (6), 881–897.

Bosker, F.J., Gladkevich, A.V., Pietersen, C.Y., Kooi, K.A., Bakker, P.L., Gerbens, F., den Boer, J.A., Korf, J., te Meerman, G., 2012. Comparison of brain and blood gene expression in an animal model of negative symptoms in schizophrenia. Prog. Neuropsychopharmacol. Biol. Psychiatry 38 (2), 142–148.

Brown, A.S., 2006. Prenatal infection as a risk factor for schizophrenia. Schizophr Bull 32 (2), 200–202.

Cai, Y., Xiong, K., Chu, Y., Luo, D.W., Luo, X.G., Yuan, X.Y., Struble, R.G., Clough, R.W., Spencer, D.D., Williamson, A., Kordower, J.H., Patrylo, P.R., Yan, X.X., 2009. Doublecortin expression in adult cat and primate cerebral cortex relates to immature neurons that develop into GABAergic subgroups. Exp. Neurol. 216 (2), 342–356.

Cajal, S., 1901a. Estudios sobre la corteza cerebral humana IV: estructura de la corteza cerebral olfativa del hombre y mamiferos. Trab. Lab. Invest. Biol. Univ. Madrid 1, 1–140.

Cassella, S.N., Hemmerle, A.M., Lundgren, K.H., Kyser, T.L., Ahlbrand, R., Bronson, S.L., Richtand, N.M., Seroogy, K.B., 2016. Maternal immune activation alters glutamic acid decarboxylase-67 expression in the brains of adult rat offspring. Schizophr. Res. 171 (1–3), 195–199.

Clancy, B., Silva-Filho, M., Friedlander, M.J., 2001. Structure and projections of white matter neurons in the postnatal rat visual cortex. J. Comp. Neurol. 434 (2), 233–252.

D'Arcangelo, G., Miao, G.G., Chen, S.C., Soares, H.D., Morgan, J.I., Curran, T., 1995. A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. Nature 374 (6524), 719–723.

Duchatel, R.J., Jobling, P., Graham, B.A., Harms, L.R., Michie, P.T., Hodgson, D.M., Tooney, P.A., 2016. Increased white matter neuron density in a rat model of maternal immune activation - Implications for schizophrenia. Prog. Neuropsychopharmacol. Biol. Psychiatry 65, 118–126.

Duchatel, R.J., Meehan, C.L., Harms, L.R., Michie, P.T., Bigland, M.J., Smith, D.W., Jobling, P., Hodgson, D.M., Tooney, P.A., 2018a. Increased complement component 4 (C4) gene expression in the cingulate cortex of rats exposed to late gestation immune activation. Schizophr. Res.

Duchatel, R.J., Meehan, C.L., Harms, L.R., Michie, P.T., Bigland, M.J., Smith, D.W., Walker, F.R., Jobling, P., Hodgson, D.M., Tooney, P.A., 2018b. Late gestation immune activation increases IBA1-positive immunoreactivity levels in the corpus callosum of adult rat offspring. Psychiatry Res. 266, 175–185.

Duchatel, R.J., Shannon Weickert, C., Tooney, P.A., 2019. White matter neuron biology and neuropathology in schizophrenia. NPJ Schizophr. 5 (1), 10.

Duncan, C.E., Webster, M.J., Rothmond, D.A., Bahn, S., Elashoff, M., Shannon Weickert, C., 2010. Prefrontal GABA(A) receptor alpha-subunit expression in normal postnatal human development and schizophrenia. J. Psychiatr. Res. 44 (10), 673–681.

Freudenberg, F., Alttoa, A., Reif, A., 2015. Neuronal nitric oxide synthase (NOS1) and its adaptor, NOS1AP, as a genetic risk factors for psychiatric disorders. Genes Brain Behav. 14 (1), 46–63.

Fung, S.J., Fillman, S.G., Webster, M.J., Shannon Weickert, C., 2014. Schizophrenia and bipolar disorder show both common and distinct changes in cortical interneuron markers. Schizophr. Res. 155 (1–3), 26–30.

Guidotti, A., Auta, J., Davis, J.M., Di-Giorgi-Gerevini, V., Dwivedi, Y., Grayson, D.R., Impagnatiello, F., Pandey, G., Pesold, C., Sharma, R., Uzunov, D., Costa, E., 2000. Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. Arch. Gen. Psychiatry 57 (11), 1061–1069.

Hashimoto, T., Arion, D., Unger, T., Maldonado-Aviles, J.G., Morris, H.M., Volk, D.W., Mirnics, K., Lewis, D.A., 2008a. Alterations in GABA-related transcriptome in the dorsolateral prefrontal cortex of subjects with schizophrenia. Mol. Psychiatry 13 (2), 147–161.

Hashimoto, T., Bazmi, H.H., Mirnics, K., Wu, Q., Sampson, A.R., Lewis, D.A., 2008b. Conserved regional patterns of GABA-related transcript expression in the neocortex of subjects with schizophrenia. Am. J. Psychiatry 165 (4), 479–489.

Hashimoto, T., Volk, D.W., Eggan, S.M., Mirnics, K., Pierri, J.N., Sun, Z., Sampson, A.R., Lewis, D.A., 2003. Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. J. Neurosci. 23 (15), 6315–6326.

Ikeda, K., Ikeda, K., Iritani, S., Ueno, H., Niizato, K., 2004. Distribution of neuropeptide Y interneurons in the dorsal prefrontal cortex of schizophrenia. Prog. Neuropsychopharmacol. Biol. Psychiatry 28 (2), 379–383.

Joshi, D., Fung, S.J., Rothwell, A., Weickert, C.S., 2012. Higher gamma-aminobutyric acid neuron density in the white matter of orbital frontal cortex in schizophrenia. Biol. Psychiatry 72 (9), 725–733.

Judaš, M., Sedmak, G., Pletikos, M., Jovanov-Milošević, N., 2010. Populations of subplate and interstitial neurons in fetal and adult human telencephalon. J. Anat. 217 (4), 381–399.

Lewis, D.A., Curley, A.A., Glausier, J.R., Volk, D.W., 2012. Cortical parvalbumin interneurons and cognitive dysfunction in schizophrenia. Trends Neurosci. 35 (1), 57–67.

Lewis, D.A., Hashimoto, T., Volk, D.W., 2005. Cortical inhibitory neurons and schizophrenia. Nat. Rev. Neurosci. 6 (4), 312–324.

Macedo, D.S., Araujo, D.P., Sampaio, L.R., Vasconcelos, S.M., Sales, P.M., Sousa, F.C., Hallak, J.E., Crippa, J.A., Carvalho, A.F., 2012. Animal models of prenatal immune challenge and their contribution to the study of schizophrenia: a systematic review. Braz. J. Med. Biol. Res. 45 (3), 179–186.

Meehan, C., Harms, L., Frost, J.D., Barreto, R., Todd, J., Schall, U., Shannon Weickert, C., Zavitsanou, K., Michie, P.T., Hodgson, D.M., 2016. Effects of immune activation during early or late gestation on schizophrenia-related behaviour in adult rat offspring. Brain Behav. Immun.
Meyer, G., Goffinet, A.M., 1998. Prenatal development of reelin-immunoreactive neurons in the human neocortex. J. Comp. Neurol. 397 (1), 29–40.
Meyer, G., Schaaps, J.P., Moreau, L., Goffinet, A.M., 2000. Embryonic and early fetal development of the human neocortex. J. Neurosci. 20 (5), 1858–1868.
Meyer, U., Feldon, J., 2010. Epidemiology-driven neurodevelopmental animal models of schizophrenia. Prog. Neurobiol. 90 (3), 285–326.
Meyer, U., Feldon, J., 2012. To poly(I:C) or not to poly(I:C): advancing preclinical schizophrenia research through the use of prenatal immune activation models. Neuropharmacology 62 (3), 1308–1321.
Meyer, U., Feldon, J., Fatemi, S.H., 2009. In-vivo rodent models for the experimental investigation of prenatal immune activation effects in neurodevelopmental brain disorders. Neuroscience & Biobehavioral Reviews 33 (7), 1061–1079.
Meyer, U., Feldon, J., Schedlowski, M., Yee, B.K., 2005. Towards an immuno-precipitated neurodevelopmental animal model of schizophrenia. Neuroscience & Biobehavioral Reviews 29 (6), 913–947.
Meynert, T., 1867. Der bau der gross-hirnrinde und seine ortlichen verschiedenheiten, nebst einem pathologisch-anato-mischen corollarium. Vierteljahrsschr Psychiatr 1, 77–93.
Mirnics, K., Middleton, F.A., Marquez, A., Lewis, D.A., Levitt, P., 2000. Molecular characterization of schizophrenia viewed by microarray analysis of gene expression in prefrontal cortex. Neuron 28 (1), 53–67.
Morris, H.M., Hashimoto, T., Lewis, D.A., 2008. Alterations in somatostatin mRNA expression in the dorsolateral prefrontal cortex of subjects with schizophrenia or schizoaffective disorder. Cerebral Cortex 18 (7), 1575–1587.
Ozawa, K., Hashimoto, K., Kishimoto, T., Shimizu, E., Ishikura, H., Iyo, M., 2006. Immune activation during pregnancy in mice leads to dopaminergic hyperfunction and cognitive impairment in the offspring: a neurodevelopmental animal model of schizophrenia. Biol. Psychiatry 59 (6), 546–554.
Piontkewitz, Y., Arad, M., Weiner, I., 2011. Abnormal trajectories of neurodevelopment and behavior following in utero insult in the rat. Biol. Psychiatry 70 (9), 842–851.
Piontkewitz, Y., Assaf, Y., Weiner, I., 2009. Clozapine administration in adolescence prevents postpubertal emergence of brain structural pathology in an animal model of schizophrenia. Biol. Psychiatry 66 (11), 1038–1046.
Richetto, J., Calabrese, F., Riva, M.A., Meyer, U., 2014. Prenatal immune activation induces maturation-dependent alterations in the prefrontal GABAergic transcriptome. Schizophr. Bull. 40 (2), 351–361.
Salinas, E., Sejnowski, T.J., 2001. Correlated neuronal activity and the flow of neural information. Nat. Rev. Neurosci. 2 (8), 539–550.
Scarr, E., Udawela, M., Dean, B., 2018. Changed frontal pole gene expression suggest altered interplay between neurotransmitter, developmental, and inflammatory pathways in schizophrenia. NPJ Schizophr. 4 (1), 4.
Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by the comparative C(T) method. Nat. Protoc. 3 (6), 1101–1108.
Thompson, M., Weickert, C.S., Wyatt, E., Webster, M.J., 2009. Decreased glutamic acid decarboxylase(67) mRNA expression in multiple brain areas of patients with schizophrenia and mood disorders. J. Psychiatr Res. 43 (11), 970–977.
Torres-Reveron, J., Friedlander, M.J., 2007. Properties of persistent postnatal cortical subplate neurons. J. Neurosci. 27 (37), 9962–9974.
Yang, Y., Fung, S.J., Rothwell, A., Tianmei, S., Weickert, C.S., 2011. Increased interstitial white matter neuron density in the dorsolateral prefrontal cortex of people with schizophrenia. Biol. Psychiatry 69, 63–70.
Zhao, Q., Wang, Q., Wang, J., Tang, M., Huang, S., Peng, K., Han, Y., Zhang, J., Liu, G., Fang, Q., You, Z., 2019. Maternal immune activation-induced PPARgamma-dependent dysfunction of microglia associated with neurogenic impairment and aberrant postnatal behaviors in offspring. Neurobiol. Dis. 125, 1–13.
Zuckerman, L., Rehavi, M., Nachman, R., Weiner, I., 2003. Immune activation during pregnancy in rats leads to a postpubertal emergence of disrupted latent inhibition, dopaminergic hyperfunction, and altered limbic morphology in the offspring: a novel neurodevelopmental model of schizophrenia. Neuropsychopharmacology 28 (10), 1778–1789.
Zuckerman, L., Weiner, I., 2005. Maternal immune activation leads to behavioral and pharmacological changes in the adult offspring. J. Psychiatr Res. 39 (3), 311–323.