Progress in Neuro-Psychopharmacology & Biological Psychiatry 65 (2016) 118–126

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Increased white matter neuron density in a rat model of maternal immune activation — Implications for schizophrenia

Ryan J. Duchatel [a,c,d,e], Phillip Jobling [a,c,d], Brett A. Graham [a,c,d], Lauren R. Harms [b,e], Patricia T. Michie [b,e], Deborah M. Hodgson [b,e], Paul A. Tooney [a,c,d,e,*]

[a] Preclinical Neurobiology Research Group, School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, University of Newcastle, Callaghan, NSW 2308, Australia
[b] School of Psychology, Faculty of Science and IT, University of Newcastle, Callaghan, NSW 2308, Australia
[c] Centre for Translational Neuroscience and Mental Health, The University of Newcastle, Callaghan, NSW 2308, Australia
[d] Hunter Medical Research Institute, New Lambton Heights, NSW 2305, Australia
[e] Schizophrenia Research Institute, Sydney, NSW, Australia

ARTICLE INFO

Article history:
Received 17 July 2015
Received in revised form 4 September 2015
Accepted 11 September 2015
Available online 15 September 2015

Keywords:
Somatostatin
Interstitial white matter neuron
Schizophrenia
Maternal immune activation
Polyinosinic:polycytidylic acid
Prenatal immune challenge

ABSTRACT

Interstitial neurons are located among white matter tracts of the human and rodent brain. Post-mortem studies have identified increased interstitial white matter neuron (IWMN) density in the fibre tracts below the cortex in people with schizophrenia. The current study assesses IWMN pathology in a model of maternal immune activation (MIA); a risk factor for schizophrenia. Experimental MIA was produced by an injection of polyinosinic:polycytidylic acid (PolyI:C) into pregnant rats on gestational day (GD) 10 or GD19. A separate control group received saline injections. The density of neuronal nuclear antigen ($NeuN^+$) and somatostatin (SST^+) IWMNs was determined in the white matter of the corpus callosum in two rostrocaudally adjacent areas in the 12 week old offspring of GD10 ($n = 10$) or GD19 polyI:C dams ($n = 18$) compared to controls ($n = 20$). $NeuN^+$ IWMN density trended to be higher in offspring from dams exposed to polyI:C at GD19, but not GD10. A subpopulation of these $NeuN^+$ IWMNs was shown to express SST. PolyI:C treatment of dams induced a significant increase in the density of SST^+ IWMNs in the offspring when delivered at both gestational stages with more regionally widespread effects observed at GD19. A positive correlation was observed between $NeuN^+$ and SST^+ IWMN density in animals exposed to polyI:C at GD19, but not controls. This is the first study to show that MIA increases IWMN density in adult offspring in a similar manner to that seen in the brain in schizophrenia. This suggests the MIA model will be useful in future studies aimed at probing the relationship between IWMNs and schizophrenia.

1. Introduction

Schizophrenia is thought to have neurodevelopmental origins where genes and environmental factors alter brain development leading to symptoms that emerge in late adolescence/early adulthood (Brown, 2006). The search for an underlying mechanism has identified cortical pathology including alterations to gamma-aminobutyric acid (GABA)ergic interneurons in the brains of people with schizophrenia (Palaniyappan et al., 2012). For example a number of studies in post-mortem tissue observed decreased levels of mRNA and protein for 67 kDa isoform of glutamic acid decarboxylase (GAD67) — a key synthetic enzyme for GABA production (Akbarian et al., 1995; Duncan et al., 2010; Guidotti et al., 2000; Hashimoto et al., 2008a; Thompson et al., 2009). Decreased levels of mRNA for other GABAergic markers including parvalbumin (Fung et al., 2014a; Hashimoto et al., 2003, 2008b), somatostatin (SST) (Fung et al., 2014a; Hashimoto et al., 2008a, 2008b; Morris et al., 2008), and neuropeptide Y (Fung et al., 2014a; Hashimoto et al., 2008a), within prefrontal cortex areas, suggest an inhibitory interneuron pathology in schizophrenia.

Meynert (reviewed in Judas et al. (2010)) first described a population of 'interstitial cells' among the fibre tracts of the adult white matter below the cortex. Dramatic changes in the density of these interstitial white matter neurons (IWMN) have been observed in schizophrenia. Of the 13 studies reported, eight show an increase in IWMN density in the superficial white matter (Akbarian et al., 1996; Anderson et al., 1996; Connor et al., 2009; Eastwood and Harrison, 2003, 2005; Joshi et al., 2012; Kirkpatrick et al., 2003; Yang et al., 2011), three described changes in the deep white matter (Akbarian et al., 1993; Ikeda et al., 2004; Rioux et al., 2003), and two reported no changes (Beasley et al., 2002; Molnar et al., 2003) in IWMN density below the cortex in

Abbreviations: GD, gestational day; GAD, glutamic acid decarboxylase; IL, interleukin; IWMN, interstitial white matter neuron; MIA, maternal immune activation; PolyI:C, polyinosinic:polycytidylic acid; NeuN, neuronal nuclei protein; SST, somatostatin.

* Corresponding author at: School of Biomedical Sciences and Pharmacy, University of Newcastle, University Drive, Callaghan, NSW 2308, Australia.
E-mail addresses: ryan.duchatel@uon.edu.au (R.J. Duchatel), Phillip.Jobling@newcastle.edu.au (P. Jobling), Brett.Graham@newcastle.edu.au (B.A. Graham), Lauren.Harms@newcastle.edu.au (L.R. Harms), Pat.Michie@newcastle.edu.au (P.T. Michie), Deborah.Hodgson@newcastle.edu.au (D.M. Hodgson), paul.tooney@newcastle.edu.au (P.A. Tooney).

http://dx.doi.org/10.1016/j.pnpbp.2015.09.006
0278-5846/

postmortem brains in schizophrenia. This work has focussed largely on IWMN density in frontal brain regions implicated in schizophrenia, including the dorsolateral prefrontal cortex (Akbarian et al., 1993, 1996; Anderson et al., 1996; Eastwood and Harrison, 2003, 2005; Yang et al., 2011), orbitofrontal cortex (Joshi et al., 2012) and anterior cingulate white matter (Connor et al., 2009).

All five studies utilising neuronal nuclei protein (NeuN) to identify IWMNs reported increased density in the brains of schizophrenia subjects (Connor et al., 2009; Eastwood and Harrison, 2003, 2005; Joshi et al., 2012; Yang et al., 2011). Furthermore, approximately 25% of IWMNs are believed to be GABAergic and increased density of GAD65/67 positive (GAD65/67$^+$) (Joshi et al., 2012) and SST$^+$ (Yang et al., 2011) IWMNs were observed in the superficial white matter of the orbitofrontal cortex and dorsolateral prefrontal cortex respectively in schizophrenia. Recent evidence also suggests that schizophrenia subjects with a high inflammatory profile categorised by the overexpression of interleukin (IL; e.g. IL-1β, IL-6 and IL-8) mRNAs have a more pronounced increase in NeuN$^+$ and GAD65/67$^+$ IWMN density in the orbitofrontal cortex compared to those schizophrenia subjects with a low inflammatory gene expression profile (Fung et al., 2014b).

This raises the possibility that neuroinflammation could have a role in the increased density of IWMN observed in schizophrenia. Indeed, there is a growing body of evidence supporting changes in the expression of genes involved in the immune system and inflammation in the blood and brains of people with schizophrenia (Fillman et al., 2013; Gardiner et al., 2013; Kumarasinghe et al., 2013). In addition, epidemiological studies suggest prenatal exposure to infectious agents including viral pathogens (e.g. influenza (Brown et al., 2004; Mednick et al., 1988), rubella (Brown et al., 2001) and measles (Torrey et al., 1988)), bacterial pathogens (Sorensen et al., 2009), the protozoan *Toxoplasma gondii* (Brown et al., 2005; Mortensen et al., 2007) and reproductive tract infections (Babulas et al., 2006) significantly increases the offspring's risk of developing schizophrenia. Furthermore, animal models that mimic these environmental insults replicate behavioural, structural and cellular brain changes, with strong face and construct validity to schizophrenia (reviewed in Meyer (2014)).

One of these models, maternal immune activation (MIA), involves the administration of the viral mimic polyriboinosinic–polyribocytidylic acid (polyI:C) to pregnant dams. Offspring of dams exposed to polyI:C exhibit numerous neurochemical and brain morphological abnormalities similar to people with schizophrenia (Meyer and Feldon, 2010; Meyer et al., 2009a; Piontkewitz et al., 2011), and with the same maturational delay (Ozawa et al., 2006; Piontkewitz et al., 2011). Rodent models of MIA display alterations in sensorimotor gating, social behaviour, and working memory, as well as increased sensitivity to psychotomimetic drugs (Meyer and Feldon, 2010, 2012; Meyer et al., 2009a). Animals exposed to MIA also exhibit structural brain abnormalities such as increased lateral ventricle volume (Piontkewitz et al., 2011) and decreased cortical brain volumes (Piontkewitz et al., 2009). The gestational timing of maternal immune activation has been shown to be a critical factor in the development of behavioural and cellular changes displayed by the offspring (Boksa, 2010; Meyer and Feldon, 2012). MIA paradigms have primarily focused on early/middle vs. late gestation insults (Meyer and Feldon, 2012). In mouse models, early/middle (gestational day — GD9) polyI:C exposure leads to impaired sensory motor gating and reduced dopamine D1 receptor levels in the prefrontal cortex, whilst late gestation (GD17) exposure potentiates NMDA receptor antagonist locomotor effects and reduced hippocampal NMDA NR1 subunit expression (Meyer et al., 2008). These gestational stages are homologous to the end of the first trimester and middle to end of the second trimester in humans and are thought to model positive and negative/cognitive symptoms of schizophrenia, respectively (Macedo et al., 2012). In contrast, reduction in neurons expressing reelin and parvalbumin, and enhancement of amphetamine-induced locomotion was observed in mice exposed to prenatal polyI:C at both gestational stages (Meyer et al., 2008).

In light of these findings we hypothesised that maternal infection may trigger a change in density of IWMNs in the adult offspring, similar to reports in schizophrenia. It was our expectation that MIA at both gestational stages would alter the density of IWMNs in adult offspring compared to control offspring. To test this, we used polyI:C to induce MIA in pregnant rats, at two different gestational days and studied the effects on IWMNs density in the corpus callosum from the brains of the adult offspring.

2. Methods and materials

2.1. Animals and maternal immune activation using polyI:C

The use and monitoring of animals 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 purposes, and with approval from the University of Newcastle Animal Care and Ethics Committee, Newcastle, Australia (Numbers A-2009-108 and A-2013-319).

Wistar rats were housed individually under a 12-h light/dark cycle, with food and water available ad libitum. Female Wistar rats in the proestrous phase (determined by vaginal impedance reading >3 Ω) were time-mated and the morning of successful mating (determined by presence of sperm using a vaginal smear) designated as GD0. Pregnant females were allocated to one of four groups and received either polyI:C (Sigma-Aldrich, Sydney, AUS) or Phosphate-buffered saline (PBS, Sigma-Aldrich) on GD10 or GD19. Pregnant rats were anaesthetised with isofluorane (Abbott Animal Health, Illinois, USA) and injected with 4 mg/kg body weight polyI:C or an equivalent volume of PBS into the tail vein. MIA was confirmed by measuring IL-6 levels from serum extracted from whole blood taken from the saphenous vein of the dam 2 h after injection of polyI:C using the IL-6 enzyme-linked immunosorbent assay (ELISA) standard protocol (Biorad; Gladesville, AUS). Circulating IL-6 was significantly increased in all dams receiving polyI:C but not dams that received PBS (Supplementary Fig. 1). Offspring were kept in full litters until postnatal day 21 when they were removed from their mothers and kept in same-sex littermate pairs until 12 weeks of age.

2.2. Preparation of brain tissue

At 12 weeks of age, rats were deeply anaesthetised by intraperitoneal injection of 160 mg/kg sodium pentobarbitone (Virbac Animal Health, Milperra, AUS), then transcardially perfused with saline followed by 4% paraformaldehyde (Sigma-Aldrich) in 0.1% Phosphate Buffer. Brains were extracted, post-fixed in 4% paraformaldehyde for 24 h before being stored in Phosphate Buffered Saline (PBS) containing 0.01% sodium azide (Sigma-Aldrich) at 4 °C. Brains were then submerged in PBS with 12.5% sucrose (ChemSupply, Port Adelaide, AUS) overnight at 4 °C, mounted in TissueTek Optimal Temperature Compound (ProSciTech, Kirwan, AUS), frozen at -20 °C and sectioned at 30 μm using a Leica SM2000R microtome (Leica Biosystems, North Ryde, AUS).

2.3. Immunohistochemistry

For diaminobenzidine immunohistochemistry, rat brain sections were processed essentially as described previously (Tooney and Chahl, 2004). Briefly, rat brain sections were treated with 50% ethanol containing 0.9% H_2O_2 for 20 min at room temperature (RT). Sections were washed in triton diluent (0.1% Triton X-100 + 1% normal donkey serum) for 15 min, blocked using 10% normal donkey serum in PBS, and incubated with primary antibodies either mouse anti-NeuN (1:1000, Millipore, California, USA; Cat. #MAB377) or rabbit anti-SST (1:500, Santa Cruz Biotechnology, California, USA; Cat. #sc-139,099) in Triton diluent for 48 h at 4 °C. Sections were next washed in PBS (3 × 15 min) and incubated in donkey anti-mouse (for NeuN — 1:1000,