Effects of maternal immune activation on adult brain neurobiology

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Abstract

Prenatal immune challenge is an environmental risk factor for the development of psychiatric illnesses including schizophrenia. Modelling this epidemiological link in animals shows that maternal immune activation (MIA) is capable of inducing long-lasting changes in brain structure, function and behaviour in the offspring; which is very promising for elucidating the underlying mechanisms for schizophrenia. Indeed, one interesting anatomical finding within schizophrenia research is an increased density of neurons residing in the white matter under grey matter cortical regions. These interstitial white matter neurons (IWMNs) have been hypothesised to have a number of neural origins, but the role they play in the underlying schizophrenia aetiology is unknown. Chapter 1 introduces schizophrenia and presents an in-depth literature review on the changes to IWMNs in schizophrenia. Chapter 1 then presents the evidence for MIA and its relationship to schizophrenia. However, to fully elucidate their role in disease pathogenesis an animal model is necessary to study IWMNs in an environment other than human post-mortem brain.

In Chapter 2, the aim was to characterise IWMNs subjacent to the frontal cortex of the adult rodent brain, including markers and location, and then examine if MIA affected the density of IWMNs in this model. MIA was induced by early or late gestation exposure of pregnant rats to polyriboinosinic-polyribocytidylic acid (PolyI:C) with IWMN density assessed in the adult rat offspring. While NeuN+ IWMNs trended to be increased by this model, both early (gestational day 10; GD10) and late (GD19) gestation MIA induced a significant increase in somatostatin positive (SST+) IWMN density in the white matter of the corpus

callosum. Interestingly the increase in SST+ IWMN density was regionally more widespread in those rats exposed to MIA at GD19. These changes are similar to that observed in post-mortem brain studies of schizophrenia. This established an animal model of increased white matter neuron density induced by a known risk factor for schizophrenia. Then in Chapter 3 the aim was to determine if other IWMN subtypes were altered by MIA. The density of both NPY+ IWMNs and GAD+ IWMNs was examined but neither were affected by MIA – suggesting that SST+ IWMNs are particularly susceptible to MIA. Chapter 3 also provided a gene expression analysis to determine if MIA affected cortical GABAergic gene expression.

The role of an abnormal immune system in schizophrenia has recently come to light. Alterations in immune related genes within the cortex of people with schizophrenia has provided an underlying “immune signature” in the disease. Furthermore, studies of post-mortem brains in schizophrenia have identified changes to glia, the brain’s immune cells. I hypothesised that the MIA-induced increase in IWMNs reported in Chapter 2 may be driven by inflammation in the cortex reflected by alterations in glial cells and inflammatory gene expression. In Chapter 4 I tested this hypothesis by examining inflammatory gene expression and immunohistochemistry for microglia and astrocytes in the brains of rats exposed to MIA induced by PolyI:C. Whilst there were no changes in cortical inflammatory gene expression, a significant increase in microglia (Iba1+) immunoreactivity was observed in the white matter of the corpus callosum, but not the cingulate cortex, suggesting that disrupted microglia were specific to the white matter. Furthermore, no alterations in astrocyte (GFAP+) immunoreactivity

was identified in rats exposed to MIA, which is congruent with current literature on their role in schizophrenia.

Further links to immune dysfunction in schizophrenia came from a ground-breaking discovery by Sekar et al. (2016), who identified a significant association of the complement component 4 (*C4*) gene with schizophrenia and that people with schizophrenia have a significant increase in the expression of the complement component 4 (*C4*) gene. In Chapter 5 I showed that this alteration in cortical *C4* gene expression was also present in the cingulate cortex of rats exposed to MIA at late gestation.

In summary the data in this thesis provides a link between white matter pathology, including increased white matter neurons, and increased microglia reactivity, with cortical innate immune system gene expression changes. Finally, this thesis provides a discussion, summarising the work presented within, linking MIA with increased IWMN density, increased microglial reactivity in the white matter and cortical changes in *C4* gene expression.

Table of Contents

List of Abbreviations

BA	Brodmann's area
ChR	channelrhodopsin
CC	cingulate cortex
Cr	calretinin
CWM	cingulate white matter
DA	dopamine
DCX	doublecortin
DLPFC	dorsal lateral prefrontal cortex
DSM-V	diagnostic and statistical manual, fifth version
DTI	diffusor tensor imaging
eGFP	enhanced green fluorescent protein
EPS	extrapyramidal syndrome
GABA	γ-aminobutyric Acid
GAD	glutamic acid decarboxylase
GAT-1	GABA transporter
GD	gestational day
GSEA	gene set enrichment analysis
GWAS	genome wide association study
ICD10	tenth revision of the international classification of disease
IPC	inferior parietal cortex
IWMN	interstitial white matter neuron
MAP2	microtubule associated protein 2
MIA	maternal immune activation
mPFC	medial prefrontal cortex
MRI	magnetic resonance imaging
NADPH	nicotinamide–adenine dinucleotide phosphate-diaphorase
NeuN	neuronal nuclei
NMDA	N-methyl-D-aspartate
nNOS	neuronal nitric oxide synthase
NR	not reported
NPY	neuropeptide Y
OFC	orbitofrontal cortex

PET	positron emission topography
PHG	para hippocampal gyrus
PND	postnatal day
PolyI:C	polyriboinosinic-polyribocytidylic acid
Pv	parvalbumin
RelN	reelin
SD	standard deviation
SST	somatostatin
vGAT	vesicular GABA transporter
vGLUT	vesicular glutamate transporter