The Role of Early Versus Late Gestational Maternal Immune Activation in the Aetiology of Schizophrenia: Establishing a Rat Model with a Focus on Cognitive Symptomology and Neuroinflammation.

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~ Psychology (Science)

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Statement of Originality

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository**, subject to the provisions of the Copyright Act 1968.

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Crystal Lea Meehan

Statement of Collaboration

I hereby certify that the work embodied in this thesis has been done in collaboration with other researchers. I have included as a part of this thesis the below statement clearly outlining the extent of collaboration, with whom, and under what auspices.

Chapter 1 consists of a literature review written by myself and edited by Dr Lauren Harms, Emeritus Professor Patricia Michie, and Professor Deborah Hodgson. I was responsible for the data collection and analysis of data presented in Chapter 2. Statistical assistance for the weight data analysis in Chapter 2 was provided by statistician Kim Colyvas. Chapter 2 was written by myself and edited by Dr Lauren Harms, Emeritus Professor Patricia Michie, and Professor Deborah Hodgson. Chapter 3A is a published manuscript that I am a co-author of. Full details of the contributions to Chapter 3A are detailed in the *'Statement of Authorship'* found on page ix. I and Dr Lauren Harms are responsible for conducting the experimental the work presented in all figures in Chapter 3B. Dr Ross Fulham and Emeritus Professor Patricia Michie contributed to data processing and data analysis. Chapter 3B was written by myself and edited by Dr Lauren Harms, Emeritus Professor Patricia Michie, and Professor Deborah Hodgson. The introduction to Chapter 3 was written by myself and edited by Dr Lauren Harms, Emeritus Professor Patricia Michie, and Professor Deborah Hodgson. Chapter 4 is a published manuscript of which I am first author, in conjunction with Dr Lauren Harms. Full details of the contributions to Chapter 4 are detailed in the *''Statement of Authorship'* found on page ix. The work in Chapter 5 is a manuscript of which I am joint first author, in conjunction with Mr Ryan Duchatel. This manuscript is a final draft that was submitted to the journal *Progress in Neuropsychopharmaoclogy and Biological Psychiatry* for peer review without success. A revised version of the manuscript presented in this thesis was submitted to the journal *Psychiatry Research* for peer review on 17 November 2017, revisions were requested by the journal on 22 January 2018, and a further revised version was re-submitted to *Psychiatry Research* on 20 March 2018. Full details of the contributions to Chapter 5 are detailed in the *''Statement of Authorship'* found on page ix. Chapter 6 was written by myself and

edited by Dr Lauren Harms, Emeritus Professor Patricia Michie, and Professor Deborah Hodgson.

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Crystal Lea Meehan

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Professor Deborah Hodgson

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Dr Lauren Harms

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Emeritus Professor Patricia Michie

Statement of Authorship

I hereby certify that the work embodied in this thesis contains published papers/scholarly work of which I am a joint author. I have included as part of the thesis, the below statement, endorsed by my supervisor, attesting to my contribution to the joint publications/scholarly work.

Chapter 3A is a published manuscript of which I am a co-author. I contributed to performing the experiments in conjunction with Dr Lauren Harms. I also contributed to the preparation of the manuscript for publication in conjunction with Dr Lauren Harm, Dr W Ross Fullham, Dr Juanita Todd, Dr Timothy W Budd, Conjoint Associate Professor Mick Hunter, Adjunct Professor Markku Penttonen, Professor Ulrich Schall, Dr Katerina Zavitsanou, Professor Deborah Hodgson, and Emeritus Professor Patricia Michie. Emeritus Professor Patricia Michie, Professor Deborah Hodgson, Dr Lauren Harm, Dr W Ross Fullham, Dr Juanita Todd, Dr Timothy W Budd, Conjoint Associate Professor Mick Hunter, Adjunct Professor Markku Penttonen, Professor Ulrich Schall, Dr Katerina Zavitsanou contributed to the conception and design of the experiments. While, Dr Lauren Harm, Dr W Ross Fullham, and Adjunct Professor Markku Penttonen were responsible for analysis of the data.

Chapter 4 is a published manuscript which myself and Dr Lauren Harms are joint first authors. I was responsible for performing the experiments detailed in the publication, with assistance from Dr Lauren Harms and Jade Frost. The qPCR was conducted by Rafael Barreto, on tissue samples prepared by myself. I and Dr. Lauren Harms were responsible for data analysis. The manuscript was written by myself and Dr Lauren Harms, and edited by Jade Frost, Dr Rafael Barreto, Dr Juanita Todd, Professor Ulrich Schall, Professor Cynthia Shannon Weickert, Dr Katerina Zavitsanou, Emeritus Professor Patricia Michie, and Professor Deborah Hodgson. I along with Dr Lauren Harms, Dr Juanita Todd, Professor Ulrich Schall, Professor Cynthia Shannon Weickert, Dr Katerina Zavitsanou, Emeritus Professor Patricia Michie, and Professor Deborah Hodgson contributed to the conception and design of the experiments.

Chapter 5 is a manuscript of which I am joint first author with Mr Ryan Duchatel. The manuscript wasill be submitted to the journal Progress in Neuropsychopharmaoclogy and Biological Psychiatry without success. A revised

version of the manuscript presented in this thesis was submitted to the journal Psychiatry Research for peer review on 17 November 2017, revisions were requested by the journal on 22 January 2018, and a further revised version was re-submitted to Psychiatry Research on 20 March 2018in the near future.. The experimental work presented in all figures of Chapter 5 was conducted by myself in collaboration with Mr. Ryan Duchatel from the School of Biomedical Sciences, The University of Newcastle. Assistance with immunohistochemistry data processing and use of laboratory equipment was provided by Associate Professor Rohan Walker. Assistance with qPCR analysis was provided by Mr Mark Bigland and Associate Professor Douglas Smith. Data analysis was undertaken by myself and Mr Ryan Duchatel. Chapter 5 was written by myself and Mr Ryan Dutchatel, and edited by Associate Professor Paul Tooney, Dr Lauren Harms, Emeritus Professor Patricia Michie, Associate Professor Rohan Walker, Mr Mark Bigland, Associate Professor Douglas Smith, Dr Phillip Jobling, and Professor Deborah Hodgson. I along with Mr. Ryan Dutchatel, Dr Lauren Harms, Associate Professor Paul Tooney, Emeritus Professor Patricia Michie, and Professor Deborah Hodgson contributed to the conception and design of the experiment.

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Crystal Lea Meehan

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Professor Deborah Hodgson

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Dr Lauren Harms

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Emeritus Professor Patricia Michie

Published Works Incorporated in this Thesis

1. **Harms, L.,** Fulham, W. R., Todd, J., Budd, T. W., Hunter, M., Meehan, C., Penttonen, M., Schall, U., Zavitsanou, K., Hodgson, D. M., Michie, P. T. (2014). Mismatch negativity (MMN) in freely-moving rats with several experimental controls. *PLoS One, 9*(10), e110892.

2. **Meehan, C., Harms, L.,** Frost, J. D., Barreto, R., Todd, J., Schall, U., Shannon Weickert, C., Zavitsanou, K., Michie, P. T., Hodgson, D. M. (2017). Effects of immune activation during early or late gestation on schizophrenia-related behaviour in adult rat offspring. *Brain Behaviour and Immunity, 63*, 8-20.

3. **Duchatel, R. J., Meehan C. L.,** Harms L., Michie P. T., Bigland M. J., Smith D. W., Walker F. R., Jobling, P., Hodgson D. M., Tooney, P. A. Maternal immune activation increases microglia levels in the white matter of the corpus callosum of adult rat offspring. [Submitted to *Progress in Neuropsychopharmacology and Biological Psychiatry* without success. A revised version of the manuscript presented in this thesis was submitted to the journal *Psychiatry Research* for peer review on 17 November 2017, revisions were requested by the journal on 22 January 2018, and a further revised version was re-submitted to *Psychiatry Research* on 20 March 2018]

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And finally, to my grandmother, Sandra Glover. Thank you for being a part of my life. I only wish I could have had more time with you. You were an inspiration to have lived with the disease of which this thesis focus is. I miss you greatly and I wish you had lived to see me complete this body of work, as you inspired me to research this topic.

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Abstract

Schizophrenia is a debilitating disorder of neurodevelopmental origins that likely stems from the cumulative action of a range of genetic and environmental factors. Epidemiological evidence has identified maternal infection during gestation as one significant environmental risk factor for the development of the disorder. Evidence from animal models has further validated the link between maternal immune activation (MIA) in the absence of an active infection and the later life development of schizophrenia-like pathology in the offspring. In particular, work in mouse models has suggested that the gestational time at which MIA occurs can alter the behavioural and neurobiological phenotype displayed. Specifically, that MIA in late gestation is involved in schizophrenia-relevant cognitive dysfunction and altered NMDA receptor expression, whereas MIA in early gestation is more closely associated with behavioural deficits reminiscent of positive symptomology and dopaminergic neurotransmission. The aim of the current thesis was to extend the mouse findings to another species, the rat, and further explore the effects of MIA. In addition to producing a reliable rat model of schizophrenia where distinct behavioural and neurological phenotypes associated with schizophrenia are produced following MIA at either early or late gestational time-points (gestational day 10 or 19, respectively), the current thesis extends on previous work by examining the schizophrenia biomarker of mismatch negativity and assessing the neuroinflammatory state of offspring.

Behavioural assessments revealed that MIA in either early or late gestation produced transient impairments in working memory and reductions in PPI. In these behavioural studies, there was no clear distinction between a dopamine and glutamate-related behavioural phenotype based on the gestational timing of exposure. However,

early but not late gestation MIA did produce alterations in the dopaminergic system of males, as indicated by increased dopamine 1 receptor mRNA in the nucleus accumbens. EEG experiments demonstrated that although the male rat brain is able to generate human-like (adaptation-independent) mismatch responses (MMRs), and although MIA (regardless of gestational timing) does alter MMRs, it does not do so in a manner comparable with schizophrenia. Immunohistochemical techniques revealed that MIA does result in subtle neuro-immune changes in adult offspring, with an increase in microglial immunoreactivity identified in the frontal white matter of late, but not early, gestation MIA animals. Furthermore, a strong trend towards increased astrocyte immunoreactivity that approached significance was identified in the prefrontal cortex of late, but not early MIA offspring.

The combined results have demonstrated that MIA during the chosen gestational time-points are sufficient to disrupt neurodevelopmental processes producing long-term alterations in behavioural and neuropathological measures relevant to schizophrenia. However, the phenotype characterised here deviates slightly from previous findings from mouse models indicating potential differences in the critical periods of neurodevelopmental susceptibility to MIA exposure between the rat and mouse. Importantly this research has provided insights into the underlying neuro-immune changes which may contribute to the behavioural abnormalities seen in adult MIA offspring and has provided evidence that MIA in rats can alter the prominent schizophrenia relevant electrophysiological biomarker of adaptation-independent MMRs, providing a basis to further investigate these measures and their underlying mechanisms.

Abbreviations

Abbreviation	Description
µg	micrograms
µl	microliter
µm	micrometre
µV	microvolts
♀	female
♂	male
11β-HSD	11β-hydroxysteroid-dehydrogenase
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPH	amphetamine
ANOVA	Analysis of Variance
BMI	body mass index
cDNA	complimentary DNA
cm	centermeter
CNS	central nervious system
CON	control
CORT	corticosterone
CPu	caudate putamen
D1r	dopamine 1 receptor
D2r	dopamine 2 receptor
D3r	dopamine 3 receptor
D4r	dopamine 4 receptor
D5r	dopamine 5 receptor
DA	dopamine
DAr	dopamine receptor
DAT	dopamine transporter
dBL	decibels
DEV	deviant
DISC1	disrupted-in-schizophrenia-1
EDTA	Ethylenediaminetetraacetic acid
EEG	electroencephalogram

ELISA	enzyme-linked immunosorbent assay
ERP	event related potential
g	grams
GABA	gamma-aminobutyric-acid
GD	gestational day
h	hours
HPA	hypothalamic-pituitary-adrenal
Hz	hertz
i.p.	intraperitoneal
ITI	intertrial interval
kg	killogram
kHz	kilohertz
LAC	left auditory cortex
L-DOPA	dihydroxyphenylalanine
LFC	left frontal cortex
LML	left of midline
LMM	linear mixed model
LPS	lipopolysaccharide
m	meter
M	mole
mg	milligram
MIA	maternal immune activation
min	minute
MK-801	Dizocilpine
mL	milliliter
MLR	mid latency response
MMN	mismatch negativity
MMR	mismatch response
mPFC	medial prefrontal cortex
mRNA	messenger ribonucleic acid
ms	milliseconds
MSC	Many-standards control
mV	millivolts

NAc	nucleus accumbens
ng	nanograms
NMDA	N-methyl-D-aspartate
NMDAr	N-methyl-D-aspartate receptor
PBS	phosphate buffered saline
PCP	phencyclidine
PET	positoron emission tomography
PFC	prefrontal cortex
pg	picograms
PND	postnatal day
Poly (I:C)	polyriboinosinic-polyribocytidilic acid
PPI	prepulse inhibition
qPCR	real-time quantitative polymerase chain reaction
RAC	right auditory cortex
RFC	right frontal cortex
RNA	ribonucleic acid
RNase	Ribonuclease
s.c.	subcutanious
SEM	standard error of the mean
SN	substantia nigra
SOA	stimulus onset asynchrony
SPSS	statistical package for the social sciences
STD	standard
TH	tyrosine hydroxilaise
TLR3	toll like receptor 3
TLR4	toll like receptor 4
VHL	ventral hippocampal lesion
VTA	ventral tegmental area

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