PERINATAL PROGRAMMING - INTEGRATION OF BRAIN, BEHAVIOUR AND IMMUNITY:
IMPLICATIONS FOR REPRODUCTIVE FITNESS

Presented By

LUBA SOMINSKY

MA (Psychobiology)

Submitted in fulfilment of the requirements of the degree of

Doctor of Philosophy

School of Psychology,
Faculty of Science and Information Technology
The University of Newcastle, Australia

September, 2013
Declaration

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 this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

I hereby certify that the work embodied in this thesis has been done in collaboration with other researchers. I have included as part of the thesis a statement clearly outlining the extent of collaboration, with whom and under what auspices. I hereby certify that this thesis is submitted in the form of a series of published papers of which I am a joint author. I have included as part of the thesis a written statement from each co-author; and endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.

I warrant that I have obtained, where necessary, permission from the copyright owners to use any of my own published work (i.e. journal articles) in the thesis in which the copyright is held by another party (i.e. publisher).

_________________

Luba Sominsky
Acknowledgements

This thesis was made possible due to the many people who have provided encouragement, help and support throughout my candidature. Firstly and mostly, I would like to thank my supervisor, Professor Deborah Hodgson, for her never ending support, inspiration, mentorship and friendship. I am very grateful for all the opportunities I have had throughout my PhD. Thank you Deb, for encouraging me to be not only a better researcher and academic, but more importantly so, a better person. Thank you for all your patience and for always being there for me, no matter how busy you are.

I would like to thank Miss Donna Catford for her dedicated animal care and unswerving dedication to the research process.

I would like to acknowledge all the many collaborators and their contributions to the publications that comprise this thesis. Especially thank you to Dr. Rohan Walker, Prof. Peter Dunkley, Dr. Larisa Bobrovskaya, Dr. Lin Ong and A. Prof. Eugene Nalivaiko. A special thanks goes to Professor Eileen McLaughlin for her advice and insightful guidance. Thank you for the opportunity to learn from you and your group.

Adam, I cannot thank you enough for all your incredible help, advice, encouragement and friendship. I would not be able to complete my PhD without your support.

Erin and Crystal, a huge thank you for all the hard work and dedication, especially during your honours year, but most importantly thank you for being such good friends.

Thank you Javad and Mahta for all your invaluable help in the lab, I am grateful for the opportunity to have met you.

I would like to thank my family and friends, for their continued love and encouragement, and for always believing in me. Thank you Regev for everything that you have given and done for me, and for all your support. And lastly, this thesis is undoubtedly dedicated to my dad, Dr. Vitaly Sominsky, who has inspired me with his willingness to learn and “infected” me with his passion for research.
List of publications included as part of the thesis


# Table of Contents

Thesis abstract .......................................................................................................................... 6  
Introduction and literature review .......................................................................................... 10  
1. Developmental Origins of Health and Disease: Implications for perinatal programming .......... 10  
   1.2 The impact of perinatal stress on adult health outcomes ................................................. 12  
      1.2.1 Perinatal programming of pathology ........................................................................ 13  
      1.2.2 Perinatal programming of psychopathology .............................................................. 14  
2. Mechanisms underpinning perinatal programming ............................................................. 16  
   2.1 The Hypothalamic-Pituitary-Adrenal (HPA) Axis ......................................................... 18  
      2.1.1 Programming of the HPA axis ................................................................................. 19  
   2.2 The Autonomic Nervous System (ANS) ............................................................................. 21  
      2.2.1 Programming of the ANS ....................................................................................... 24  
   2.3 The Hypothalamic-Pituitary-Gonadal (HPG) axis ............................................................. 25  
      2.3.1 Programming of the HPG axis ................................................................................ 28  
   2.4 The immune system ......................................................................................................... 31  
      2.4.1 Programming of the immune system ...................................................................... 34  
      2.4.2 Programming of the immune response via neural-endocrine-immune interactions ... 36  
3. An animal model of early life stress ..................................................................................... 38  
   3.1 Lipopolysaccharide - An Immunological Stressor .............................................................. 39  
   3.2 Neonatal immune challenge by administration of LPS as a model of early life stress ... 42  
      3.2.1 Impact of Neonatal LPS on Metabolic Function ..................................................... 43  
      3.2.2 Impact of Neonatal LPS on Endocrine Function ..................................................... 44  
      3.2.3 Impact of Neonatal LPS on Immunity ...................................................................... 45  
      3.2.4 Impact of Neonatal LPS on behaviour .................................................................... 47  
      3.2.5 Impact of Neonatal LPS on Reproduction ............................................................... 50  
4. Aim and Rationale of Thesis ............................................................................................... 52  
5. Overview of papers ............................................................................................................. 53  
Published Papers ..................................................................................................................... 57  
   Paper 1: Increased microglial activation in the rat brain following neonatal exposure to a 
   bacterial mimetic ................................................................................................................... 57
Paper 2: Functional programming of the autonomic nervous system by postnatal immune challenge: implications for anxiety .................................................................64

Paper 3: Neonatal lipopolysaccharide exposure impairs sexual development and reproductive success in the Wistar rat.................................................................78

Paper 4: Neonatal immune challenge alters reproductive development in the female rat ...90

Paper 5: Immune regulation of ovarian development: programming by neonatal immune challenge........................................................................................................102

Discussion................................................................................................................................................123

1. General Discussion ................................................................................................................................123

2. Activation of neural pathways by neonatal LPS challenge .................................................................125

3. Programming of the HPA axis and ANS by neonatal LPS challenge ........................................130

4. Anxiety-like phenotype: a broad behavioural spectrum ..................................................................133

5. Programming of reproductive development by neonatal LPS challenge ......................................136

6. Long term alterations in gonadal physiology: an emphasis on ovarian function ...138

7. Conclusions........................................................................................................................................142

7.1 Summary ........................................................................................................................................142

7.2 A new perspective: Brain-Immune-Gonadal (BIG) axis .................................................................143

8. Implications ........................................................................................................................................145

References .............................................................................................................................................151
**Thesis abstract**

Events occurring in early life can induce long-term physiological and behavioural changes through the process of perinatal programming. The concept of perinatal programming has an adaptive value, preparing the foetus for specific extra-uterine demands. As such, early life adversity is thought to enhance an immediate survival via physiological adaptation when the postnatal environment is similar to the prenatal environment. However, under conditions of discrepancy between the early and later life environment, this adaptation may prove disadvantageous, leading to physiological and psychological changes that may predispose the organism to poorer long term health outcomes. Early life adversity, elicited by changes in the nutritional environment, or due to an exposure to stressful and traumatic events, has received increasing recent attention. One model of early life adversity that has been useful in modelling developmental outcomes associated with the early life environment is the model of “neonatal immune challenge”. Specifically, previous research has identified the early microbial environment as a critical factor in the development of mood and behaviour, with increased immune activation during neonatal life having been linked to an emergence of anxiety behaviours in adulthood. The primary aim of the current thesis was to investigate the immediate and long term effects of neonatal immune challenge on the neuroimmune and neuroendocrine pathways, which are proposed to underpin the altered behavioural phenotype. To achieve this aim the Wistar strain rat model was employed. To simulate an immune challenge, these animals were intraperitoneally administered lipopolysaccharide (LPS; *Salmonella enterica*, serotype *enteritidis*), on postnatal days (PNDs) 3 and 5 (birth = PND 1). Importantly, an established framework of an anxiety-like phenotype was expanded to encompass a wider range of behavioural changes. Thus, in addition to anxiety-like
behaviours, sexual behaviour was examined, along with the underlying regulatory
mechanisms of reproductive development and function.

The first paper (Sominsky et al., 2012b) in this thesis reported that neonatal LPS exposure is
associated with increased microglial activation in the adult brain, corresponding to an
increase in anxiety-like behaviours. Given the mediating role of microglia in inflammation-
induced psychopathology, the results of this study suggest a neuroimmune pathway which
may underpin the long term behavioural changes observed in adulthood following neonatal
LPS challenge. Moreover, the increase in microglial activation was specific to the
hippocampal areas of the brain, suggesting a susceptibility of this primary HPA axis-
regulatory region to neonatal immune challenge and thus supporting previous research which
has demonstrated programming of the HPA axis activity by neonatal LPS exposure.

The second paper (Sominsky et al., 2013a) investigated the neurocircuitry of the anxiety
observed in relation to early life exposure to LPS, specifically by examining the central gene
expression in association with peripheral endocrine and autonomic activity. The data
indicated that neonatal LPS induces an altered expression of the GABA-A receptor α2
subunit, CRH receptor type 1, CRH binding protein, and glucocorticoid receptor mRNA
levels in the prefrontal cortex, hippocampus and hypothalamus of adult rats. These changes
were associated with a persistent elevation of circulating corticosterone. Furthermore, the
long term effects of neonatal LPS exposure were examined for the first time on autonomic
function. The data indicate that neonatal LPS exposure results in increased autonomic
arousal, as indicated by increased activity of tyrosine hydroxylase in the adrenal glands and
increased respiratory rate in response to mild sensory stress. The findings of Paper 2 therefore
suggest that neonatal immune challenge produces a prolonged alteration in both central and
peripheral measures of the HPA axis activity, associated with a persistent change in autonomic function, and potentially contributing to the anxiety-like phenotype.

Given the link between anxiety and reproductive outcomes a subsequent paper further characterised the behavioural and reproductive profile of neonatally treated rats. Sexual behaviour as well as reproductive capacity were assessed in Paper 3 (Walker et al., 2011). Outcomes of this study revealed that neonatally treated rats exhibited impaired mating behaviours, accompanied by persistent HPG suppression. In addition, morphological assessment of the male gonads revealed immediate and long term alterations in the testicular morphology of LPS-treated males. A follow-up Paper 4 (Sominsky et al., 2012a) continued to explore these outcomes with a focussed analysis of reproductive development in the female rat, including ovarian morphology. In addition to alterations in the timing of pubertal onset and endocrine function, diminished ovarian follicular reserve was observed in LPS-treated females when compared to non-treated animals. Taken together the findings of Papers 3 and 4 suggest that neonatally LPS-treated rats demonstrate a subfertile phenotype in adulthood, and this is mediated by functional and morphological changes to the gonads, indicating for the first time a specific susceptibility of the developing gonads to an immune challenge. Therefore the aim of the final Paper 5 (Sominsky et al., 2013b) was to assess whether neonatal LPS may have a direct impact on ovarian development via alteration of the ovarian immune milieu. The results of this paper indicated that neonatal LPS exposure induces activation of inflammatory signalling in the ovary, potentially mediated via increased expression of Toll-like receptor (TLR) 4. Given that common bacterial infections, such as E.Coli and Chlamydia, are associated with increased TLR4 expression in reproductive tissues, which is thought to result in impaired fertility, the findings presented in Paper 5 provide a valuable insight into the link between early life infection and reproductive fitness.
Taken together, the papers presented in this thesis demonstrate that neonatal immune challenge contributes to long term programming of physiology and behaviour, fundamentally influencing reproductive fitness and success. The novel insights presented in this thesis, particularly those related to programming of autonomic function and reproductive development, significantly contribute to the understanding of a critical role of the early microbial environment in determining the developmental trajectories of an organism and advance the current knowledge in the perinatal programming field. The observed effects of neonatal immune challenge may be placed into a wider perspective, integrating the continued interaction between the immune system, the brain, the gonads, and the behavioural outcomes of this interaction, reflective of phenotypic plasticity in response to the changing environment.