

PERINATAL PROGRAMMING OF FEMALE  
SUBFERTILITY:  
THE IMPACT OF NEONATAL IMMUNE  
ACTIVATION ON BEHAVIOUR, OVARIAN  
DEVELOPMENT, AND THE BRAIN



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## **Declaration**

I hereby certify that the work embodied in the thesis is my own original work, conducted under normal supervision. The thesis contains published scholarly work of which I am a co-author. For each such work a written statement, endorsed by the other authors, attesting to my contribution to the joint work, has been included. The thesis contains no material which has been accepted, or is being examined, 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 and any approved embargo.

Signed:

***Erin Alexandra Fuller,***

December 14<sup>th</sup>, 2017

This thesis consist of an introduction comprised of a written literature review and a published review paper. Experimental chapters are presented as both published works and traditional chapters, with figures and tables embedded throughout.

### Published Works Incorporated in this Thesis

1. Sominsky, L., **Fuller, E.A.**, Hodgson, D.M. (2015). Factors in early-life programming of reproductive fitness. *Neuroendocrinology*, 102 (3): 216-225. DOI: 10.1159/000431378
2. **Fuller, E.A.**, Sominsky, L., Sutherland, J.M., Redgrove, K.A., Harms, L., McLaughlin, E.A., Hodgson, D.M. (2017). Neonatal immune activation depletes the ovarian follicle reserve and alters ovarian acute inflammatory mediators in neonatal rats. *Biology of Reproduction*. Accepted 7<sup>th</sup> October, 2017. DOI--: 10.1093/biolre/iox123
3. Ong, L.K., **Fuller, E.A.**, Sominsky, L., Hodgson, D.M., Dunkley, P.R., Dickson, P.W. (2017). Early life peripheral lipopolysaccharide challenge reprograms catecholaminergic neurons. *Scientific Reports* (7), DOI: 10.1038/srep40475.

## Table of Contents

Acknowledgments.....	ii
Declaration.....	iv
List of Published Works.....	v
Thesis Abstract.....	xii
List of Abbreviations.....	xiv
List of Figures.....	xix
List of Tables.....	xxii
<b>Chapter 1. Introduction and Literature Review.....</b>	<b>1</b>
1.1 Developmental Origins of Health and Disease.....	1
1.2 Perinatal Programming.....	3
1.3 The Impact of Perinatal Stress on Adult Health Outcomes.....	5
1.3.1 Perinatal Programming of Pathology.....	7
1.3.2 Perinatal Programming of Psychopathology.....	8
1.3.3 The Role of Stress in Perinatal Programming.....	11
1.3.4 Prenatal Stressors.....	13
1.3.5 Post-Natal Stressors.....	15
1.4 Mechanisms of Perinatal Programming.....	17
1.4.1 The Autonomic Nervous System (ANS).....	18
1.4.1.1 Programming of the ANS.....	20
1.4.2 The Hypothalamic-Pituitary-Adrenal (HPA) Axis.....	22
1.4.2.1 Programming of the HPA Axis.....	23
1.4.3 The Hypothalamic-Pituitary-Gonadal (HPG) Axis.....	25
1.4.3.1 Programming of the HPG Axis.....	28
1.4.4 The Immune System.....	32
1.4.4.1 Immune Mediation of Female Reproductive Parameters.....	40
1.4.4.2 Perinatal Programming of the Immune System.....	43
1.4.4.3 Perinatal Programming of the Immune System via Neural-Endocrine-Immune Interactions.....	45
1.5. Animal Models of Early Life Stress.....	48
1.5.1 Lipopolysaccharide (LPS): An Immunological Stressor.....	49
1.5.2 Lipopolysaccharide: Animal Models of Neonatal Immune Activation (NIA).....	51
1.5.2.1 Impact of Neonatal LPS on Metabolic Function.....	52

1.5.2.2 <i>Impact of Neonatal LPS on Endocrine Function</i> .....	53
1.5.3 <i>Impact of LPS Administration on Behaviour</i> .....	53
1.5.3.1 <i>Anxiety-like behaviours</i> .....	53
1.5.3.2 <i>Sickness behaviours and depressive-like behaviours</i> .....	54
1.5.4 <i>Impact of Neonatal LPS on Immune Function</i> .....	57
1.5.5 <i>Impact of LPS on Reproductive Parameters</i> .....	59
1.5.5.1 <i>Endocrine alterations</i> .....	60
1.5.5.2 <i>Morphological alterations</i> .....	61
1.5.5.3 <i>Ovarian alterations and reproductive aging</i> .....	62
1.5.5.4 <i>Central alterations</i> .....	64
<b>1.6. Mechanisms of the LPS Inflammatory Response: Involvement in female</b>	
<b>Reproduction</b> .....	<b>65</b>
1.6.1 <i>Cytokines</i> .....	66
1.6.1.1 <i>Interleukin 1 (IL-1)</i> .....	67
1.6.1.2 <i>Interleukin 2 (IL-2)</i> .....	68
1.6.1.3 <i>Interleukin-6 (IL-6)</i> .....	68
1.6.1.4 <i>Tumour Necrosis Factor alpha (TNF<math>\alpha</math>)</i> .....	69
1.6.2 <i>Toll-Like Receptors (TLRs)</i> .....	71
1.6.2.1 <i>Toll-like receptors and female reproductive function</i> .....	73
1.6.3 <i>Prostaglandins and Cyclooxygenase (COX) Enzyme Pathways</i> .....	75
1.6.3.1 <i>Prostaglandins</i> .....	75
1.6.3.2 <i>Cyclooxygenase (COX) Enzyme Pathways</i> .....	76
<b>1.7 Conclusion: Rational Summary and Aim of Thesis</b> .....	<b>78</b>
<b>1.8 Overview of papers</b> .....	<b>81</b>
<b>Publication 1</b> .....	<b>85</b>
<b>Chapter 2. General Methods</b> .....	<b>95</b>
<b>2.1 Animal Ethics Approval</b> .....	<b>95</b>
<b>2.2 Animals and Housing</b> .....	<b>95</b>
2.2.1 <i>Housing</i> .....	96
2.2.2 <i>Breeding</i> .....	96
2.2.3 <i>Housing of Experimental Animals</i> .....	97
<b>2.3 Animal Weights and Monitoring</b> .....	<b>97</b>
2.3.2 <i>Monitoring During Experimental Procedures</i> .....	98
<b>2.4 Early life Stress Paradigm: Neonatal Lipopolysaccharide Administration</b> .....	<b>98</b>

<b>2.5 Neonatal Blood and Tissue Collection.....</b>	<b>100</b>
2.5.1 Blood Sampling.....	100
2.5.2 Tissue Collection.....	101
<b>2.6 Adult Blood and Tissue collection.....</b>	<b>101</b>
2.6.1 Non-terminal and Terminal Blood Sampling.....	101
2.6.2 Tissue Collection.....	102
<b>2.7 Tissue Preparation and Analysis.....</b>	<b>103</b>
2.7.1 Ovarian tissue.....	103
2.7.1.1 <i>Histological Evaluation of Ovaries</i> .....	104
2.7.2 Frozen Tissue.....	106
2.7.2.1 <i>RNA extraction</i> .....	106
2.7.2.2 <i>Reverse Transcription</i> .....	106
2.7.2.3 <i>Quantitative Real Time PCR</i> .....	106
2.7.2.4 <i>ELISA and Corticosterone RIA Assays</i> .....	107
<b>2.8 Determination of Puberty Onset.....</b>	<b>107</b>
<b>2.9 Female Reproductive Anatomy, Oestrus Cycle and Oestrus Monitoring.....</b>	<b>108</b>
<b>2.10 Adult Behavioural Tests.....</b>	<b>113</b>
2.10.1 Sucrose Preference Test.....	113
2.10.1.1 <i>Sucrose Preference Test Protocol</i> .....	114
2.10.2 Social Interaction Test.....	116
2.10.2.1 <i>Social Interaction Test Protocol</i> .....	117
2.10.3 Female Sexual Behaviour Testing.....	119
2.10.3.1 <i>Paced Mating Protocol</i> .....	120
2.10.4 Restraint Stress.....	121
2.10.4.1 <i>Restraint Stress Protocol</i> .....	122
<b>2.11 Data Analysis.....</b>	<b>123</b>
<b>Chapter 3. Neonatal Immune Activation Alters the Female Behavioural Phenotype: Motivational, Social, and Reproductive Behaviours.....</b>	<b>125</b>
<b>3.1 Introduction.....</b>	<b>125</b>
<b>3.2 Methods.....</b>	<b>133</b>
3.2.1 Animals.....	133
3.2.2 Behavioural Testing.....	134
3.2.2.1 <i>Sucrose preference</i> .....	135
3.2.2.2 <i>Social interaction</i> .....	136
3.2.2.3 <i>Paced mating</i> .....	137



3.2.3 Blood and Tissue Sampling.....	139
3.2.4 Statistical Analysis.....	140
<b>3.3 Results.....</b>	<b>140</b>
3.3.1 Neonatal Weight Gain.....	140
3.3.2 Neonatal Circulating Tumour Necrosis Factor Alpha (TNF $\alpha$ ).....	140
3.3.3 Developmental Weight Gain.....	141
3.3.4 Day of Vaginal Opening, Weight at Puberty and Oestrus Cyclicity.....	142
3.3.5 Sucrose Preference Assay.....	143
3.3.6 Social Interaction Behaviours.....	146
3.3.6.1 Analysis of complete duration behavioural totals.....	146
3.3.6.2 Time bin analysis of social interaction behaviours.....	147
3.3.6.3 Time bin analysis of social interaction sniffing behaviours.....	149
3.3.6.4 Social interaction circulating corticosterone levels.....	150
3.3.7 Paced Mating Behaviours.....	152
3.3.7.1 Motivational, proceptive and receptive behaviours.....	152
3.3.7.2 Anxiety-like and hypervigilance behaviours.....	153
3.3.7.3 Sperm plug detection.....	154
3.3.7.4 Female HPG axis assessment during paced mating: luteinising hormone and follicle stimulating hormone.....	154
<b>3.4 Discussion.....</b>	<b>157</b>
<b>Chapter 4. Neonatal Immune Activation Depletes the Ovarian Follicle Reserve and Alters Ovarian Acute Inflammatory Mediators in Neonatal Rats.....</b>	<b>171</b>
4.1 Publication Introduction.....	171
Publication 2.....	174
<b>Chapter 5. Neonatal Immune Activation Leads to Sustained Ovarian Reserve Depletion and Altered Peripheral Inflammatory Mediators.....</b>	<b>187</b>
5.1 Introduction.....	187
5.2 Method.....	194
5.2.1 Animals.....	194
5.2.2 Oestrus cycle monitoring.....	195
5.2.3 Acute stress protocol.....	195
5.2.4 Blood and Tissue Collection.....	196
5.2.4.1 Blood collection and assessment.....	196
5.2.4.2 Tissue collection.....	197

5.2.5 Tissue Preparation and Analysis.....	199
5.2.5.1 <i>Fixed ovarian tissue</i> .....	199
5.2.5.2. <i>RNA extraction, Reverse Transcription and qRT-PCR</i> .....	199
5.2.6 Data Analysis.....	200
<b>5.3 Results.....</b>	<b>202</b>
5.3.1 Neonatal Weight Gain.....	202
5.3.2 Neonatal Circulating Inflammation.....	202
5.3.3 Developmental Weight Gain.....	203
5.3.4 Day of Vaginal Opening, Weight at Puberty and Oestrus Cyclicity.....	204
5.3.5 Adult Circulating Inflammation.....	205
5.3.5.1 <i>Circulating Interleukin-6 (IL-6)</i> .....	205
5.3.5.1 <i>Circulating Interleukin-2 (IL-2) 24 hours post restraint</i> .....	206
5.3.6 Adulthood Ovarian Follicle Quantification 24 Hours Post-Restraint.....	207
5.3.6.1 <i>Early ovarian follicle populations</i> .....	207
5.3.6.2 <i>Late ovarian follicle populations</i> .....	209
5.3.7 Ovarian mRNA expression 24 hours post restraint.....	210
<b>5.4 Discussion.....</b>	<b>212</b>
<b>Chapter 6. Neonatal Immune Activation and a ‘Second Hit’ of Adult</b>	
<b>Psychological Stress Alters Central Inflammatory Mediators: Implications for</b>	
<b>Female Reproduction.....</b>	<b>226</b>
<b>6.1 Introduction.....</b>	<b>226</b>
<b>6.2 Methods.....</b>	<b>231</b>
6.2.1 Brain Dissection.....	231
6.2.2 Brain Sectioning.....	232
6.2.3 RNA extraction, Reverse Transcription and qRT-PCR.....	234
6.2.4 Data Analysis.....	236
<b>6.3 Results.....</b>	<b>236</b>
6.3.1 Hippocampus.....	236
6.3.2 Hypothalamus.....	241
6.3.3 Medial Preoptic Area.....	246
<b>6.4 Discussion.....</b>	<b>250</b>
<b>Chapter 7. Future Directions: The Kynurenine Pathway and the Catecholaminergic</b>	
<b>System.....</b>	<b>263</b>
<b>7.1 Introduction.....</b>	<b>263</b>

<b>7.2 Perinatal programming of the kynurenine pathway: Potential role in female NIA induced subfertility.....</b>	<b>265</b>
<b>7.3 Methods.....</b>	<b>271</b>
7.3.1 Animals and neonatal treatment.....	271
7.3.2 Tissue collection.....	272
7.3.3 RNA extraction, reverse transcription and qRT-PCR.....	272
7.3.4 Data Analysis.....	273
<b>7.4 Results.....</b>	<b>274</b>
7.4.1 Neonatal weight.....	274
7.4.2 Peripheral tissue examination.....	274
7.4.3 Central tissue examination.....	275
<b>7.5 Discussion.....</b>	<b>278</b>
<b>8. General Discussion.....</b>	<b>296</b>
<b>8.1 Introduction.....</b>	<b>296</b>
<b>8.2 Defining the Female Behavioural Phenotype.....</b>	<b>298</b>
<b>8.3 Perinatal Programming of Reproductive Development.....</b>	<b>304</b>
<b>8.4 Perinatal Programming of Peripheral Inflammation and Immune Vulnerability.....</b>	<b>306</b>
<b>8.5 Perinatal Programming of the Ovarian Reserve.....</b>	<b>307</b>
8.5.1 Acute impact of neonatal immune activation.....	307
8.5.2 Sustained impact of neonatal immune activation.....	309
<b>8.6 Mediators of Acute and Sustained Ovarian Follicle Depletion.....</b>	<b>311</b>
<b>8.7 Perinatal Programming of Central Mediators: Contribution to Behaviour.....</b>	<b>315</b>
<b>8.8 Conclusions, Future directions, and Implications.....</b>	<b>318</b>
8.8.1 General Summary.....	318
8.8.2 Future Directions.....	319
8.8.3 Implications.....	321
<b>References.....</b>	<b>325</b>

## **Thesis Abstract.**

### **Perinatal programming of female subfertility: Impact of neonatal immune activation on behaviour, ovarian development, and the brain.**

The early life environment prescribes long-term health and disease outcomes. Accumulating evidence suggests that female reproductive health is shaped by perinatal factors, such as immune status. The fundamentals of female reproductive success and longevity are established in early life, where the dynamics of ovarian development are co-regulated via immune pathways to establish the ovarian reserve. Additionally, the immune system is known to be especially sensitive to perinatal stressors. This suggests that the early life environment plays an important role in sustained ovarian health and female fertility. Thus, inflammatory stressors during this critical period may permanently modify female ovarian development and immune-drive reproductive functioning, altering sexual behaviour and leading to a suboptimal female phenotype.

Using a rat model, we have previously demonstrated that neonatal immune activation (NIA) with bacterial mimetic lipopolysaccharide (LPS) is associated with; altered immune milieu, hypothalamic-pituitary-adrenal axis dysfunction, adult stress vulnerability, and an anxiety-like phenotype in males. The current thesis aimed to examine both the acute and long-term alterations in reproductive parameters in female rats exposed to an intraperitoneal injection of saline (control) or LPS (0.05mg/kg) to induce NIA on postnatal days 3 and 5.

Firstly, the behavioural phenotype of females in this model was examined in order to confirm and refine previous findings pertaining to female mating behaviour deficits, and establish if these alterations were driven by altered motivational states. The results of this study indicate that NIA leads to impairments in proceptive and receptive mating behaviours and an altered reproductive developmental trajectory. Secondly, the acute effects of NIA on

female rats was analysed, where by NIA treatment was demonstrated to significantly deplete early ovarian follicle populations and increase ovarian inflammation, suggesting that immune-mediated development of the ovary is perturbed by NIA in the female rat. Thirdly, the long term ramifications of neonatal bacterial exposure was examined in the adult female rat, demonstrating that NIA led to significantly advanced puberty onset, sustained ovarian reserve depletion, exaggerated peripheral inflammatory responses to stress, and increased ovarian inflammatory pathway gene expression. Lastly, the central gene expression of mediators associated with inflammation, stress regulation, and reproductive function were examined to elucidate on potential central mechanisms that may contribute to behavioural alterations and ovarian inflammation and reserve depletion. Furthermore, prospective mechanisms are suggested and data is presented demonstrating the potential of these for investigation in a female rat model of subfertility. The findings presented in this thesis suggest that NIA has the potential to perinatally program long-term central and ovarian immune functioning to a proinflammatory bias. This may detrimentally affect female reproductive fitness, fecundity, and stress responsivity, and as such, have implications for both physiological and psychological female health.

## Abbreviations

5-HT	Serotonin
AA	Arachidonic acid
ABS	Australian Bureau of Statistics
ACEC	Animal Ethics Committee
ACTH	Adrenocorticotrophic hormone
ADHD	Attention deficit/hyperactivity disorder
AG	Anogenital
ANOVA	Analysis of variance
aNS	Adulthood no stress
ANS	Autonomic nervous system
APAF	Australian Proteome Analysis Facility
APC	Antigen presenting cells
ARC	Animal Resources Centre
ARTs	Assisted reproductive therapies
aST	Adulthood restraint stress
BBB	Blood-brain barrier
CASP3	Caspase 3
cDNA	Complementary deoxyribonucleic acid
CORT	Corticosterone
COX	Cyclooxygenase
CRH	Corticotropin releasing hormone
CRHR1/2	Corticotropin releasing hormone receptor 1/2
C-RP	C-reactive protein
CSF	Colony stimulating factor
CTCF	Corrected total cell florescence
DA	Dopamine
DAB	3, 3-diaminobenzidine
DAPI	4',6-diamidino-2-phenylindole
DEHP	Di(2-ethylhexyl)phthalate
DEPc	Diethyl pyrocarbonate

DES	Diethylstilboestrol
DEX	Dexamethasone
DG	Dentate gyrus
DNA	Deoxyribonucleic acid
DOHaD	Developmental Origins and Health and Disease
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, version 5
DVO	Day of vaginal opening
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EPI	Epinephrine
EPM	Elevated plus maze
ER	Oestrogen receptor
Foxo3	Forkhead box O3
FSH	Follicle stimulating hormone
FWD	Forward
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GD	Gestation day
Gdf	Growth differentiation factor
GFAP	Glial fibrillary acidic protein
GnRH	Gonadotropin releasing hormone
GnRHR	Gonadotropin releasing hormone receptor
GR	Glucocorticoid receptor
H&E	Hematoxylin and eosin
HC	Hippocampus
HD	Habituation day
HPA	Hypothalamic-pituitary-adrenal
HPG	Hypothalamic-pituitary-gonadal
HTH	Hypothalamus
Iba-1	Ionized calcium-binding adapter molecule 1
IDA	Information dependent acquisition
IDO	Indolamine-2,3-Dioxygenase

IFN	Interferon
IgE	Immunoglobulin E
IL	Interleukin
ip	Intraperitoneal
IPA	Ingenuity Pathway Analysis
IRAK	Interleukin-1 receptor-associated kinase 1
IUGR	Intrauterine growth restriction
IVF	In vitro fertilisation
JAK/STAT	Janus kinase/signal transducers and activators of transcription
KP	Kynurenine pathway
Kyn	Kynurenine
LBP	LPS-binding protein
LC3	Light chain 3
LC	Locus coeruleus
L-Dopa	3,4-dihydroxy-l-phenylalanine
LH	Luteinizing hormone
LPS	Lipopolysaccharide
LSV	Lateral saphenous vein
LXR/RXR	Liver X receptor/retinoic X receptor
MANOVA	Multivariate analysis of variance
MAPK	Map kinase
Mapk8/Jnk1	Mitogen activated protein kinase 8/Jun N-terminal kinase
MD	Myeloid differentiation protein
MDD	Major Depressive Disorder
MHC	Major histocompatibility complex
MIA	Maternal immune activation
mPOA	Medial pre optic area
MR	Mineralocorticoid
mRNA	Messenger ribonucleic acid
MS	Mass spectrometry
mTOR	Mechanistic target of rapamycin



MyD88	Myeloid differentiation primary response 88
NCRIS	National Collaborative Research Infrastructure Strategy
NE	Norepinephrine
NF	Nuclear factor
NHMRC	National Health and Medical Research Council of Australia
NIA	Neonatal immune activation
NK	Natural killer
nLPS	Neonatal lipopolysaccharide
NMDA	N-methyl-D-aspartate
Nos1	Nitric oxide synthase-1
NSAI	Nonsteroidal anti-inflammatory pharmaceutical
nSAL	Neonatal saline
PAH	Polycyclic aromatic hydrocarbon
PAMP	Pathogen-associated molecular patterns
PBS	Phosphate buffered saline
PCB	Polychlorinated biphenyl
PCOS	Polycystic ovarian syndrome
PFC	Prefrontal cortex
PG	Prostaglandin
PMT	Paced-mating test
PND	Post-natal day
PNS	Parasympathetic nervous system
POF	Premature ovarian failure
POI	Primary ovarian insufficiency
Poly I:C	Polyinosinic:polycytidylic acid
Prkc	Protein kinase C
PRR	Pattern-recognition receptor
PTSD	Post-traumatic stress disorder
PVC	Polyvinyl chloride
PVN	Paraventricular nucleus
qRT-PCR	Quantitative reverse transcription polymerase chain reaction

REV	Reverse
RNA	Ribonucleic acid
SDS	Sodium dodecyl sulfate
SIT	Social interaction test
SN	Substantia nigra
SNS	Sympathetic nervous system
SPSS	Statistical Package for the Social Sciences
SPT	Sucrose preference test
STI	Sexually transmitted infection
TB	Time bin
TBS	Tris-buffered saline
T <sub>C</sub>	T cytotoxic
TCDD	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin
TDO	Tryptophan-2,3-Dioxygenase
TGF	Transforming growth factor
T <sub>H</sub>	T helper
TH	Tyrosine hydroxylase
TLR	Toll-like receptor
TNF	Tumour necrosis factor
TOFMS	Time of flight mass spectrometry
TRAF	Tumour necrosis factor receptor associated factor
Trp	Tryptophan
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labelling
TX	Thromboxane
VTA	Ventral tegmental area

## List of Figures

<b>Figure 1.1</b> Perinatal programming of long term health and disease.....	2
<b>Figure 1.2</b> Perinatally programmed developmental alterations when mismatch occurs.....	4
<b>Figure 1.3</b> Pathway for catecholamine synthesis and enzymatic steps.....	20
<b>Figure 1.4</b> HPA axis cascade.....	23
<b>Figure 1.5</b> Schematic representation of the Female HPG axis.....	28
<b>Figure 1.6</b> Functional flow of immunity following antigen detection.....	38
<b>Figure 1.7</b> LPS immune activation via toll-like receptors, cytokines and prostaglandins.....	50
<b>Figure 1.8</b> Identification and corresponding postnatal development of ovarian follicle pool.....	62
<b>Figure 2.1</b> The model of early life immune activation, critical periods of developmental plasticity for the immune system, the HPA and HPG axis.....	100
<b>Figure 2.2</b> Pictorial representation of rat ovarian follicles for histological quantification.....	105
<b>Figure 2.3</b> Schematic representation of the H & E stained ovarian sections mounted on a microscope slide.....	105
<b>Figure 2.4</b> Schematic representation of ovarian follicle recruitment in the female rat.....	110
<b>Figure 2.5</b> Graphical representation of fluctuations in hormone levels during the rat female oestrus cycle.....	111
<b>Figure 2.6</b> Photomicrograph at 10x magnification showing stages of the rodent oestrus cycle.....	112
<b>Figure 2.7</b> Top view of individual sucrose preference test (SPT) cage setup.....	115
<b>Figure 2.8</b> Schematic of the social investigation test (SIT) arena.....	118
<b>Figure 2.9</b> Photographic representation of the social interaction test (SIT) arena.....	118
<b>Figure 2.10</b> Diagram representing dimensions and layout of the paced mating test (PMT) apparatus.....	121
<b>Figure 3.1</b> Average neonatal female weights and circulating TNF- $\alpha$ levels.....	141
<b>Figure 3.2</b> Difference in weight gain between saline and LPS animals and absolute developmental weights.....	142
<b>Figure 3.3</b> Day of vaginal opening, weights and first proestrus.....	143

<b>Figure 3.4</b> Sucrose preference and consumed (%) over a 3 day habituation period and 4 hour test phase.....	145
<b>Figure 3.5</b> Counts of rearing and kicks during social interaction test (SIT).....	146
<b>Figure 3.6</b> Mean frequency of approach and avoidance behaviours of test rat in time bins.....	148
<b>Figure 3.7</b> Mean frequency of follow and rearing behaviour of test rat in time bins.....	148
<b>Figure 3.8</b> Mean frequency of grooming behaviour by test rat.....	149
<b>Figure 3.9</b> Sniffing behaviour performed by test rat in social interaction test (SIT).....	150
<b>Figure 3.10</b> Corticosterone (CORT) levels across saline/treatment, pre/post social interaction test (SIT).....	151
<b>Figure 3.11</b> Mean frequency of behaviours in the paced mating test (PMT).....	153
<b>Figure 3.12</b> Counts of rears and grooming during the PMT.....	154
<b>Figure 3.13</b> Mean circulating luteinising hormone (LH) pre/post paced mating test (PMT).....	155
<b>Figure 5.1</b> Representation of treatment allocations.....	196
<b>Figure 5.2</b> Flowchart of experimental protocol.....	198
<b>Figure 5.3</b> Average female neonatal weights on PND 3 and 5.....	202
<b>Figure 5.4</b> Circulating proinflammatory cytokines on PND 5 after LPS treatment.....	203
<b>Figure 5.5</b> Average female developmental weights.....	204
<b>Figure 5.6</b> Average DVO, DVO average weight and day of 1 <sup>st</sup> oestrus.....	205
<b>Figure 5.7</b> Circulating IL-6 levels pre and post restraint stress.....	206
<b>Figure 5.8</b> Circulating IL-2 24 hours following restraint stress (terminal bleed).....	207
<b>Figure 5.9</b> Adult mean ovarian counts of early follicles.....	208
<b>Figure 5.10</b> Adult mean ovarian counts for late ovarian follicles.....	209
<b>Figure 5.11</b> Normalised fold change mRNA expression in the adult ovary.....	211
<b>Figure 6.1</b> lateral sagittal visual representation of brain sections.....	233
<b>Figure 6.2</b> Coronal representations of brain sections (rat atlas).....	233
<b>Figure 6.3</b> Fold change expression of hippocampal inflammatory genes.....	239
<b>Figure 6.4</b> Fold change expression of hippocampal stress and neuropeptide genes.....	240
<b>Figure 6.5</b> Fold change expression of hypothalamic inflammatory genes.....	244
<b>Figure 6.6</b> Fold change expression of hypothalamic stress and neuropeptide genes.....	245
<b>Figure 6.7</b> Fold change expression of mPOA inflammatory genes.....	248

<b>Figure 6.8</b> Fold change expression of mPOA stress and neuropeptide genes.....	249
<b>Figure 7.1</b> Simplified schematic of the Kynurenine metabolic pathway (KP).....	267
<b>Figure 7.2</b> Mean neonatal weights on PND 5.....	274
<b>Figure 7.3</b> Fold change expression of inflammatory and KP pathway genes in the spleen and liver of male pups following LPS (6hr post).....	276
<b>Figure 7.4</b> Fold change expression of inflammatory and KP pathways genes in whole brain tissue of male pups following LPS (6hr post).....	277

## List of Tables

<b>Table 1.1</b> Toll-like receptor expression and specificity.....	73
<b>Table 2.1</b> Summary of oestrus phase and duration, behaviour and vaginal cell morphology.....	121
<b>Table 3.1</b> Definition of social interaction behavioural variables measured.....	137
<b>Table 3.2</b> Definition of paced mating behavioural variables measured.....	138
<b>Table 3.3</b> Means, SEM and SD of frequency of social interaction total variables.....	151
<b>Table 3.4</b> Mean, SEM and SD of paced mating variables.....	156
<b>Table 5.1</b> Primer forward and reverse sequence for ovarian qRTPCR.....	200
<b>Table 5.2</b> ANOVA summary of statistics for late follicle types.....	209
<b>Table 5.3</b> ANOVA statistics for normalised ovarian mRNA expression.....	210
<b>Table 6.1</b> Brain section Bregma levels.....	232
<b>Table 6.2</b> Primer forward and reverse sequences for Central qRTPCR.....	235
<b>Table 6.3</b> ANOVA statistics for hippocampal mRNA expression.....	238
<b>Table 6.4</b> ANOVA statistics for hypothalamic mRNA expression.....	243
<b>Table 6.5</b> ANOVA statistics for medial preoptic area mRNA expression.....	247
<b>Table 7.1</b> qRTPCR gene targets and gene assay IDs.....	273