PREGNANCY COMPROMISES: ROLE OF NEUROSTEROIDS IN NEURODEVELOPMENT AND BEHAVIOUR

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

February 2017

DECLARATION

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Signed:_____ Date:_____

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This thesis is dedicated to my mother,

Karin Kneis.

Without your love and support

I would not be where I am

or who I am today.

ACKNOWLEDGEMENTS

Undertaking this PhD has been my biggest challenge to date, but it has been the most amazing experience of my life. I have met some truly remarkable people, and come out of this a better person for it. There are so many people I have to thank, and I feel this will not begin to cover just how grateful I am to everyone who has been on this journey with me but I'll give it my best shot!

First and foremost, I'd like to thank my supervisors Jon Hirst and Hannah Palliser for giving me the opportunity to complete my PhD in their lab. Words cannot convey just how wonderful the pair of you are, as supervisors and mentors. This thesis would not have gotten to this point without both you to nudge me in the right direction when I needed it. Jon, I've always admired your enthusiasm and optimism. Your invaluable knowledge and problem-solving has made it a joy to learn the field. Hannah, I am so grateful for all your support and guidance. You have been the voice of logic, the troubleshooting guide and a shoulder to cry on when experiments didn't work out the way I wanted them to. You have also given me the lab and life skills I need to get anywhere in the world of science. I look forward to future collaborations with the both of you from wherever I end up!

To my beautiful support group Celine, Minoo and Poonam. I would not have made it through my PhD without you there to lift me up and make me laugh when I needed, to be my sounding board when I was confused and to listen to me vent about the most insignificant things. Celine, we're both two crazy peas in a pod. This PhD has given me some incredible memories because of you. I love that I found someone who would laugh and sing as loud as me in the lab, and loved my eclectic taste in music in return. Minoo, thank you for the tea breaks, the hugs and being the best desk neighbour a person could ask for. I wish you the best of luck with the rest of your work, I know you'll go far. And last but not least, Poonam. Thank you for your help in the lab, your endless patience and the laughs. I cherish everything I've learned from all three of you, be it science, language or food.

To my fellow students; thank you for making this experience so much fun, and providing caramel slices when things went wrong. Julia, I can't wait for the day, 30 years from now when we'll be the leaders in our field, I can stand up and say I knew you when. I have always admired your dedication and spirit in your work. That is what makes you a great scientist, not just a good one. Kirsten, thank you for appreciating my truly terrible, and more times than not inappropriate, jokes. I wish you the best of luck in everything you do. Gabby, it's been such an honour to work with you and watch you learn and grow as a scientist.

A big thank you to the Pringle group for being some of the most wonderful people to work with. Kirsty, you became my unofficial mentor throughout my PhD. your knowledge has been invaluable. Sarah, you are the best hug-giver out of everyone. To Riaz, Sam, Saije and Yu Xi, I didn't get to know you all as well as I would have liked, but I have truly enjoyed every moment in your company. The lab and office has been so much fun with all of you. I hope to see you around the conference circuit.

Thank you to Kayla Friedman and Malcolm Morgan of the Centre for Sustainable Development, University of Cambridge, UK for producing the Microsoft Word thesis template used to produce this document.

Thank you my friends, Mandy, Steph and Ange for understanding that being a PhD student meant having little down time and that plans would happen at short notice. Yet you still made time for me just to hang out and gave me something outside of science to appreciate.

Thank you to my fellow PhD buddies, and close friends Tim and Heather. This journey has been made easier knowing I had both of you there with me, from (high school with you Tim) undergrad, through honours to our PhDs. It's going to be so amazing seeing the world through our research.

Thank you to my loving, crazy and amazing family. Nanna, thank you for providing food, comfort, endless support and laughs throughout it all. And finally the biggest thank you to my mum. This thesis is just as much yours as it is mine. I could not have even considered attempting a PhD without you there to support me. There is nothing I can say that will express just how much your unwavering love and understanding has made this journey so much smoother. Thank you for trying so hard to understand my research, and being enthusiastic about my work even when I felt there wasn't much to be enthusiastic about. I am the luckiest daughter in the world to have you as my biggest cheerleader, I could not have done this without you.

CONTENTS

ABSTRACT

Pregnancy compromises impact a number of infants annually around the world. These include intrauterine growth restriction (IUGR), maternal psychological stress (prenatal stress; PS) and preterm birth (PTB). These compromises can result in alterations to the normal neurodevelopmental process in key brain regions, including the hippocampus, cerebellum and amygdala. These changes in fetal development are being increasingly attributed to the programming of fetal systems *in utero* towards negative *ex utero* events. In fetal life, IUGR, PS and PTB cause a delay and/or reduction in mature myelin, concurrent with an alteration in astrocyte activation. Infants born following IUGR, PS and PTB are more likely to develop cognitive deficits, anxiety, behaviours disorders and schizophrenia.

The progesterone metabolite, allopregnanolone is a key neurosteroid involved in fetal development. Allopregnanolone promotes myelination, inhibits astrocytosis, and has allosteric action at the γ-aminobutyric acid type A ($GABA_A$) receptor to modulate neural excitability. Allopregnanolone synthesis is upregulated following various acute neurological insults, including hypoxia and stress, protecting against excitotoxic cell death. Inhibition of allopregnanolone synthesis in late gestation, as may occur in chronic pregnancy compromises, reduces myelination and up-regulates astrocyte activation. In later life, dysregulation of allopregnanolone is implicated in several psychological disorders such as depression, and premenstrual dysphoric disorder. Little information exists on the effect of major pregnancy compromises, their contribution to neurosteroid dysfunction, and neurodevelopmental and behavioural outcomes of the offspring.

The effect of inhibiting allopregnanolone synthesis, using finasteride, in late gestation on postnatal cerebellar development was investigated in a guinea pig model. At 8 days postnatal age there was increased astrocyte activation and decreased expression of the allopregnanolone-sensitive $GABA_AR$ α6 subunit in the cerebella of neonates exposed to finasteride. This demonstrates the ongoing effects of a low neurosteroid environment in pregnancy extending into childhood. At 21 days postnatal age, females with *in utero* finasteride exposure displayed

increased neophobia-like responses to changes in their environment. This was without ongoing effects on myelin, astrocyte or GABAergic enzyme expression in the hippocampus or amygdala. These observations suggest that prenatal loss of neurosteroids programs an anxious phenotype in females, possibly by inducing deficits in the end-point targets of allopregnanolone action, and does not impact male anxiety development.

A model of combined IUGR+PS was used to investigate neurodevelopmental changes of offspring exposed to multiple pregnancy compromises at term. Circulating allopregnanolone and hippocampal myelination in males, whilst reduced in both IUGR and IUGR+PS, was not cumulatively affected by the combination of stressors. Interestingly, the addition of PS to IUGR had a potentially positive effect on subcortical myelination, suggesting the triggering of a protective mechanism, occurring in the fetal neurodevelopment, to preserve an already compromised brain.

The placenta contains all the essential enzymes and is the major contributor to fetal allopregnanolone for neurodevelopment Thus the health of the placenta is critical for the development of a healthy fetus. Many preterm infants do not survive the immediate neonatal period, with no overt indicators as to poor health. The placenta of surviving and non-surviving preterm guinea pigs were investigated to determine expression of the allopregnanolone synthesis enzymes. The expression of 5α-reductase type 2 was greatest in placentae from neonates that did not survive to 24 hours. This may indicate an upregulation of protective actions to increase allopregnanolone exposure, suggesting these neonates experienced an adverse *in utero* environment and were therefore more vulnerable to the insult of premature birth.

The current body of work indicates that fetal allopregnanolone plays a role in programming $GABA_A$ receptor subunit expression as well as juvenile female behaviour, and thus impaired supply of this steroid *in utero* may be a predisposing factor in the development of depression and anxiety. Allopregnanolone is implicated in the poor development of myelination following IUGR, yet PS may

have a neuroprotective action on myelin development in IUGR male brains. Based on this work, it is also postulated that placental expression of neurosteroid producing enzymes provide identification of neonates at risk of poor outcomes in the immediate neonatal period. Further studies investigating the protective effects of PS in IUGR, and their impacts on later behavioural development are warranted. Future work should investigate the potential of perinatal neurosteroid replacement for the improvement of mental health outcomes following pregnancy compromises.

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LIST OF ABBREVIATIONS AND ACRONYMS

0.1M	0.1MOLAR
11β-HSD2	11β-HYDROXYSTEROID DEHYDROGENASE TYPE 2
17β-DIOL	17β-ESTRADIOL
3α-DIOL	3α-ANDROSTANEDIOL
5α-DHDOC	5α-DIHYDRODEOXYCORTICOSTERONE
5α, 3α-THDOC	5α, 3α- TETRAHYDRODEOXYCORTICOSTERONE
5α-DHP	5α-DIHYDROPROGESTERONE
5α-DHT	5α-DIHYDROTESTOSTERONE
5αR	5α-REDUCTASE
ADHD	ATTENTION DEFICIT HYPERACTIVITY DISORDER
AGA	APPROPRIATE FOR GESTATIONAL AGE
ANOVA	ANALYSIS OF VARIANCE
B_0	TRACER ANTISERA BINDING
BAT	BROWN ADIPOSE TISSUE
BLA	BASOLATERAL AMYGDALA
BLR	BRAIN-TO-LIVER RATIO
BSA	BOVINE SERUM ALBUMIN
CA	*CORNU AMMONIS*
CC	CORPUS CALLOSUM
CCAS	CEREBELLAR COGNITIVE AFFECTIVE DISORDER
CEA	CENTRAL AMYGDALOID NUCLEUS
CNS	CENTRAL NERVOUS SYSTEM
CPAP	CONTINUOUS POSITIVE AIRWAY PRESSURE
CREDITTS	CRITICAL RESEARCH DESIGN, INFORMATION TECHNOLOGY AND STATISTICAL SUPPORT
CRH	CORTICOTROPHIN RELEASING HORMONE

CSF	CEREBROSPINAL FLUID
CT	CYCLE THRESHOLD
DAB	3, 3'-DIAMINOBENZIDINE TETRAHYDROCHLORIDE
DG	DENTATE GYRUS
DHEA	DEHYDROEPIANDOSTERONE
DOHAD	DEVEVELOPMENTAL ORIGINS OF HEALTH AN DISEASE
DWM	DEEP WHITE MATTER
ECL	ENHANCED CHEMILUMINESCENCE
EDTA	ETHYLENEDIAMINETETRAACETIC ACID
EGL	EXTERNAL GRANULE LAYER
ELISA	ENZYME-LINKED IMMUNOSORBENT ASSAY
EPM	ELEVATED PLUS MAZE
FMRI	FUNCTIONAL MAGENTIC RESONANCE IMAGING
GA	GESTATIONAL AGE
GABA	γ-AMINOBUTYRIC ACID
$GABA_AR$	γ-AMINOBUTYRIC ACID TYPE A RECEPTOR
GAD67	GLUTAMIC ACID DECARBOXYLASE ISOFORM 67KDA
GAT1	GABA TRANSPORTER TYPE 1
GDNA	GENOMIC DEOXYNUCLEIC ACID
GEE	GENERALISED ESTIMATING EQUATIONS
GFAP	GLIAL FIBRILLARY ACIDIC PROTEIN
HPA	HYPOTHALAMIC-PITUITARY AXIS
HRP	HORSERADISH PEROXIDASE
HSD	HYDROXYSTEROID DEHYDROGENASE (3α, 17α ETC)
IGG	IMMUNOGLOBULIN G
IGL	INTERNAL GRANULE LAYER
IQ	INTELLIGENCE QUOTIENT

IUGR	INTRAUTERINE GROWTH RESTRICTION
IUGR+PS	INTRAUTERINE GROWTH RESTRICTION WITH PRENATAL STRESS
MAG	MYELIN ASSOCIATED GLYCOPROTEIN
MAP2	MICROTUBULE ASSOCIATED PROTEIN 2
MBP	MYELIN BASIC PROTEIN
ML	MOLECULAR LAYER
MRI	MAGNETIC RESONANCE IMAGING
mRNA	MESSENGER RIBONUCLEIC ACID
NCBI	NATIONAL CETNRE FOR BIOTECHNOLOGY INFORMATION
NeuN	NEURONAL NUCLEI
NMDA	N-METHYL-D-ASPARTATE
NSB	NON-SPECIFIC BINDING
NTC	NO-TEMPLATE CONTROL
OF	OPEN FIELD
Olig2	OLIGODENDROCYTE TRANSCRIPTION FACTOR
$P450_{17\alpha}$	17α-HYDROXYLASE
$P450_{AROM}$	P450 ENZYME COMPLEX, AROMATASE ENZYME
$P450_{SCC}$	P450 ENZYME COMPLEX, SIDE-CHAIN CLEAVAGE
PBS	PHOSPHATE BUFFERED SALINE
PCR	POLYMERASE CHAIN REACTION
PDGFRα	PLATELET-DERIVED GROWTH FACTOR RECEPTOR α
PLP	MYELIN PROTEOLIPID PROTEIN
PMDD	PREMENSTRUAL DYSPHORIC DISORDER
PMS	PREMENSTRUAL SYNDROME
PND	POSTNATAL DAY
PS	PRENATAL STRESS
PTB	PRETERM BIRTH

PTSD	POST-TRAUMATIC STRESS DISORDER
RF	RHINAL FISSURE
RIPA	RADIOIMMUNOPRECIPITATION ASSAY
RNA	RIBONUCLEIC ACID
RNASE	RIBONUCLEASE
RT	REVERSE TRANSCRIPTION
S100B	S100 CALCIUM-BINDING PROTEIN B
SCWM	SUBCORTICAL WHITE MATTER
SGA	SMALL FOR GESATIONAL AGE
TBS-T	TRIS-BUFFERED SALINE WITH TWEEN
TC	TOTAL COUNTS
TH	THALAMUS
TMB	TETRAMETHYLBENZIDINE
UV	ULTRAVIOLET
WWII	WORLD WAR II

PUBLICATIONS FOR INCLUSION

The work in this thesis describes the effects of intrauterine allopregnanolone loss via pharmacological inhibition on postnatal neurodevelopment, behavioural outcomes. It also investigates the impact of combined intrauterine compromises in fetal neurodevelopment and neurosteroid profiles at term, as well as placental expression of neurosteroid enzymes in relation to survival following premature delivery. As such, this thesis is divided into four publications, beginning with the loss of allopregnanolone in late gestation on postnatal cerebellar development, and then impact of low gestational allopregnanolone on behavioural outcomes at juvenility. The subsequent paper investigates the individual impacts of intrauterine growth restriction and the combined effects with prenatal stress on neurodevelopmental markers, and finally the mRNA expression of the 5α-reductases within the placenta of premature neonates who did not survive the immediate 24 hours period, comapred to preterm survivors and term neonates.

Cumberland AL, Palliser HK, Walker DW, Hirst JJ. Cerebellar Changes in Guinea Pig Offspring Following Suppression of Neurosteroid Synthesis During Late Gestation.
The Cerebellum. 2016:1-8. DOI: 10.1007/s12311-016-0802-0.
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Cumberland AL, Crombie GC, Palliser KH, Hirst JJ. Increased Anxiety-like Phenotype in Female Guinea Pigs Following suppression of Neurosteroid Synthesis In Utero. Submitted to *Developmental Neuroscience*.

Cumberland AL, Palliser KH, Rani P, Hirst JJ. Combination of IUGR and Prenatal Stress on Fetal Brain Development. Prepared for submission to *Journal of Developmental Origins of Health and Disease.*

Cumberland AL, Palliser HK, Hirst JJ. Increased placental neurosteroidogenic gene expression precedes poor outcome in the preterm guinea pig. *Journal of Developmental Origins of Health and Disease*. 2014;5(02):74-78. DOI: 10.1017/S2040174413000573.

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ADDITIONAL PUBLICATIONS

Palliser HK, Bennett GA, Kelleher MA, **Cumberland AL**, Walker DW, Hirst JJ. Models of Perinatal Compromises in the Guinea Pig: Their Use in Showing the Role of Neurosteroids in Pregnancy and the Newborn. Book Chapter, Chapter 10. Prenatal and Postnatal Determinants of Development. *Neuromethods*. New York, NY: Springer; 2016. p. 221-243. DOI: 10.1007/978-1-4939-3014-2_11.

Hirst JJ, **Cumberland AL**, Shaw JC, Bennett GA, Kelleher MA, Walker DW, Palliser HK. Loss of neurosteroid-mediated protection following stress during fetal life. Review. *The Journal of Steroid Biochemistry and Molecular Biology*. 2016;160:181-188. DOI: 10.1016/j.jsbmb.2015.09.012.