EFFECT OF PRE AND POSTNATAL NEUROSTEROID THERAPY ON NEURODEVELOPMENT AND BEHAVIOUR

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DECLARATION

I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision.

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This thesis is dedicated to mama bear and papa bear,

Jacqueline and Steven Shaw.

Thank you for doing all that you do for me.

This would not have been possible without you.

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CONTENTS

ABSTRACT

Children that are born preterm are at an increased risk of developing late onset cognitive problems and behavioural disorders, such as attention deficit hyperactivity disorder (ADHD) and anxiety, with males being particularly vulnerable. The mechanisms by which this happens are poorly understood; however, actions and modulation of $GABA_A$ receptor signalling by the neurosteroid allopregnanolone has a major role in late gestation neurodevelopment, and we believe that the early loss of placentally-derived allopregnanolone following preterm birth is pivotal to the development of these disorders. There is increasing interest in the development of the hippocampus and cerebellum following preterm birth and the potential involvement of GABAergic pathways in neurodevelopmental disorders. In these studies, we propose that the early loss of the intrauterine trophic environment as a result of preterm birth alters the development of the hippocampus and cerebellum, contributing to ongoing neurobehavioral disorders. We anticipate that neurosteroid-replacement therapy with ganaxolone (GNX) following preterm birth may prevent the deficits in neonatal development that contribute to these disorders. We further propose that maternal administration of progesterone, which is commonly administered prophylactically to women at risk of preterm labour, may have unforeseen effects on fetal neurodevelopment due to the ability of progesterone to be metabolized to a number of steroids with varying effects on development including allopregnanolone and cortisol.

We found that there is an adaptive increase in the mRNA levels of $GABA_A$ receptors involved in neurosteroid action after term birth in the guinea pig neonate,

but not after preterm birth. The increased levels in the term neonate may compensate for the dramatic decline in allopregnanolone levels following separation from the placenta, and this lack of an adaptive increase in the preterm neonate may heighten the adverse effect of the premature decline in neurosteroid exposure. Preterm neonates also had deficits in myelination of the hippocampus, subcortical white matter, and cerebellum. At juvenile age these deficits remained in the hippocampus, subcortical white matter, and female cerebellum. Interestingly, increased myelination of the male cerebellum at juvenility, suggesting a deficit in axonal pruning, was observed in conjunction with a dysregulation of the cerebellar GABAergic system. In addition to the white matter alterations at juvenility, male guinea pigs that were born preterm exhibited a hyperactive, ADHD-type phenotype, whilst females had anxious behaviour. Unexpectedly, maternal progesterone therapy did not affect fetal allopregnanolone or cortisol steroid levels, nor did it have an effect on myelination of the hippocampus. Circulating maternal cortisol was increased, but fortunately the placental enzymatic barrier protected the fetus from this potentially damaging rise in cortisol. Novel neurosteroid-therapy replacement therapy using ganaxolone during the immediate neonatal period following preterm birth improved the neurobehavioural outcomes of the male juvenile offspring by ameliorating myelination deficits and returning behavioural phenotypes to term-born levels.

Therefore, through utilizing our guinea pig model of preterm birth, we were able to conclude that the GABAergic system and its effects on myelination are disrupted following preterm birth and that this occurs in parallel with a hyperactive phenotype in males, and conversely an anxious phenotype in females. Furthermore, whilst prenatal progesterone therapy does not influence fetal allopregnanolone levels,

restoring the *in utero* neurosteroid environment for preterm neonates following preterm birth may be a viable therapy to prevent the onset of behavioural and learning disorders.