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

Presented By

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Declaration

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List of publications included as part of the thesis

1. **Sominsky L.**, Walker A.K., Ong L.K., Tynan R.J., Walker F.R., Hodgson D.M. (2012) Increased microglial activation in the rat brain following neonatal exposure to a bacterial mimetic. *Behavioural Brain Research.* 226(1), 351-356.

2. **Sominsky L.,** Fuller E.A., Bondarenko E., Ong L.K., Averell L., Nalivaiko E., Dunkley P.R., Dickson P.W., Hodgson D.M. (2013) Functional programming of the autonomic nervous system by postnatal immune challenge: implications for anxiety. *PLOS ONE* 8(3): e57700. doi:10.1371/journal.pone.0057700

3. Walker A.K., Hiles, S.A., **Sominsky L.,** McLaughlin E.A., Hodgson D.M. (2011) Neonatal lipopolysaccharide exposure impairs sexual development and reproductive success in the Wistar rat. *Brain, Behaviour & Immunity,* 25 (4), 674-684

4. **Sominsky L.,** Meehan C.L., Walker A.K., Bobrovskaya L., McLaughlin E.A., Hodgson D.M. (2012) Neonatal immune challenge alters reproductive development in the female rat. *Hormones and Behavior*. 62(3), 345-355

5. **Sominsky L.,** Sobinoff A.P., Jobling M.S., Pye V., McLaughlin E.A., Hodgson D.M. (2013) Immune regulation of ovarian development: programming by neonatal immune challenge. *Frontiers of Neuroscience.* 7(100). doi: 10.3389/fnins.2013.00100

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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 enteri*ca, 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.