PRIMING OF INFLAMMATORY PAIN RESPONSES
BY A NEONATAL IMMUNE CHALLENGE:
IMPLICATIONS OF NEUROIMMUNE-ENDOCRINE
COMMUNICATION FOR PAIN

Presented By

Ihssane Zouikr

MSc (Biomedical Sciences)

MSc (Neurosciences)

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

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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.

Ihssane Zouikr

Ihssane Zouikr
Laboratory of Neuroimmunology,
School of Psychology,
The University of Newcastle,
Callaghan, New South Wales,
Australia.
22\textsuperscript{nd} July 2014

This letter outlines the contribution made by Ihssane Zouikr to the following paper which will comprise his PhD Thesis by publication.


We as co-authors of the published manuscript, attest that Research Higher Degree candidate Ihssane Zouikr was the primary contributor to the publication. Ihssane was involved in the planning, experimental design, data collection and analysis, and drove the composition of the manuscript.

Dr. Melissa A Tadros
School of Biomedical Sciences & Pharmacy,
The University of Newcastle, Australia

A/Prof. Vicki L Clifton
Robinson Institute,
The University of Adelaide, Adelaide Australia

Prof. Kenneth W Beagley
Institute of Health, Biomedical Innovation,
Queensland University of Technology
Brisbane, Australia

Prof. Deborah M Hodgson
Laboratory of Neuroimmunology,
School of Psychology,
The University of Newcastle, Australia

A/Prof. Jenny Cameron
Acting Assistant Dean (Research Training)
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Dr. Melissa A Tadros
School of Biomedical Sciences & Pharmacy, The University of Newcastle, Australia

A/Prof. Vicki L Clifton
Robinson Institute, The University of Adelaide, Adelaide Australia

Dr. Javad Barouei
School of Psychology, The University of Newcastle, Australia

Prof. Robert J Callister
School of Biomedical Sciences & Pharmacy, The University of Newcastle, Australia

Prof. Kenneth W Beagley
Institute of Health, Biomedical Innovation, Queensland University of Technology, Brisbane, Australia

Prof. Deborah M Hodgson
School of Psychology, The University of Newcastle, Australia

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Dr. Morgan H James
School of Biomedical Sciences & Pharmacy, The University of Newcastle, Australia

A/Prof. Vicki L Clifton
Robinson Institute, The University of Adelaide, Adelaide Australia

Miss Erin J Campbell
School of Biomedical Sciences & Pharmacy, The University of Newcastle, Australia

Dr. Christopher V Dayas
School of Biomedical Sciences & Pharmacy, The University of Newcastle, Australia

Prof. Kenneth W Beagley
Institute of Health, Biomedical Innovation, Queensland University of Technology, Brisbane, Australia

Prof. Deborah M Hodgson
School of Psychology, The University of Newcastle, Australia

A/Prof. Jenny Cameron
Acting Assistant Dean (Research Training)

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Mr Abdulrzag F Ahmad 
School of Biomedical Sciences & Pharmacy, The University of Newcastle, Australia

Dr. Rick F Thorne 
School of Biomedical Science and Pharmacy, The University of Australia

Dr. Jay C Horvat 
School of Biomedical Sciences & Pharmacy, The University of Newcastle, Australia

Miss. Allyson Ray 
School of Psychology, The University of Newcastle, Australia

Prof. Kenneth W Beagley 
Institute of Health, Biomedical Innovation, Queensland University of Technology
Brisbane, Australia

A/ Prof. Vicki L. Clifton 
Robinson Institute, The University of Adelaide, Australia

A/Prof. Jenny Cameron 
Acting Assistant Dean (Research Training)

Prof. Deborah Hodgson 
School of Psychology, The University of Newcastle, Australia
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Thesis Abstract

The perinatal period, which encompasses both in utero and neonatal life, represents a time of significant plasticity during which many physiological systems including the immune, endocrine, and nociceptive systems are undergoing fine-tuning and maturation. Thus, an exposure to environmental stimuli during this sensitive period of development can interfere with the normal developmental trajectory of these physiological systems, leading to maladaptive responses later in life. Several animal and human studies have documented that exposure to a variety of stressors such as psychological, physiological, or social stress, can critically influence how organisms evolve and respond to their environment later in life. One factor that has recently received considerable interest is exposure to bacteria during the neonatal period. Exposure to the bacterial mimic, Lipopolysaccharide (LPS), is an established model of early life immune-mediated stress. Our laboratory has previously shown that neonatal LPS exposure is associated with altered behavioural, endocrine, and immune responses later in life. However, the impact of neonatal LPS exposure on nociceptive responses later in life is less known. The primary aim of the current thesis was to develop a profile of the formalin-induced behavioural alterations that are associated with neonatal LPS exposure, as well as characterizing the neuroendocrine, neuroimmune, spinal, and supraspinal changes associated with this behavioural profile. To achieve this aim, we have subjected Wistar rats to intraperitoneal administration of LPS (LPS, Salmonella enterica, serotype enteritidis) on postnatal days (PNDs) 3 and 5 (birth = PND 1) and subjected them to formalin injection at PNDs 7, 13, 22, and 80-97.

The first manuscript (Zouikr et al., 2013) examined the impact of low formalin concentrations, not previously used in the literature, on formalin-induced nociceptive responses (i.e. flinching and licking) during the first three postnatal weeks in rats. The results indicated that low formalin concentrations (0.3-2.25%) induced developmentally regulated pain responses. The characteristic biphasic nociceptive response appeared as early as PND 13 following an intraplantar injection of 0.8% formalin. Additionally, we demonstrated that PNDs 7, 13, 22 and adult rats displayed fine-tuned responses with
low formalin concentrations including appearance of licking responses in one week old rats.

Following the optimization of the formalin test, in the second manuscript (Zouikr et al., 2014b), we investigated the behavioural profile of infant and preadolescent rats following exposure to an immune challenge during the neonatal period. We demonstrated for the first time that dual exposure to LPS at PNDs 3 and 5 exerts long-term effects on inflammatory pain responses in a developmentally regulated manner. An increased susceptibility (i.e. hyperalgesia) to formalin-induced licking (at PND 13) and flinching (at PND 22) responses was observed following neonatal LPS exposure. Neonatal LPS exposure did not alter formalin-induced nociceptive response in PND 7 rats. We further characterized the neuroendocrine changes associated with this age-dependent behavioural hyperalgesia. LPS-treated rats displayed an increased plasma corticosterone levels at PND 22, but not PND 13, and a shift in the balance of glucocorticoid and mineralocorticoid receptor mRNA in the hypothalamus at PND 22 following formalin injection. We have also investigated the impact of neonatal LPS challenge on spinal dorsal horn neuronal changes and found significant changes in the intrinsic properties of spinal dorsal horn (SDH) neurons in PND 22 rats after neonatal LPS exposure as indicated by decreased input resistance and decreased Action Potential (AP) amplitude in LPS-treated rats. These data provided the first evidence that neonatal immune challenge produces developmentally regulated changes in formalin-induced nociception, HPA axis function, and SDH neuronal properties.

We then focused on the supraspinal changes associated with the behavioural hyperalgesia in preadolescent rats. The third manuscript (Zouikr et al., 2014a) investigated whether the increased formalin-induced behaviour observed in preadolescent rats treated with LPS as neonates is due to decreased neuronal activation of the PAG, a substrate known to mediate analgesia. cFos was used as a marker of neuronal activation. We demonstrated that the LPS-induced hyperalgesia in PND 22 rats was associated with distinct recruitment of supra-spinal regions involved in analgesia as indicated by significantly attenuated Fos-protein induction in the rostral dorsal periaqueductal grey (DPAG) as well as rostral and caudal axes of the ventrolateral PAG (VLPAG). Formalin injections were associated with increased Fos-protein labelling in lateral habenula (LHb) as compared to medial habenula (MHb),
however the intensity of this labelling did not differ as a result of neonatal immune challenge.

In the fourth manuscript (Zouikr et al., 2014c, in press), we were interested in determining the long-term effects of neonatal LPS challenge on formalin-induced nociceptive behaviour. Specifically, whether the LPS-induced hyperalgesia persists into adulthood. We were also interested in determining and characterizing the neuroimmune alterations to be driven such behavioural, neuroendocrine, spinal, and supraspinal alterations observed in manuscript 2 and 3 (Zouikr et al., 2014a; Zouikr et al., 2014b). The fourth manuscript demonstrated that neonatal LPS exposure induces increased formalin-induced nociceptive behaviour in both preadolescent (i.e. PND 22) and adult rats (i.e. PNDs 80-97). This behavioural hyperalgesia was accompanied by developmentally regulated changes in peripheral and central immune responses as indicated by enhanced plasma levels of IL-1β and enhanced mast cell degranulation in LPS-treated preadolescent rats as well as increased hippocampal IL-1β in LPS-treated adult rats.

Taken together, these studies demonstrate that the early microbial environment plays an eminent role in determining inflammatory pain sensitivity later in life via the action on behaviour, immune, neuroendocrine, spinal, and supraspinal systems. Because of the well regulated interaction between all of these physiological systems and their vulnerability during sensitive windows of development, particularly during the neonatal period when the neurocircuitry underlying the nociceptive and the immune system are undergoing significant plasticity, it is important to guarantee a healthy and infection-free environment to vulnerable infants such as preterm infants. This will help reduce possible interference with the normal developmental trajectory of the brain and the nociceptive system. The findings from this thesis also emphasize on the importance of the neuroimmune interface in modulating pain sensitivity. If we are to combat this stubborn condition that is chronic pain, future therapeutic approaches should take into account the critical aspect and the critical role of the neuroimmune interface by targeting component of the immune system (e.g. antagonizing the effects of pro-inflammatory cytokines) in addition to targeting component of the nervous system because these two physiological systems constitute one overarching, highly modulated system.