An Examination of the Cellular and Inflammatory Response in Rats After Spinal Cord Injury; the Effects of Age and Survival Time

A thesis submitted in fulfilment of the requirements of the degree of

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By

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DECLARATION OF AUTHORSHIP/ORIGINATLITY

I, Theresa Sutherland, declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Life Sciences at the University of Technology Sydney. This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis. This document has not been submitted for qualifications at any other academic institution. This research is supported by the Australian Government Research Training Program.

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- *Sutherland, TC. and Gorrie, CA. (2018)* ***Paediatric Spinal Cord Injury: The Challenges of Injury Modelling and Translation to the Clinic.*** *Journal of Pediatrics and Infants. 1(1): 34-36*

ASSOCIATED PUBLICATIONS

- *Nguyen, T., Mao, Y., Sutherland, T., and Gorrie, CA (2017)* ***Neural progenitor cells but not astrocytes respond distally to a rat thoracic spinal cord injury****. Neural Regeneration Research 12(11): 1885-1894*
- *Mao, Y., Nguyen, T., Sutherland, TC., and Gorrie, CA. (2016)* ***Endogenous neural progenitor cells in the repair of the spinal cord****. Neural Regeneration Research 11(7): 1075-1076*

CONFERENCE PROCEEDINGS ARISING FROM THIS THESIS

- **ASMR NSW Meeting 2018**
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- **Neuroscience 2017**
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- **ANS Annual Meeting 2017**
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- **Neuroscience 2016**
    - Theresa Sutherland, Arjun Sapkota and Catherine Gorrie: Comparison of the injury and consequent inflammatory response after spinal cord injury in neonates and mature rats may lead to a new therapeutic avenue. In: Neuroscience: 2016; San Diego, US; 2016
- **ANS Annual Meeting 2016**
    - Theresa Sutherland, Alison Ricafrente and Catherine Gorrie: A comparison of the histology and neuroinflammatory response after spinal cord injury in adult and infant rats shows significant differences. In: Australasian Neuroscience Society Annual Meeting: 2016; Hobart, Aus; 2016

- **Inter-University Neuroscience and Mental Health Conference**
    - Theresa Sutherland, Yilin Mao, Tara Nguyen, Catherine Gorrie: Comparison of the injury and innate immune cell response over a 6 week period after spinal cord injury in neonate, juvenile and adult rats. In: Interuniversity Neuroscience and Mental Health: 2015; Sydney, Australia; 2015.
- **ISN-APSN-ANS International Conference 2015**
    - Theresa Sutherland, Yilin Mao, Tara Nguyen, Catherine Gorrie: Comparing innate immune cell and progenitor cell responses 24 hours after spinal cord injury in neonate, juvenile and adult rats. In: ISN-APSN-ANS Joint Conference: 2015; Cairns, Australia; 2015.
- **New Horizons Conference 2014**
    - Theresa Sutherland, Yilin Mao, Tara Nguyen, Catherine Gorrie: A comparison of the endogenous neural progenitor cell reaction 24 hours after spinal cord injury in adult, juvenile and infant rats. In: New Horizons: 2014; Kolling Institute, Sydney, Australia; 2014.

TABLE OF CONTENTS

CHAPTER 3: THE PROGRESSION OF THE CELLULAR INFLAMMATORY, REACTIVE ASTROCYTIC AND ENDOGENOUS NEURAL PROGENITOR CELL RESPONSES TO SPINAL CORD INJURY IN DIFFERENT AGES OF RATS

APPENDIX

LIST OF FIGURES

CHAPTER 1:

CHAPTER 2:

CHAPTER 4:

CHAPTER 5:

APPENDIX:

LIST OF TABLES

CHAPTER 2:

CHAPTER 3:

CHAPTER 4:

APPENDIX:

ABBREVIATIONS

AD……..Alzheimer's disease

ANOVA……Analysis of variance (statistic)

ASIA……American spinal injury association (impairment scale)

BBB……Blood brain barrier

BBB……Basso, Beattie and Bresnahan (behavioural score)

BDNF……Brain derived neurotrophic factor

BMDM……Bone marrow derived macrophages

BMP……Bone morphogenic protein

BMSC……Bone marrow stromal cells

BSCB……Blood spinal cord barrier

CBS……Combined behavioural score

CNS……Central nervous system

CNTF……Ciliary neurotrophic factor

CXCL……(C-X-C motif) ligand

EAE……Experimental autoimmune encephalomyelitis

ELISA……Enzyme-linked immunosorbent assay

eNPC……Endogenous neural progenitor cell

GDNF……Glial derived neurotrophic factor

GFAP……Glial fibrillary acidic protein

hECS……Human embryonic stem cells

IGF-1……Insulin-like growth factor-1

IFN-γ……Interferon-gamma

IL-……..Interleukin-

INC……Intermediate nucleus of Cajal

IRLS……Infant rat locomotor score

M1……Pro-inflammatory macrophage/monocyte

M2……Anti-inflammatory macrophage/monocyte

MBP……Myelin basic protein

MDM……Monocyte-derived macrophages

MS……...Multiple Sclerosis

MSC……Mesenchymal Stem Cells

MVA……Motor vehicle accident

NGF……Nerve growth factor

NPC……Neural progenitor cells

NSPC……..Neural stem/progenitor cell

NT-3……..Neurotrophin 3

NTF……Neurotrophic Factors

OEC……..Olfactory ensheathing cells

OPC……..Oligdendrocyte progenitor cells

P7……Post-natal day 7

P10……Post-natal day 10

PD……Parkinson's disease

PNS……..Peripheral nervous system

RT-PCR……Reverse transcription polymerase chain reaction

ROS……Reactive oxygen species

RNS……..Reactive nitrogen species

SCI……Spinal cord injury

SCIWORA……..Spinal cord injury without radiographic anomaly

SEM……Standard error of the mean

SEZ……Subependymal zone

SNI……Spinal nerve injury

SPC……Stem/progenitor cell

SVZ……Subventricular zone

T10……10^{th} thoracic vertebral level

TBI……Traumatic brain injury

TGF-β……Transforming growth factor-beta

TNF-α……Tumour necrosis factor-alpha

TNF-R……Tumour necrosis factor receptor

TLR……Toll-like receptor

TSCI……Traumatic spinal cord injury

ABSTRACT

Spinal cord injury (SCI) is a complex and devastating condition that has a life-long effect on patients' quality of life, their family, carers and society. Currently there is no cure for SCI, and no proven treatment in the acute phases of SCI. Tissue loss and varying degrees of functional impairment result from a SCI, and only limited repair is exhibited. A great deal of research has focused on reducing the degenerative effects that occur during the secondary injury phase of injury to order to promote tissue repair and regeneration. The immune and inflammatory response is thought to play a significant role in this process, albeit with both beneficial and detrimental responses reported. Most research to date has concentrated on adult SCI, yet it has been suggested that the young show better functional recovery compared to adults both for humans and in a variety of animal models.

The current research project used an animal model of contusive SCI to compare adult (9wk), juvenile (5wk) and infant (P7) Sprague-Dawley rats. One cohort (n=108) was assessed over a 6 week post-injury period for 1) locomotor function using established and newly developed scoring systems, 2) injury progression using histology, and 3) inflammatory cell changes using immunohistochemistry. A second cohort (n=97) was assessed acutely (1h, 24h and 1wk post-injury) for inflammatory mediators using flow cytometry on the injured tissue homogenate and multiplex cytokine ELISA on the tissue supernatant. Finally, an in vitro study was conducted to

explore the possibility of modulating different macrophage populations using conditioned media to create a more anti-inflammatory microenvironment.

The results described in this thesis show that following a SCI of comparative severity there were significant differences between adult and infant injury progression and presentation, inflammatory responses, and behavioural recovery. This research reinforced the inherent difficulties in modelling infant conditions for comparative studies, but it has also highlighted two important avenues of research to be pursued. 1) A better understanding of SCI progression in the young is needed to inform how paediatric SCI is treated and managed, and 2) targeted modulation of the inflammatory response in adult SCI patients may be a promising avenue for better functional recovery.