

## **Final Report**

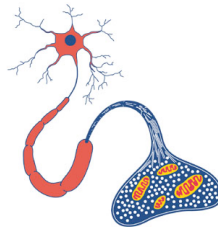
# Pre-Conditioned Lesion: Inflammatory Effects on CNS Regeneration

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A thesis submitted for the degree of  
Doctor of Philosophy



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*Dedicated to*

*My Wife*

*Dorani Aguilar*

*and*

*My Parents*

*Oscar and Marta de Aguilar*

## **INSPIRATIONAL QUOTES**

There are no small problems. Problems that appear small are large problems that are not understood.

*Santiago Ramon y Cajal*

In the middle of difficulty lies opportunity.

*Albert Einstein*

“De todas las reacciones posibles ante una injuria, la mas habil y economica es el silencio”. Once development was ended, the founts of growth and regeneration of the axons and dendrites dried up irrevocably. Everything may die, nothing may be regenerated. It is for the science of the future to change, if possible, this harsh decree.

*Santiago Ramon y Cajal*

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## **THESIS SUMMARY**

In the adult central nervous system (CNS) several factors are implicated in the failure of neurons to regenerate after spinal cord injury (SCI). However, this reduced ability of injured CNS neurons to regenerate can be improved by under certain conditions. For instance, in adult dorsal root ganglion (DRG) neurons, injury to its peripheral branch (unilateral conditioning lesion) prior to injury of its central DRG branch (dorsal column cut) enhances the intrinsic capability of some but not all CNS afferent neurons to regenerate. The exact mechanism mediating this type of response is not known. However, previous studies by other groups have proposed that the regeneration of these CNS afferent neurons might be associated with the inflammatory response following injury to the peripheral DRG branch. Our general aim, was to examine the involvement of the immune response in the regeneration of the CNS DRG branch, as part of the pre-conditioned lesion model. To test this, three questions/hypotheses were investigated.

Firstly, we investigated the effects of vaccination in pre-conditioned lesion animals using a peripheral nerve homogenate (PNH, sciatic nerve) as the immunogen. Given the regenerative capabilities of peripheral nerves, we proposed that exposure to this homogenate could enhance the limited regeneration of CNS fibres, after pre-conditioning of DRG neurons. Our results showed that in adult and/or neonatal Sprague Dawley (SD) rats PNH-vaccinated, had greater number of regenerated fibres, as compared



to injury matched saline-vaccinated controls. Conversely, passive exposure to PNH through parental vaccination resulted in the suppression of this regenerative trigger. This suppressed competence of CNS fibres to regenerate was indirectly correlated with a reduced number of macrophage cells throughout the SCI epicentre, as compared to greater macrophage numbers found in the adult and/or neonatal treated groups.

Secondly, we explored the possibility that a systemic inflammatory effect originating from the peripheral conditioning lesion, might be able to contribute to the regeneration of other injured neurons within the matured CNS. Again, using adult SD rats, we pre-conditioned the peripheral DRG branch as previous and changed the location of the CNS injury from the spinal cord to the optic nerve. Where alike any other injured neuron within the CNS, fails to regenerate. Unfortunately, our results from anterograde or retrograde labelling did not find any regenerated optic nerve fibres, although, we did find macrophage numbers to be higher in pre-conditioned lesion animals as compared to sham-operated animals. Therefore, it is possible that the pre-conditioning peripheral lesion might be allowing for a greater macrophage infiltration into the CNS compartment.

Finally, we determined whether an early macrophage infiltration into the CNS compartment could be correlated with the observed CNS regeneration, characteristic of the pre-conditioned lesion model. To test this, we temporarily depleted macrophages before, during and after peripheral nerve lesion, via liposomal clodronate delivery. Our results from

anterograde and retrograde labelling of spinal cord fibres revealed no regenerated CNS fibres in macrophage depleted animals, only in injury matched controls.

In conclusion, macrophage cells play a beneficial role in the regeneration of CNS afferent fibres of pre-conditioned lesion DRG neurons. This most likely occurs through activation of intrinsic somatic DRG responses, as well as, an increased macrophage activation. We believe this inflammatory response to be of favourable phenotypic characteristic to the regeneration of injured CNS neurons, especially those in proximity to the DRG cell body. In addition, we propose that the conditioning peripheral lesion permits an influx of macrophage cells into the CNS compartment before injury of the CNS DRG branch, which is also likely to be supporting regeneration of afferent fibres. Future studies should evaluate the possibility that activated inflammatory cells might be infiltrating into the CNS under minimal blood-brain barrier disruption. It is clear that a complex communication between the nervous and immune system is occurring after the initial peripheral injury.

## **DECLARATION**

I certify that this thesis does not incorporate, without acknowledgment, any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief, it does not contain any material previously published or written by another person except where due reference is made in the text.

Ernest Aguilar Salegio

## **SUPERVISOR DECLARATION**

It is my opinion that this thesis is properly presented, conforms to the specifications for the university and is of sufficient standard to be, *prima facie*, worthy of examination.

Prof Xin-Fu Zhou

Principle Supervisor

## **PERSONAL ACKNOWLEDGEMENTS**

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A huge acknowledgement goes to my wife Dorani, for her support, encouragement and understanding during my years of study, especially

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## **MANUSCRIPTS**

1. Wang YJ, Valadares D, Sun Y, Wang X, Zhong JH, Liu XH, Majd S, Chen L, Gao CY, Chen S, Lim Y, Pollard A, **Aguilar E**, Gai WP, Yang M, Zhou XF (2010). Effects of proNGF on Neuronal Viability, Neurite Growth and Amyloid-beta Metabolism. *Neurotox Res* **17**: 257-267
2. **Aguilar E**, Pollard A, Smith M, Zhou XF. Macrophage Presence is Essential for the Regeneration of Ascending Afferent Fibres Following a Conditioning Sciatic Nerve Lesion in Adult Rats. *Submitted to BMC Neuroscience*
3. **Aguilar E**, Pollard A, Smith M, Zhou XF. Sciatic Nerve Conditioning Lesion Increases Macrophage Response but it Does Not Promote the Regeneration of Injured Optic Nerves. *Submitted to Brain Research*
4. Pollard A, **Aguilar E**, Zhou FH, Zhong JH, Oliver J, Zhou XF (2008). Intranasal delivery of ciliary neurotrophic factor to the rat brain along olfactory pathways. *In Preparation*
5. Pollard A, **Aguilar E**, Wang YJ, Zhou FH, Zhou XF. Adenoviral vector gene delivery of enhanced-GFP to the brain via the olfactory receptor neurons. *In Preparation*

## **CONFERENCE PROCEEDINGS**

1. **Aguilar E**, Rush RA, Smith M, Zhou XF (2007): Effects of Sciatic Nerve Vaccination on Axonal Regeneration in the Adult Rat Spinal Cord following a Conditioning Lesion. *In Proceedings of the Society for Neuroscience, San Diego, California (poster)*.
2. **Aguilar E**, Rush RA, Smith M, Zhou XF (2008): Conditioning Lesion: Possibility for Systemic CNS Regeneration in Adult Rats. *In Proceedings of the Australian Neuroscience Society, Hobart, Tasmania (poster)*.



**AWARDS RECEIVED DURING PhD**

- 2008 Australian Neuroscience Society Travel Award**  
to attend 28<sup>th</sup> Annual ANS conference held in Hobart, Tasmania
- 2008 Health Science Student Conference Travel Grant**  
to attend 28<sup>th</sup> Annual ANS conference held in Hobart, Tasmania
- 2007 Flinders Medical Centre Travel Award**  
to attend 37<sup>th</sup> Annual Society for Neuroscience conference held in San Diego, California, USA
- 2007 Health Science Student Conference Travel Grant**  
to attend 37<sup>th</sup> Annual Society for Neuroscience conference held in San Diego, California, USA
- 2007 Flinders University Student Conference Travel Grant**  
to attend 37<sup>th</sup> Annual Society for Neuroscience conference held in San Diego, California, USA
- 2004 Flinders University PhD Research Scholarship Grant**  
Competitive PhD program

## **ABBREVIATIONS**

<b>AD</b>	Alzheimer's Disease
<b>ANOVA</b>	Analysis of Variance
<b>BBB</b>	Blood Brain Barrier
<b>BCA</b>	Bicinchoninic Acid
<b>BDA</b>	Biotinylated Dextran Amine
<b>BDNF</b>	Brain-Derived Neurotrophic Factor
<b>C</b>	Caudal
<b>cAMP</b>	Cyclic Adenosine Monophosphate
<b>CD68</b>	Cluster Differentiation 68
<b>CD4</b>	Cluster Differentiation 4
<b>CFA</b>	Complete Freund's Adjuvant
<b>cGMP</b>	Cyclic Guanosine Monophosphate
<b>CL</b>	Contralateral
<b>CNS</b>	Central Nervous System
<b>CNTF</b>	Ciliary Neurotrophic Factor
<b>CSPG/s</b>	Chondroitin Sulfate Proteoglycan/s
<b>CSF</b>	Corticospinal Fluid
<b>CST</b>	Corticospinal Tract
<b>CXCL10</b>	Chemokine (C-X-C motif) Ligand 10
<b>D</b>	Dorsal, Distal
<b>DAB</b>	3'3-Diaminobenzidine Tetrahydrochloride
<b>DAPI</b>	4',6-Diamidino-2-Phenylindole
<b>DC</b>	Dorsal Column
<b>DRG</b>	Dorsal Root Ganglion
<b>EAE</b>	Experimental Autoimmune Encephalomyelitis
<b>EAN</b>	Experimental Autoimmune Neuritis
<b>ECM</b>	Extracellular Matrix
<b>ELISA</b>	Enzyme-Linked Immunosorbent Assay
<b>FB</b>	Fast Blue
<b>FGF</b>	Fibroblast Growth Factor
<b>FR</b>	Fluoruby, Dextran Tetramethylrhodamine
<b>GAP-43</b>	Growth-Associated Protein 43
<b>GDNF</b>	Glial-Derived Neurotrophic Factor
<b>GFAP</b>	Glial Fibrillary Acid Protein
<b>HCl</b>	Hydrochloric Acid
<b>HRP</b>	Horseradish Peroxidase
<b>Hz</b>	Hertz
<b>H<sub>2</sub>O<sub>2</sub></b>	Hydrogen Peroxide
<b>IFA</b>	Incomplete Freund's Adjuvant

<b>IGF-1</b>	Insulin Growth Factor-1
<b>IgG</b>	Immunoglobulin G
<b>IgM</b>	Immunoglobulin M
<b>IHC</b>	Immunohistochemistry
<b>IL</b>	Ipsilateral
<b>IL-1</b>	Interleukin 1
<b>IL-1<math>\beta</math></b>	Interleukin 1-beta
<b>IL-6</b>	Interleukin 6
<b>IL-10</b>	Interleukin 10
<b>i.p.</b>	Intraperitoneal
<b>i.v.</b>	Intravenous
<b>KSPG/s</b>	Keratan Sulfate Proteoglycan/s
<b>LC</b>	Lateral Column
<b>LIF</b>	Leukemia Inhibitory Factor
<b>LS</b>	Left Side
<b>L4-5</b>	Lumbar 4-5
<b>M</b>	Molar
<b>MAG</b>	Myelin-Associated Glycoprotein
<b>MBP</b>	Myelin Basis Protein
<b>MCP-1</b>	Macrophage Chemoattractant Protein 1
<b>MS</b>	Multiple Sclerosis
<b>MW</b>	Molecular Weight
<b>M<math>\phi</math>/s</b>	Macrophage/s
<b>NHS</b>	Normal Horse Serum
<b>NF-200</b>	Neurofilament 200
<b>NGF</b>	Nerve Growth Factor
<b>NiSO<sub>4</sub></b>	Nickel Sulfate
<b>NT/s</b>	Neurotrophin/s
<b>NT-3</b>	Neurotrophin 3
<b>NaNO<sub>2</sub></b>	Sodium Nitrite
<b>OEC</b>	Olfactory Ensheathing Cell
<b>OGD</b>	Oligodendrocyte
<b>ONC</b>	Optic Nerve Crush
<b>OMGP</b>	Oligodendrocyte Myelin Glycoprotein
<b>OPC/s</b>	Oligodendrocyte Progenitor Cell/s
<b>P</b>	Proximal
<b>PB</b>	Phosphate Buffer
<b>PBS</b>	Phosphate Buffered Saline
<b>PBST</b>	Phosphate Buffered Saline Tween-20
<b>PFA</b>	Paraformaldehyde
<b>PNH</b>	Peripheral Nerve Homogenate
<b>PNS</b>	Peripheral Nervous System
<b>R</b>	Rostral

<b>RAG/s</b>	Regeneration-Associated Gene/s
<b>RGC/s</b>	Retinal Ganglion Cells
<b>RS</b>	Right Side
<b>RT</b>	Room Temperature
<b>SC</b>	Superior Colliculus
<b>SCH</b>	Spinal Cord Homogenate
<b>SCI</b>	Spinal Cord Injury
<b>SD</b>	Sprague Dawley
<b>SE</b>	Standard Error
<b>SEMA-3</b>	Semaphorin-3
<b>SNH</b>	Sciatic Nerve Homogenate
<b>SNI</b>	Sciatic Nerve Injury
<b>SS</b>	Sterile Saline
<b>TGF<math>\beta</math></b>	Transforming Growth Factor-beta
<b>TMB</b>	3,3',5,5'-tetramethylbenzidine
<b>TNF<math>\alpha</math></b>	Tumour Necrosis Factor-alpha
<b>T9-10</b>	Thoracic 9-10
<b>V</b>	Ventral
<b>VC</b>	Ventral Column
<b>VEGF</b>	Vascular Endothelial Growth Factor
<b>WD</b>	Wallerian Degeneration