Final Report

Pre-Conditioned Lesion: Inflammatory Effects on CNS Regeneration

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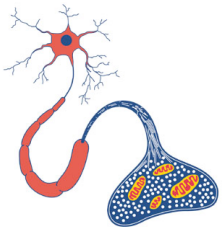

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Dedicated to

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

First, I would like to thank my supervisors, Prof Xin-Fu Zhou and Prof Malcolm Smith. Xin-Fu thank you very much for all your help and encouragement during my PhD, I always felt I could come and talk to you about anything. You always understood my passion for research and you supported me through some very hard times, especially after changing projects half way through my PhD studies.

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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 28th Annual ANS conference held in Hobart, Tasmania

2008 Health Science Student Conference Travel Grant
to attend 28th Annual ANS conference held in Hobart, Tasmania

2007 Flinders Medical Centre Travel Award
to attend 37th Annual Society for Neuroscience conference held in San Diego, California, USA

2007 Health Science Student Conference Travel Grant
to attend 37th Annual Society for Neuroscience conference held in San Diego, California, USA

2007 Flinders University Student Conference Travel Grant
to attend 37th Annual Society for Neuroscience conference held in San Diego, California, USA

2004 Flinders University PhD Research Scholarship Grant
Competitive PhD program

ABBREVIATIONS

AD	Alzheimer's Disease
ANOVA	Analysis of Variance
BBB	Blood Brain Barrier
BCA	Bicinchoninic Acid
BDA	Biotinylated Dextran Amine
BDNF	Brain-Derived Neurotrophic Factor
C	Caudal
cAMP	Cyclic Adenosine Monophosphate
CD68	Cluster Differentiation 68
CD4	Cluster Differentiation 4
CFA	Complete Freund's Adjuvant
cGMP	Cyclic Guanosine Monophosphate
CL	Contralateral
CNS	Central Nervous System
CNTF	Ciliary Neurotrophic Factor
CSPG/s	Chondroitin Sulfate Proteoglycan/s
CSF	Corticospinal Fluid
CST	Corticospinal Tract
CXCL10	Chemokine (C-X-C motif) Ligand 10
D	Dorsal, Distal
DAB	3'3-Diaminobenzidine Tetrahydrochloride
DAPI	4',6-Diamidino-2-Phenylindole
DC	Dorsal Column
DRG	Dorsal Root Ganglion
EAE	Experimental Autoimmune Encephalomyelitis
EAN	Experimental Autoimmune Neuritis
ECM	Extracellular Matrix
ELISA	Enzyme-Linked Immunosorbent Assay
FB	Fast Blue
FGF	Fibroblast Growth Factor
FR	Fluoruby, Dextran Tetramethylrhodamine
GAP-43	Growth-Associated Protein 43
GDNF	Glial-Derived Neurotrophic Factor
GFAP	Glial Fibrillary Acid Protein
HCl	Hydrochloric Acid
HRP	Horseradish Peroxidase
Hz	Hertz
H_2O_2	Hydrogen Peroxide
IFA	Incomplete Freund's Adjuvant

IGF-1	Insulin Growth Factor-1
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHC	Immunohistochemistry
IL	Ipsilateral
IL-1	Interleukin 1
IL-1β	Interleukin 1-beta
IL-6	Interleukin 6
IL-10	Interleukin 10
i.p.	Intraperitoneal
i.v.	Intravenous
KSPG/s	Keratan Sulfate Proteoglycan/s
LC	Lateral Column
LIF	Leukemia Inhibitory Factor
LS	Left Side
L4-5	Lumbar 4-5
M	Molar
MAG	Myelin-Associated Glycoprotein
MBP	Myelin Basis Protein
MCP-1	Macrophage Chemoattractant Protein 1
MS	Multiple Sclerosis
MW	Molecular Weight
Mø/s	Macrophage/s
NHS	Normal Horse Serum
NF-200	Neurofilament 200
NGF	Nerve Growth Factor
$NiSO_4$	Nickel Sulfate
NT/s	Neurotrophin/s
NT-3	Neurotrophin 3
$NaNO_2$	Sodium Nitrite
OEC	Olfactory Ensheathing Cell
OGD	Oligodendrocyte
ONC	Optic Nerve Crush
OMGP	Oligodendrocyte Myelin Glycoprotein
OPC/s	Oligodendrocyte Progenitor Cell/s
P	Proximal
PB	Phosphate Buffer
PBS	Phosphate Buffered Saline
PBST	Phosphate Buffered Saline Tween-20
PFA	Paraformaldehyde
PNH	Peripheral Nerve Homogenate
PNS	Peripheral Nervous System
R	Rostral

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