# *Axon stretch growth: Towards functional repair of the spinal cord – An early translational exercise*


**Malcolm Philip Brinn**

**B.HlthSc (Life Sc) Flinders University**

**B.HlthSc (Hons Anat) University of Adelaide**

**Discipline of Anatomy and Pathology**

**Adelaide Medical School**



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## LANGUAGE: OXFORD UK ENGLISH (MODIFIED)

# Abstract


## *Axon stretch growth: Towards functional repair of the spinal cord – An early translational exercise*

**Background**

Injury to the spinal cord often visually presents as a local injury, damaging neurons that reside in the spinal cord, their projecting axons and supporting infrastructure such as oligodendrocytes. However, damage also occurs to ascending and descending axons that communicate with the brain. Fundamentally, repair of these injuries requires two distinct restorative approaches. The local injury will require stabilisation of the local environment, the rescue of injured neurons and support infrastructure, replacement of lost cells and restoration of intra-spinal communication. The latter ascending and descending axon injury will require proximal and distal axon reunification to restore supra-spinal communication with the brain.

This thesis presents the results of an early translational exercise that takes a non-linear approach to facilitate investigation into axon stretch growth (ASG) - an intrinsic mechanism that allows axons to adapt to body height and size throughout life. Pioneering research has shown that in-vitro exploitation of ASG has the potential to bridge significant gaps associated with injuries to long supra-spinal nerve tracts within the spinal cord.

Although translational science can be applied across the research spectrum, the traditional practice is to intervene once the research has matured. Here, the intervention occurs early, in an environment of limited funding within a progressive school of basic sciences. At the time of intervention, no infrastructure or experience in ASG research was evident within the faculties.

## Translational Methods

The absence of a robust in-vitro adult motor neuron culture was identified as a potential barrier in ASG translation. Collaborations in anatomy, neuroscience, and toxicology were formed. Three separate animal ethics applications were required. Publication of a protocol followed.

The infrastructure required to conduct necessary in-vitro investigations into ASG was determined. Multidisciplinary collaborations were formed with mechanical, electrical and electronics engineers. Design, engineering, and commissioning of the equipment followed.

The lack of a definitive translational animal model has been previously identified as a significant barrier to spinal cord injury research. Specifically, a suitable large animal model has yet to be clearly defined for ASG research. Collaborations with comparative anatomy, a large animal research centre, and a senior spinal surgeon progressed development of a sheep model. Separate multi-institutional animal ethics applications were also required.

## Results

A robust peer reviewed method was established to hydraulically extrude the spinal cord of adult Sprague-Dawley rats in under 60 seconds, and a serum free culture protocol simplified to maximise the yield of motor neurons and reduce culture costs. Adult motor neurons harvested and cultured using this protocol are capable of in-vitro survival for periods exceeding 21 days.

A decommissioned infant humidicrib was successfully converted into a portable temperature (32 – 39$^{o}$C ± 0.1$^{o}$C), and carbon dioxide controlled imaging incubator. Additional modifications incorporating umbilical support for multiple tailored bioreactors was also developed. A tailored ASG bioreactor was prototyped, tested, and commissioned. Axon stretch growth of motor neurons has been initiated in the bioreactor.

The literature review suggested that non-human primates were the optimal model for final translational confirmation. However, there was sufficient evidence to indicate that ungulates (i.e. sheep or pig) may be an alternative for ASG research. Relevant information on the sheep was collated, and basic investigation on their anatomy progressed.

**Conclusion** Early applied translational science (as practised here) is strategic and cost effective, showing that the overall strategy facilitates research, while potentially identifying barriers that could delay progress or cause late translational failure. The introduction of an “off the shelf” early intervention funding model allocated to translational scientists should be considered as a mechanism to progress basic science investigations that are in conceptual stages of development.

# Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Malcolm Philip Brinn

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**Modified Oxford UK/English Version**

# Research contribution

## Publications

Brinn, MP, O'Neill, K, Musgrave, I, Freeman, BJC, Henneberg, M & Kumaratilake, J 2016, 'An optimized method for obtaining adult rat spinal cord motor neurons to be used for tissue culture'*, J Neurosci Methods*, vol.273, pp. 128-137

Brinn, MP, Al-Sarawi, SF, Lu, T-F, Freeman, BJC, Kumaratilake, J & Henneberg, M. 2016, 'A portable cell culture and imaging system with optional umbilical bioreactor using a modified infant incubator', Bioengineering

Brinn, MP, Kumaratilake, J, Al-Sarawi, SF, Lu, T-F, Freeman, BJC & Henneberg, M 2016, 'An optimized method for obtaining adult rat spinal cord motor neurons to be used for tissue culture'*, J Tissue Engineering (submitted 24th January 2017)*

## Presentations

Brinn MP, Kumaratilake, J, Henneberg M, and Freeman BJC. Exploiting Axon Stretch Growth as a novel mechanism for the future repair of long nerve tracts within the spinal cord. XIII Adelaide Centre for Spinal Research Symposium 13-15 August 2015 Novotel Barossa Valley, South Australia

## Posters

Brinn MP, O'Neill K, Zhao S, Kumaratilake J, Musgrave I, Tien-Fu Lu, Al-Sarawi S, Linke I, Slater, A, Freeman BJC & Henneberg M 2016 'Axon Stretch Growth – Overcoming distance: Towards repair of long nerve tracts within the spinal cord' Poster presented at The University of Adelaide, Faculty of Health Sciences Postgraduate research conference: National Wine Centre, Adelaide, August 2014

Brinn MP, Kumaratilake J, Tien-Fu Lu, Al-Sarawi, Freeman BJC & Henneberg M. 'Axon Stretch Growth of adult primary motor neurons", Proceedings of the 25th Biennial Meeting of the ISN jointly with the 13th Meeting of the APSN in conjunction with the 35th Meeting of the ANS, 23rd - 27th August 2015, Cairns Australia, p333

# Dedication and Acknowledgements

This thesis is dedicated to Rosemary, who has been the love of my life for over 40 years. Thank you for sharing this journey and for your support even when you had good reasons not too. Thank you also for your love, your motivation and guidance during the highs and lows.

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# Ethics Statement and Permits

All experimental studies presented in this thesis were conducted per the guidelines established by the National Health and Medical Research Council

| Ethics Committee | Ethics Number | Description | Approval Date |
|---|---|---|---|
| UOA | M-2012-205 | Harvesting of rat spinal cords for motor and sensory neuron culture under serum-free conditions | 19/12/2012<br>24/09/2013 |
| UOA | M2014-159 | Long term culture of adult motor neurons obtained from the spinal cord of a Sprague Dawley rat | 14/11/2014 |
| UOA<br><br>SAHMRI | M-2014-051<br><br>SAM100 | Pilot study stimulating intrinsic growth in the injured spinal cord: Phase 1: Validation of the sheep model | 16th April 2014<br><br>4th June 2014 |

UOA = University of Adelaide

SAHMRI – South Australian Health and Medical Research Institute - PIRL

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# List of Abbreviations

| Abbreviation | Full Description |
|---|---|
| AMPA | α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid |
| ASF | Artifical Cerebro-Spinal Fluid |
| ASG | Axon Stretch Growth |
| bFGF | Basic Fibroblast Growth Factor |
| BDNF | Brain-derived neurotrophic factor |
| C1-C7 | Cervical Vertebrae (numbered) |
| $Ca^{2+}$ | Calcium |
| cAMP | 8(-4 Chlorophenylthic cyclic adenosine 3'5 monophosphate) |
| CNE | Computer Numeric Control |
| CNS | Central Nervous System |
| CNTF | Ciliary Neurotrophic Factor |
| $CO_2$ | Carbon Dioxide |
| CSF | Cerebrospinal Fluid |
| CST | Corticospinal tract |
| Cx1 – Cx4 | Coccyx Vertebrae (numbered) |
| DIV | Days in Vitro |
| DNA | Deoxyribose Nucleic Acid |
| DRG | Dorsal Root Ganglion |
| DSHB | Developmental Studies Hybridoma Bank |
| EDTA | Ethylene Diamine Tetra Acetic acid |

| Abbreviation | Full Description |
|---|---|
| ETS | Early Translational Scientist |
| GDNF | Glial-derived neurotrophic factor |
| HIB-PM | Hibernate processing medium |
| L1-L5 | Lumbar Vertebrae (numbered) |
| $Mg^{2+}$ | Magnesium |
| Neurobasal - CM | Neurobasal based conditioned medium |
| NMDA | N-methyl-D-aspartate |
| NTSC | Non-Traumatic SCI |
| $^oC$ | Degrees Celsius |
| $O_2$ | Oxygen |
| PBS | Phosphate buffered solution |
| PDL | Poly-D-Lysine |
| PEEK | PolyEtherEther Ketone |
| PEG | Polyethylene glycol |
| PET | Polyethylene terephthalate |
| PNS | Peripheral Nervous System |
| S1-S5 | Sacral Vertebrae (numbered) |
| SCI | Spinal Cord Injury |
| T1-T12 | Thoracic Vertebrae (numbered) |
| TSCI | Traumatic SCI |

# Glossary of Terms

**Primary injury:** An event that causes initial damage to the spinal cord

**Secondary injury:** A series of biochemical events that occur within the spinal cord consequential to primary injury or repair intervention.

**Wallerian Degeneration:** A specific form of degeneration that affects neurites (including axons) when separated from a neurons cell body. This can be further specified as anterograde or orthograde.

**Acute injury:** The immediate post-injury phase, where damage to the spinal cord is continuing consequential to vascular or CSF disruption, and/or secondary injury biochemical events (generally < 24 hours' post injury)

**Sub-acute injury:** The period where optimal neuroprotective interventions are in place. Importantly, no new damage is being initiated, but secondary biochemical events are potentially still active, and the fate of existing damaged cells can be influenced through cell rescue measures (24 hours > 10 days)

**Stabilised injury:** A point between sub-acute and chronic injury, where the injury to the spinal cord has not yet reached steady-state but has stabilised. Biochemical events may be continuing, but the clinical response is reactionary (10 days > 24 months)

**Chronic injury:** The stage where the patient has reached steady state, and all biochemical events have subsided to the extent that the spinal cord is no longer actively responding to the injury or post injury intervention (>24 months)

**Acute Repair:** An immediate repair intervention that occurs while the spinal cord is in either acute or early subacute phase of injury.

**Planned Repair:** A planned intervention that occurs during the mid to late sub-acute or chronic phase of injury. The aim is to restore connections but, the repair may not improve functional outcome.

**Restoration of Function:** A planned intervention that occurs during the mid to late sub-acute or chronic phase of injury and is aimed to deliver specific functional gain i.e. nerve/muscle transfer surgery.

# Motto


**The Surgical Treatise - Case 31 (Edwin Smith Papyrus)**

In 1930, renowned Egyptologist James Henry Breasted completed and published (now out of print) a comprehensive translation of the “Edwin Smith Papyrus.” The papyrus has been dated to the sixteenth century B.C and is considered to be the earliest known health record of the spinal column and spinal cord trauma. Case 31 (hieroglyphs above) old English transliteration: “If thou examines a man having a dislocation in a vertebra of his neck, shouldst thou finds him unconscious of his two arms and his two legs on account of it, while his phallus is erected on account of it and urine drops from his member without his knowing it; his flesh has received the wind; his two eyes are blood-shot; it is a dislocation of a vertebra of his neck extending to his backbone which causes him to be unconscious of his two arms and his two legs”……”An ailment not to be treated”.

https://oi.uchicago.edu/research/publications/oip/edwin-smith-surgical-papyrus

volume-1-hieroglyphic-transliteration (Accessed: 29/5/2015)