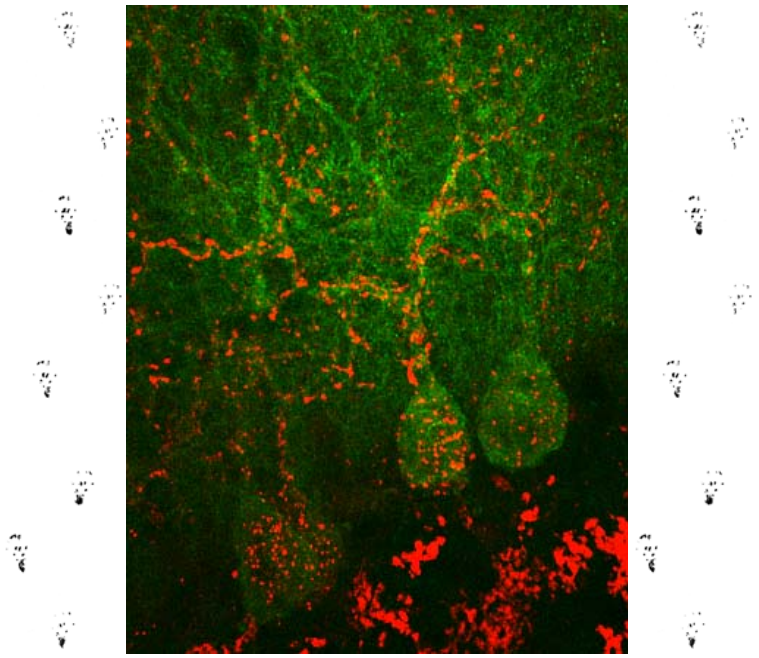
The induction and inhibition of post-lesional transcommissural climbing fibre reinnervation in the neonatal and adult rat cerebellum using Brain-derived neurotrophic factor: anatomical and functional implications



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B.App.Sci. (Biochemistry & Microbiology)
B.App.Sci. Honours I

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School of Veterinary & Biomedical Sciences
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JCU
JAMES COOK UNIVERSITY

THESIS / ANTI-THESIS / SYNTHESIS

Georg Wilhelm Friedrich Hegel, a 19th century German philosopher, was considered by his admirers to have found the key to explaining in principle, just about everything. His position was called Dialectical Idealism. "Dialectic" refers to the process of examining an idea (thesis), working out its implications, consequences and applications, and thereby finding difficulties (anti-thesis) that require the discarding of the original idea and the adoption of a modified form of it (synthesis), a new idea. We then examine the new idea (thesis) and repeat the process. The goal of the process is the final thesis, the 'Absolute'.

Georg W F Hegel (1770-1831)

PLASTICITY

The habits of an elementary particle of matter cannot change (on the principles of the atomistic philosophy), because the particle is itself an unchangeable thing; but those of a compound mass of matter can change, because they are in the last instance due to the structure of the compound, and either outward forces or inward tensions can, from one hour to another, turn that structure into something different from what it was. That is, they can do so if the body be plastic enough to maintain its integrity, and be not disrupted when its structure yields. Plasticity, then, in the wide sense of the word, means the possession of a structure weak enough to yield to an influence, but strong enough not to yield all at once. Each relatively stable phase of equilibrium in such a structure is marked by what we may call a new set of habits. Organic matter, especially nervous tissue, seems endowed with a very extraordinary degree of plasticity of this sort; so that we may without hesitation lay down as our first proposition the following, that the phenomena of habit in living beings are due to the plasticity of the organic materials of which their bodies are composed.

William James (1842 – 1910)
Exert from ‘The Principles of Psychology’ (1980) Vol 1: Ch 4

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SIGNED STATEMENT OF SOURCES

Funding for this PhD came from a variety of sources: James Cook University (project support and a 'Doctoral Research Scheme' grant), the University of Western Australia, the University of Notre Dame and Apex Foundation for research into intellectual disabilities. Kirsty also received a travel scholarship (JCU and the School Veterinary and Biomedical Sciences) and a Woodside Neurotrauma PhD Excellence Award (UWA), which allowed attendance at two international conferences and laptop upgrades to continue thesis writing.

During this PhD Kirsty was supported by an Australian Postgraduate Award (3 years, 3 months) and received a three month extension from the School of Veterinary & Biomedical Sciences (JCU). Kirsty also received a James Cook University Doctoral Completion Scholarship.

BDNF was generously donated by Amgen Inc.

Dr Cheryl Johansen (UWA) donated the chicken serum.

Lotteries West provided the confocal microscope and Dr Paul Rigby (BIAF, UWA) helped with the confocal imaging.

The C and V Ramaciotti Foundation provided the Nikon E800 fluorescence microscope.

Laboratory and desk space was provided by the School of Medicine at James Cook University (2 years, 2 months).

Laboratory and desk space was also provided by the School of Anatomy and Human Biology at the University of Western Australia (2 years).

STATEMENT ON THE CONTRIBUTION OF OTHERS

I hereby declare that this thesis is my own and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given. Furthermore, I declare that I performed all of the work in this thesis, unless stated otherwise.

Stipend support:	Australian Postgraduate Award (3 years)
Supervision:	Whilst at JCU Supervisor: Dr Sherrard (Feb 02 – Apr 04) Co-supervisor: A/Prof Bower (Feb 02 – Dec 03) Associate Supervisor: Dr Walker (Feb 02 – Mar 03) Whilst at UWA Supervisor: Dr Sherrard (Apr 04 – Nov 06) Supervisor: Dr Hodgetts (Apr 06 – Dec 06) Co-supervisor: A/Prof Fitzpatrick (Apr 04 – Dec 06)
Other collaborations:	nil
Research assistance:	nil
Any other assistance:	Dr Rachel Sherrard performed approximately 75 % of the unilateral inferior cerebellar peduncle transections. For three animals in chapter five, Dr Rachel Sherrard performed the Px30 surgery, cerebellar injections and analysed the retrograde dye transport.
Project costs:	$15 000
Infrastructure external to JCU:	Laboratory and desk space provided by School of Anatomy & Human Biology (2 years) at the University of Western Australia. Animals housed at the University of Western Australia in the Biological Sciences Animal Unit (BSAU) and the Pre-Clinical Facility (PCF).
Infrastructure external to organisational unit within JCU:	Laboratory and desk space provided by School of Medicine (2 years 2 months) at James Cook University.

_____ _____

Signature Date

DECLARATION ON ETHICS

The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the National Statement on Ethics Conduct in Research Involving Human (1999), the Joint NHMRC/AVCC Statement and Guidelines on Research Practice (1997), the James Cook University and the University of Western Australia Statement and Guidelines on Research Practice (2001). The proposed research methodology received clearance from the James Cook University Experimentation Ethics Review Committee (approval number A749_02) and the University of Western Australia Animal Ethics Committee (approval number 04/100/359).

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Signature Date

CONTRIBUTION OF AUTHORS ON THE CO-AUTHORED PAPER

I hereby declare that the published co-authored paper, which forms the basis of chapter five, was written mostly by Dr Rachel Sherrard. For this thesis I have removed the abstract and modified the introduction, methods and conclusion sections, however the majority of the results and discussion sections remain unchanged. The figures for this paper, which remain almost unchanged for the thesis (except figure 5.5), were created by myself in consultation with Dr Sherrard.

Signature

Date

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This PhD has taken four years and ten months to complete, during which time I worked with some absolutely delightful people who made carrying out this research an enjoyable task.

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Lastly, I am indebted to Amgen Inc. for generously donating the BDNF; to Google ™ for helping to find almost anything in the world; and to some incredibly good reds from the Margaret River, Barossa and M^{c}Claren Vale regions for keeping me sane.

Bunker Bay, WA

PUBLICATIONS

Dixon K.J. and Sherrard R.M. Brain-derived neurotrophic factor induces post-lesion transcommissural growth of olivary axons that develop normal climbing fibres on mature Purkinje cells (2006) *Experimental Neurology* 202:44-56.

ABSTRACTS

Refereed

Dixon K.J. and Sherrard R.M. Brain-derived neurotrophic factor induces olivocerebellar plasticity in the mature cerebellum (2006) *Federation of European Neurosciences*

Dixon K.J. and Sherrard R.M. Brain-derived neurotrophic factor induces olivocerebellar plasticity in the mature cerebellum (2006) *Australian Neuroscience Society*

Dixon K.J. and Sherrard R.M. Brain-derived neurotrophic factor and exercise induce post-lesion transcommissural olivocerebellar reinnervation of mature Purkinje cells (2005) *Australian Neuroscience Society*

Non-refereed

Dixon K.J. and Sherrard R.M. BDNF delays inferior olivary cell death following axotomy (2005) *Student Expo, School of Anatomy & Human Biology, UWA*

Dixon K.J. and Sherrard R.M. BDNF induces olivocerebellar plasticity in mature rats (2004) *Student Expo, School of Anatomy & Human Biology, UWA*

INVITED SEMINAR PRESENTATION

Dixon K.J. Brain-derived neurotrophic factor induces functional olivocerebellar plasticity in the mature cerebellum (2005) *Symposium of Western Australian Neuroscience*

MEDIA RELEASE

Researcher's brainwave (15 July 2006) *Townville Bulletin*, bi-line Johnston J, p35

ABSTRACT

In the adult mammalian central nervous system, reinnervation and recovery from trauma is limited. During development however, post-lesion plasticity generates alternate paths providing models to investigate factors that promote reinnervation to appropriate targets. Following unilateral transection of the neonatal rat olivocerebellar pathway, axons from the remaining inferior olive reinnervate the denervated hemicerebellum and develop topographically organised normal climbing fibre arbors on Purkinje cells. However, the capacity to recreate this accurate target reinnervation in a mature system remains unknown. This thesis will identify whether one factor, Brain-derived neurotrophic factor (BDNF) is involved in reinnervation during the neonatal period and whether it induces similar reinnervation in the mature system. If BDNF does induce reinnervation in the mature system, this thesis will identify any return of lost function.

In rats lesioned on postnatal days 3 (P3), P15, P20 or P30 and treated with an intracerebellar injection of brain-derived neurotrophic factor (BDNF) or a BDNF blockade 24 hours following surgery, the morphology and organisation of transcommissural olivocerebellar reinnervation was examined using neuronal tracers and immunohistochemistry. The behavioural sequela of these rats was also investigated using vestibulo-spinal reflexes, simple locomotion, complex locomotion and gait synchronisation tests. Additionally, in neonatal rats (P3) with a unilateral lesion and treated with an intraolivary injection of BDNF, the survival of the axotomised inferior olivary complex and associated ipsilateral olivocerebellar pathway was examined using histochemical dyes and neuronal tracers.

In neonatal animals (P3), intracerebellar application of a BDNF blockade prevents olivocerebellar reinnervation of target Purkinje cells in the treatment area, while addition of BDNF in the mature system induces transcommissural olivocerebellar axonal growth into the denervated hemicerebellum. The distribution of BDNF-induced reinnervating climbing fibres was not confined to the injection sites, but extended throughout the denervated hemivermis and, less densely, up to 3.5 mm into the hemisphere. Transcommissural reinnervating axons were organised into parasagittal

microzones that were almost symmetrical to those in the right hemicerebellum. Reinnervating climbing fibre arbors were predominantly normal, but in the P30-lesioned group 10 % branched within the molecular layer forming a smaller secondary arbor, and in the P15-lesion group, the reinnervating arbors extended their terminals almost to the pial surface and were larger than control arbors ($p<0.02$). Behavioural testing revealed that BDNF and extensive exercise induce olivocerebellar reinnervation and that this reinnervation provides functional recovery, although this is delayed in the vehicle-treatment group. Additionally, the behavioural testing revealed that functional recovery is dependent on the age of the animal, whereby animals lesioned prior to acquiring task specific skills were developmentally disadvantaged. Lastly, during the neonatal period intraolivary BDNF transiently prevented degeneration of the axotomised inferior olivary complex, however it was unsuccessful in inducing transcommissural axonal growth of the ipsilateral olivocerebellar pathway into the denervated hemisphere.

For the first time, data from this PhD suggests that BDNF is involved in transcommissural reinnervation of denervated areas during the neonatal period and show that BDNF promotes topographically organised morphologically correct reinnervation in the mature rat cerebellum. Additionally, this reinnervation in the mature system provides functional recovery similar to sham-operated control animals. BDNF administered intraolivary however does not maintain the persistence of axotomised olivary neurons or induce transcommissural axonal growth of the ipsilateral olivocerebellar pathway. Data from this study can one day be used to contribute to a repair mechanism for traumatic brain injury, minimising long-term disabilities and on-going costs to society.

LIST OF ABBREVIATIONS USED

15B .....Pedunculotomy at postnatal day 15 and BDNF intracerebellar injection
15V .....Pedunculotomy at postnatal day 15 and vehicle intracerebellar injection
20B .....Pedunculotomy at postnatal day 20 and BDNF intracerebellar injection
20V .....Pedunculotomy at postnatal day 20 and vehicle intracerebellar injection
30B .....Pedunculotomy at postnatal day 30 and BDNF intracerebellar injection
30V .....Pedunculotomy at postnatal day 30 and vehicle intracerebellar injection
^{0}C ..... Degrees Celsius
> ..... Greater than
< .....Less than
µg ..... Microgram
µl .....Microlitre
µm ..... Micrometre
% ..... Percent
BDNF ..... Brain-derived neurotrophic factor
BSA .....Bovine serum albumin
Ca^{2+} .....Calcium
Cbr .....Cerebellar
climbing fibre .....Climbing Fibre
cm .....Centimetre
CNS .....Central nervous system
DAO .....Dorsal accessory olive
DCN ..... Deep cerebellar nuclei
DePeX ..... Di-n-butyl phthalate polystyrene xylene
E .....Embryonic day
EGL .....External germinal layer
GAP-43 .....Growth associated protein-43
GL .....Granular Layer
GTP ..... Guanosine triphosphate
IGL .....Internal granular layer
IOC .....Inferior olivary complex

IU ..... International units
Kg ..... Kilogram
M ..... Molar
MAO ..... Medial accessory olive
mg ..... Milligram
mm ..... Millimetre
MBS ..... Modified Bouins solution
ml ..... Millilitre
ML ..... Molecular layer
mRNA ..... Messenger RNA
NGF ..... Nerve growth factor
nM ..... nanoMolar
NMDA ..... N-methyl-D-aspartate
NT-3 ..... Neurotrophoin-3
NT-4/5 ..... Neurotrophin-4/5
NT-6 ..... Neurotrophin-6
NT-7 ..... Neurotrophin-7
P ..... Postnatal day
p75 ..... Pan neurotrophin receptor 75
PBS ..... Phosphate buffered saline
PC ..... Purkinje cell
pH ..... Hydrogen-ion concentration
PI-3 kinase Phosphatidylinositol-3-kinase
PO ..... Principal olive
P15 ..... Postnatal day 15
P20 ..... Postnatal day 20
P30 ..... Postnatal day 30
Px15 ..... Pedunculotomy at postnatal day 15
Px15B ..... Pedunculotomy at postnatal day 15 and BDNF intracerebellar injection
Px15V ..... Pedunculotomy at postnatal day 15 and vehicle intracerebellar injection
Px20 ..... Pedunculotomy at postnatal day 20
Px20B ..... Pedunculotomy at postnatal day 20 and BDNF intracerebellar injection

Px20V.....Pedunculotomy at postnatal day 20 and vehicle intracerebellar injection
Px30 .....Pedunculotomy at postnatal day 30
Px30B.....Pedunculotomy at postnatal day 30 and BDNF intracerebellar injection
Px30V.....Pedunculotomy at postnatal day 30 and vehicle intracerebellar injection
Rpm.....Revolutions per minute
s .....seconds
trkA .....Tropomyosin kinase receptor A
trkB.....Tropomyosin kinase receptor B
trkC.....Tropomyosin kinase receptor C
T-PBS.....TritonX-PBS
T-PBS-G.....TritonX-PBS with gelatin
UTL.....Upper time limit
VGLUT1 .....Vesicular glutamate transporter 1
VGLUT2 .....Vesicular glutamate transporter 2

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