

1-2015

The Expression Pattern of Death Associated Protein Kinase I in Normal Dorsal Root Ganglion Neurons and Following Peripheral Nerve Injury

Maryam Sulaiman Mohammed Al Saadi

Follow this and additional works at: https://scholarworks.uaeu.ac.ae/all_theses

Part of the [Biology Commons](#)

Recommended Citation

Mohammed Al Saadi, Maryam Sulaiman, "The Expression Pattern of Death Associated Protein Kinase I in Normal Dorsal Root Ganglion Neurons and Following Peripheral Nerve Injury" (2015). *Theses*. 251.
https://scholarworks.uaeu.ac.ae/all_theses/251

This Dissertation is brought to you for free and open access by the Electronic Theses and Dissertations at Scholarworks@UAEU. It has been accepted for inclusion in Theses by an authorized administrator of Scholarworks@UAEU. For more information, please contact fadl.musa@uaeu.ac.ae.



جامعة الإمارات العربية المتحدة
United Arab Emirates University

United Arab Emirates University

College of Science

THE EXPRESSION PATTERN OF DEATH ASSOCIATED PROTEIN
KINASE1 IN NORMAL DORSAL ROOT GANGLION NEURONS AND
FOLLOWING PERIPHERAL NERVE INJURY

Maryam Sulaiman Mohammed Al Saadi

This Dissertation is submitted in partial fulfilment of the requirements for the degree of
Doctor of Philosophy

Under the Supervision of Dr. Rasheed Al Hammadi

January 2015

Declaration of Original Work

I, Maryam Sulaiman Al Saadi, the undersigned, a graduate student at the United Arab Emirates University (UAEU) and the author of the dissertation entitled "*The Expression Pattern of Death Associated Protein Kinase 1 (DAPK1) in Normal Dorsal Root Ganglion Neurons and Following Peripheral Nerve Injury*" hereby, solemnly declare that this dissertation is an original research work done and prepared by me under the supervision of Dr. Rasheed Al Hammadi in the College of Science and Professor Safa Shehab in the College of Medicine at UAEU. This work has not been previously formed as the basis for the award of any academic degree diploma or similar title at this or any other university. The materials borrowed from other sources and included in my dissertation have been properly cited and acknowledged.

Student's Signature _____



Date _____

2 Feb 2015

Copyright © 2014 by Maryam S. Al Saadi
All Rights Reserved

APPROVAL OF DOCTORATE DISSERTATION

This Doctorate Dissertation is approved by the following Examining Committee Member^s:

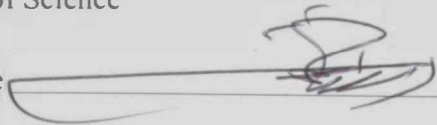
- 1) Advisor (Committee Chair): Rasheed Al Hammadi

Title: Assistant Professor

Department of Biology

College of Science

Signature



Date

12/1/2015

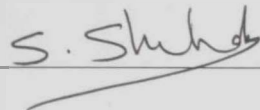
- 2) Member: Safa Shehab

Title: Professor

Department of Anatomy

College of Medicine and Health Sciences

Signature



Date

12.1.2015

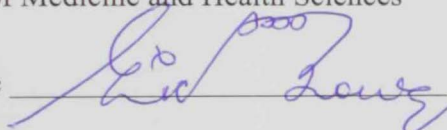
- 3) Member: Eric Brown

Title: Professor

Department of Anatomy

College of Medicine and Health Sciences

Signature



Date

12/1/15

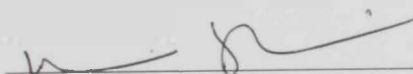
- 4) Member (External Examiner): Kazim A. Sheikh

Title: Professor

Department of Neurology

Institution: University of Texas-USA

Signature

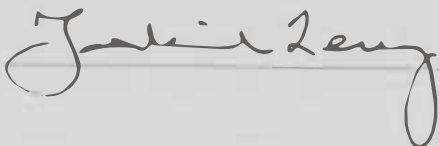


Date

12/1/15

This Doctorate Dissertation is accepted by:

- 1) Dean of the College of Science: Professor Frederick Chi-Ching Leung

Signature  Date 28-1-2015

- 2) Dean, College of the Graduate Studies: Professor Nagi T. Wakim

Signature  Date 2/2/2015

Copy 1 of 15

Abstract

Death-associated protein kinase1 (DAPK1) is a calcium/calmodulin (Ca²⁺/CaM) regulated serine/threonine kinase. An increasing body of evidence supports the significance of DAPK1 protein in cancer and CNS diseases. The role of DAPK1 in peripheral nerve regeneration and neuropathic pain remains completely unexplored. The aim of this study is to investigate the expression pattern of DAPK1 along with key pro- and anti-apoptotic cell signaling molecules (p53, Bax, Akt, ERK5, p38) and to verify the possibilities of DAPK1-NMDA NR2B relationship in dorsal root ganglion neurons (DRG) after 2 hours, 7 days and 14 days following a sciatic nerve injury. Gene expression analysis and immunohistochemistry were used to assess the effects of nerve injury. ATF3 was used as a neuronal injury marker. The results showed that DAPK1 mRNA was expressed and translated to functional protein in normal DRG neurons. Soon after a sciatic nerve injury (2 hours), DAPK1 was significantly up-regulated ($p < 0.05$, 2.2 fold) in the injured L4 and L5 DRG compared with the contralateral uninjured side. However, 7 days after axotomy a profound decrease was observed in the DAPK1 level, with a further reduction that reached its minimum level at 14 days postoperatively. In addition, 7 days after injury, most of the DAPK1 positive injured neurons (76.11%) were ATF3 positive, while after 14 days DAPK1 immunoreactivity significantly decreased (53.89%) in injured ATF3 positive neurons. Interestingly, DAPK1, p53 and Bax exhibited a similar expression pattern in axotomized lumbar DRG. The results also revealed that a sciatic nerve injury had no effects on the gene expression of ERK5, p38 and Akt at every time point. Moreover, NMDA NR2B mRNA expression increased after 7 days and continued to up-regulate significantly until 14 days postoperatively ($p < 0.05$, 3.6 fold). In contrast, our immunofluorescence results showed a decrease in the protein level in DRG neurons during this time period; but a strong positive NMDA NR2B immunoreactivity appeared in the satellite cells that surround the injured large-sized neurons in L4 and L5 DRG neurons. In addition, immunofluorescence double labelling revealed that DAPK1 and NMDA NR2B are co-localized in normal and injured DRG neurons. In conclusion, the down-regulations of DAPK1 following sciatic nerve injury,

along with other vital pro-apoptotic players promoting neuronal survival might shed light on the mechanisms of peripheral nerve regeneration. The results also suggest that NMDA NR2B might modulate neuropathic pain through satellite cells, and not through neurons, 7 and 14 days after peripheral nerve injury.

Keywords: Neurobiology; death associated protein kinase 1; peripheral nerve injury; dorsal root ganglion; pain; regeneration

Title and Abstract in Arabic

دراسة مستوى وجود البروتين كيناز (DAPK1) في الخلايا العصبية للعقد الجذرية الظهرية السليمة وبعد إصابة العصب الطرفي

ملخص البحث

يرتبط بروتين كيناز (DAPK1) عادة بالموت الخلوي ويملك وظيفة انزيمية الفسفرة (كيناز) لكل من السيرين والثريونين، خاضعة لتأثير الكالسيوم كالمودولين. تدعم دراسات عدة اليوم الدور الكبير الذي يلعبه هذا البروتين في مرض السرطان و أمراض الجهاز العصبي المركزي. إن دور هذا البروتين في الجهاز العصبي الطرفي و الألم الاعتلالي غير معروف على الاطلاق ولم يسبق دراسته بعد. نهدف في هذا البحث إلى استكشاف مختلف مسارات التعبير الجيني للمورثة (DAPK1) وعلاقتها بمختلف الجينات التي تعرف بتأثيرها في الموت الخلوي المبرمج والجينات المضادة لهذا الأخير (p53, Bax, Akt, ERK5, p38 and NMDA-NR2B). كما نهدف إلى التحقق من امكانية العلاقة بين DAPK1-NMDA NR2B في خلايا العقد الجذرية الظهرية بعد ساعتين، 7 أيام و 14 يوم من قطع عصب النسي. استخدمنا في هذه الدراسة البروتين المضاد إلي (ATF3) كعلامة لإصابة الخلايا العصبية. أجريت هذه الدراسة باستخدام تقنيتي تحليل التعبير الجيني (qRT PCR) و الكيمياء الهستولوجية المناعية (Immunohistochemistry) لتقييم آثار إصابة عصب النسي.

لقد أظهرت نتائج هذا البحث وجود نسخ RNA الرسول لمورثة DAPK1 و ترجمتها إلى بروتين في خلايا العقد الجذرية الظهرية الطبيعية السليمة. لقد تم ملاحظة ارتفاع معتبر في نسخ RNA الرسول لمورثة بأكثر من الضعف (أضعاف 2.2, $p < 0.05$) في الخلايا العصبية للعقد الجذرية الظهرية للعصبون الظهري الرابع و الخامس الموجودة جهة القطع بالمقارنة مع الجهة السليمة. غير أن بعد 7 أيام من قطع عصب النسي، شاهدنا إنخفاض معتبر في التعبير الورثي لمورثة (DAPK1) الذي بلغ أقصاه بعد 14 يوم من الإصابة.

كما جاءت نتائج تقنية الكيمياء الهستولوجية المناعية مشابهة لنتائج تحليل التعبير الجيني حيث أظهرت معظم الخلايا إيجابية (DAPK1) إيجابية أيضا في (ATF3) بعد 7 أيام من قطع عصب النسي (76.11%)، في حين قلت هذه النسبة إلي (53.89%) بعد 14 يوم من القطع. ومن المثير للاهتمام أنه قد جاء التعبير الجيني لكل من المورثتين p53 و Bax منخفضا بعد 7 أيام من الإصابة وهذا يتناسب طرديا مع انخفاض التعبير الورثي ل DAPK1 في الخلايا العصبية المصابة الموجودة في

العقد الجذرية الظهرية. في حين أظهرت النتائج أن إصابة عصب النسي لم تؤثر بشكل معتبر على التعبير الجيني من ERK5، p38 وAkt خلال مختلف الأزمنة المدروسة في هذا البحث. ومن جانب آخر ، علاوة على ارتفاع RNA الرسول للمورثة NMDA NR2B بعد 7 أيام بعد إصابة عصب النسي وملاحظة استمراره في الارتفاع حتى اليوم 14 (أضعاف 3.6, $p < 0.05$)، إلا أن نتائج الكيمياء الهيستولوجية المناعية أظهرت لأول مرة ما يوضح انخفاض مستوى بروتين المستقبل NMDA NR2B في الخلايا العصبية للعقد الجذرية الظهرية في الفترة الزمنية المدروسة وارتفاعه في الخلايا الساتلة المحيطة بالخلايا العصبية كبيرة الحجم المصابة في كل من العصبون الظهرى الرابع و الخامس في الخلايا العصبية للعقد الجذرية الظهرية. بالإضافة إلى ذلك، كشفت نتائج التآلق المناعي المزدوج التزامن في تواجد البروتينين DAPK1 والبروتين NMDA NR2B في الخلايا العصبية المصابة و السليمة للعقد الجذرية الظهرية.

في الختام، إن التراجع في مستوى DAPK1 كنتيجة لإصابة عصب النسي وتزامن هذا الانخفاض مع وسائط الموت الخلوي المبرمج لتسلط الضوء على آليات قد يكون لها تأثير في تجدد الأعصاب الطرفية. كما تأتي نتائج هذا البحث موضحة امكانية أن يلعب NMDA NR2B دور في تعديل الألم الإعتلالي من خلال وجوده في الخلايا الساتلة و ليس العصبية بعد 7 و 14 يوم من إصابة الجهاز العصبي الطرفي.

Acknowledgements

I thank Allah for giving me the ability to complete my doctorate study. I am extremely grateful to UAEU for granting me a scholarship to pursue my postgraduate study. I am truly indebted to my supervisor Dr. Rasheed Al Hammadi, College of Science, for his generous assistance and encouragement during the course of my Doctorate study and research. My sincere appreciation is due to my supervisor Professor Safa Shehab, Faculty of Medicine & Health Science, for giving me a care, patience, continuous support and guidance, as well as an opportunity to conduct my research work in his laboratory.

I would like to thank the committee for their guidance, support and assistance throughout the preparation of this dissertation. My special thanks are extended to Ahmed Taha, at the research desk in the UAEU Library for providing me with the relevant reference materials and reviewing the guidelines.

I would like to give my heartfelt thanks to Professor Regine Schneider-Stock and Dr Khuloud Bajbouj, who supported me and opened doors to primary research work. I am very thankful to Dr. Loay Lubbad, Mrs. Afaf Abdul Karim and Ms. Mehwish Anwer for technical assistance and support. Special thanks go to my parents, brothers, sisters and friends, for their continuous support, love and friendship.

My husband Sultan played a vital role in keeping me patient, optimistic and peaceful throughout the years of my study and he deserves appreciation. Big thank-you to my beloved daughter Alya.

Dedication

To my beloved parents, family and country.

Table of Contents

Declaration of Original Work	ii
Approval of the Doctorate Dissertation	iv
Abstract	vi
Title and Abstract in Arabic	viii
Acknowledgements	x
Table of Contents	xii
Lists of Tables	xiv
List of Figures	xv
List of Abbreviations	xvi
CHAPTER 1: INTRODUCTION.....	1
1.1 Overview	2
1.2 Statement of the Problem	4
1.3 Relevant Literature.....	5
1.3.1 Cellular and Molecular Changes after Peripheral Nerve Injury	5
1.3.2 DAPK1 Signal Transduction Pathways and Its Role in the Nervous System	35
1.3.3 The Role of NMDA Receptors in Neuronal Death and Neuropathic Pain	51
1.3.4 Satellite Cells and Neuropathic Pain.....	61
CHAPTER 2: MATERIALS AND METHOD.....	66
2.1 Experimental Procedures	66
2.2 Surgical Procedures and Sciatic Nerve Injury	66
2.3 Immunohistochemistry.....	68
2.4 Quantitative Real Time PCR.....	70
2.5 Morphological and Statistical Analysis	74
CHAPTER 3: RESULTS.....	76
3.1 DAPK1 Gene Expression in the Dorsal Root Ganglion	76
3.2 The Relationship between DAPK1 and Key Pro- and Anti-Apoptotic Genes Following Sciatic Nerve Injury	80
3.3 DAPK1 protein expressions in normal DRG.....	80
3.4 DAPK1 and ATF3 Protein Expressions in DRG	82
3.5 DAPK1–NMDA NR2B relationship in the DRG	87
3.6 NMDA NR2B in Satellite Cells of Large Injured Neurons	91
CHAPTER 4: DISCUSSION	95
4.1 DAPK1 Expression in Normal DRG	96
4.2 DAPK1 expression in injured PNS	97
4.3 Relationship of DAPK1 with Key Pro- and Anti-Apoptotic Genes in PNS Injury	100

4.4 DAPK1 and NMDA NR2B Relationship	101
4.5 DAPK 1-NMDA NR2B and Pain	103
4.6 The Relationship of the Expression of NMDA NR2B in Satellite Cells and Pain	105
CHAPTER 5: CONCLUSION.....	108
BIBLIOGRAPHY	112
A. Journal Articles	112
B. Book Chapters:.....	168
PUBLICATIONS	170

Lists of Tables

Table 1: List of primary antibodies used in this study.....	70
Table 2: References of genes used in this study	74
Table 3: Comparison between the number of DAPK1 +ve neurons in injured ATF3 +ve L5 DRG 7 days and 14 days after sciatic nerve transection.	83

List of Figures

Figure 1: Illustrating the characteristic features of six types of neurons in rat lumbar DRG	9
Figure 2: Anatomy of the somatosensory system, neuron cell bodies in the DRG.	11
Figure 3: p53-dependent cell death in neurons.	32
Figure 4: DAPK1 Multi-domain structure.	37
Figure 5: DAPK1 anti-apoptosis signals transduction pathways.....	41
Figure 6: DAPK1 apoptosis and autophagy signals transduction pathways.....	42
Figure 7: Proposed diagram illustrating the differences between blockade of NMDA receptor and NMDA NR2B-DAPK1 in stroke	60
Figure 8: A schematic diagram showing sciatic nerve injury as peripheral nerve injury model..	67
Figure 9: Design of gene expression analysis experiments	72
Figure 10: Real time qRT-PCR results of total DAPK1, NMDA NR2B, p53, and Bax mRNA levels following sciatic nerve injury	78
Figure 11: Real time qRT-PCR results of total Akt, p38, ERK5 mRNA levels following sciatic nerve injury	79
Figure 12: DAPK1, CGRP and IB4 triple immunostaining in normal intact L5 DRG neurons...81	
Figure 13: DAPK1 and ATF3 double immunostaining in uninjured and injured L4 DRG 2 hours, 7 days and 14 days after sciatic nerve transection.	84
Figure 14: DAPK1 and ATF3 double immunostaining in uninjured and injured L5 DRG 2 hours, 7 days and 14 days after sciatic nerve transection	85
Figure 15: DAPK1 immunoreactivity in uninjured and injured L4 DRG 2 hours and 7 and 14 days after sciatic nerve transection as revealed by peroxidase method of immunohistochemistry.	86
Figure 16: Statistical analysis of DAPK1 immunoreactivity in injured L5 DRG neurons 7 and 14 days after sciatic nerve transection	87
Figure 17: DAPK1 and NMDA NR2B double immunostaining in uninjured and injured L4 DRG 2 hours, 7 days and 14 days after sciatic nerve transection	89
Figure 18: DAPK1 and NMDA NR2B neuronal surface size of normal DRGs neurons.....	90
Figure 19: NMDA NR2B immunoreactivity 14 days after sciatic nerve injury in the ipsilateral L4 DRG compared with the control contralateral side revealed with peroxidase method of immunohistochemistry.....	92
Figure 20: Effects of sciatic nerve transection after 7 and 14 days on the expression of GFAP and NMDA NR2B in the injured L4 DRG	93
Figure 21: ATF3 and NMDA NR2B double immunostaining in uninjured and injured L4 DRG after 7 and 14 days following sciatic nerve transection	94
Figure 21: Diagrams illustrate DAPK1 and NMDA NR2B protein expression in DRG neurons following peripheral nerve injury	109
Figure 22: DAPK1 in CNS	110
Figure 23: DAPK1 suggested working model in PNS in this study	110

List of Abbreviations

Akt	Protein kinase B
AMPA	α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
ATF/CREB	Activating transcription factor / cyclic AMP response element binding protein
ATF3	Activating transcription factor 3
ATP	Adenosine-5'-triphosphate
BDNF	Brain-derived neurotrophic factor
cAMP	Cyclic 3', 5'-adenosine monophosphate
CAP-23	Cortical cytoskeleton-associated and calmodulin binding protein
CBI	Cannabanoid 1 receptor
C/EBPbeta	CCAAT enhancer binding protein-beta
cGMP	Guanosine 3',5'-monophosphate
CGRP	Calcitonin gene related peptide
CMAK2	Calcium/calmodulin-dependent kinase 2
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
CNTFRa	Ciliary neurotrophic factor receptor a
CREB	cAMP response element binding protein
CREM	cAMP-responsive element modulator
dbcAMP	Dibutyryl cyclic AMP
DAPK1	Death associated protein kinase 1
DREZ	Dorsal root entry zone

DRG	Dorsal root ganglion/dorsal root ganglia
ECM	Extracellular matrix
EGF	Epidermal growth factor
ERK	Extracellular regulated kinase
Erk5	Extracellular regulated kinase 5
ETA	Endothelin A receptor
ET1	Endothelin 1
FG	FluorGgold®
FGF-2	Fibroblast growth factor 2
FRAP	Fluoride resistant acid phosphatase
FGFR1-3	Fibroblast growth factor 2 receptors 1-3
gadd153/Chop10	Growth arrest and DNA damaging/ C/EBP-homologous protein10
GAL1, 2	Galanin receptor 1, 2
GAP-43	Growth-associated protein 43
GDNF	Glial cell line-derived neurotrophic factor
GFR α	Glial cell line-derived neurotrophic factor receptor alpha
GLUR1, and 5	Glutamate receptor1 or 5
gp130	Glycoprotein-130
HSPGs	Heparan sulfate proteoglycans
HSPs	Heat shock proteins
Hsp-27	Heat shock protein-27
IB4	Isolectin B4
IGF-1 and 2	Insulin-like growth factors 1 and 2

IGFR	In ^S ulin-like growth factor receptor
IHC	Immunohistochemistry
IL-1 β	Interleukin-1 beta
IL-6	Interleukin-6
JAK/STAT	Anu ^S kina ^{se} /signal transducer and activator of transcription
JNK	c-Jun-N-terminal kinase
JNK/SAPK	c-Jun-N-terminal kinase/stress-activated protein kinase
KOR	κ opioid receptor
LIF	leukemia inhibitory factor
LIF-R	leukemia inhibitory factor receptor
LL	large light neurons
LRF-1	liver regenerating factor-1
MAG	Myelin-associated glycoprotein
MAPK	Mitogen activated protein kinase
MCP-1	Monocyte chemotactic protein-1
MIP-1 α	Macrophage inflammatory protein-1 alpha
MOR	GDNF family coreceptor, μ opioid receptor
NF200	Neurofilament 200
NCAM	Neural cell adhesion molecule
NF-kB	Nuclear factor kappa light chain enhancer of activated B cells
NGF	Nerve growth factor
NgR	Nogo receptor
NK1	Neurokinin-1 receptor

NLS	Nuclear localization signal
NMDA	N-methyl-D-aspartate
NPY	Neuropeptide protein Y
NT-3	Neurotrophin-3
NT-4/5	Neurotrophin-4/5
OMgp	Oligodendrocyte-myelin glycoprotein
PACAP	Pituitary adenylate cyclase-activating polypeptide
PI3K	Phosphoinositide 3-kinase
PKC	Protein kinase C
PKC ϵ	Epsilon isoform of protein kinase C
PKA	Protein kinase A
PLC	Phospholipase C
PLD	Phospholipase D
PNS	Peripheral nervous system
PPT	Preprotachykinin
pSTAT3	Phosphorylated signal transducer and activator of transcription 3
P2X(3)	Purinergic receptor 2X(3)
P2Y1	Purinergic receptor 2Y1
p75NTR	p75 neurotrophin receptor
PSCs	Perisynaptic Schwann cells
Rapl	Ras-proximate-1
RGCs	Retinal ganglion cells
RNA	Ribonucleic acid

STATs	Signal transducers and activators of transcription
SD	Small dark
SSTR2a	Somatostatin receptor
SOX11	Sex determining region Y-box 11
SP	Substance P
Sp1	Specificity protein-1
STAT3	Signal transducer and activator of transcription 3
TGF- α	Transforming growth factor alpha
TGF β 1	Transforming growth factor-beta 1
TNF- α	Tumor necrosis factor alpha
TNFR	Tumor necrosis family receptor
tPA	Tissue plasminogen activator
trk (A, B, C)	Tropomyosin-related kinase
TRP	Transient receptor potential cation channels
TTX	Tetrodotoxin
VIP	Vasoactive intestinal polypeptide
WD	Wallerian degeneration

CHAPTER 1: INTRODUCTION

1.1 Overview

Death associated protein kinase1 (DAPK1) is a novel calcium/calmodulin ($\text{Ca}^{2+}/\text{CaM}$). It regulates the serine/threonine kinase that plays an important role in a wide variety of signal transduction pathways such as apoptosis, autophagy, and immune responses induced by various stimuli in different cell systems (Cohen et al., 1997, 1999; Deiss et al., 1995; Inbal et al., 1997; Jang et al., 2002; Pelled et al., 2002; Raveh et al., 2001; Yamamoto et al., 2002). These studies revealed that DAPK1 is a positive mediator of program cell death signals including interferon- α , tumor necrosis factor α ($\text{TNF}\alpha$), transforming growth factor ($\text{TGF}\beta$), Fas and detachment from the extracellular matrix (Cohen et al., 1997, 1999; Deiss et al., 1995; Inbal et al., 1997; Jang et al., 2002; Pelled et al., 2002; Raveh et al., 2001; Yamamoto et al., 2002), as well as oncogenes such as c-Myc and tumor protein p53, E2F transcription factor 1, and ceramide (Inbal et al., 2002; Lin et al., 2010; Schumacher et al., 2002).

Overexpression of the DAPK1 death domain has been reported to protect cells from death by $\text{TNF}\alpha$, $\text{TGF}\beta$ and Fas (Cohen et al., 1999; Jang et al., 2002). Furthermore, it has been well documented that DAPK1 participates in autophagosome formation, mitochondrial depolarization and permeabilisation, caspases activation, DNA fragmentation, cytoskeleton reorganization and membrane blebbing (Bialik and Kimchi, 2004).

In the central nervous system (CNS), several investigators have found that DAPK1 mRNA is particularly abundant in the adult brain (Bialik and Kimchi, 2004), with protein expression pattern limited to cortical areas, hippocampus, the olfactory bulb

and cerebellar Purkinje cells (Sakagami and Kondo, 1997; Tian et al., 2003). It is already established that DAPK1 is a key player in several modes of neuronal death injury and has been implicated in brain damage in stroke, epilepsy, Alzheimer's disease (AD) and neuronal death in cerebral ischemia (Fujita and Yamashita, 2014). In addition, Schumacher et al. (2002) demonstrated that DAPK1 may have a role in both neuronal developments as well as in recovery from injury, as an up-regulation of DAPK1 has been reported in the affected hippocampus 7 days after ischemic injury.

Furthermore, DAPK1 has been suggested to have other functional roles such as regulating exocytosis of neurotransmitter release by phosphorylation of syntaxin-1 (Tian et al., 2003) and protecting neurons during development or recovery from hypoxic-ischemic injury (Schumacher et al., 2002). The absence of DAPK1 has been shown to protect neurons from a wide variety of acute toxic insults (Fujita and Yamashita, 2014). DAPK1 has also been shown to function as a mediator of multi types of stress signals induced by deprivation of neuronal cells from Netrin-1, and stimulation of N-methyl-D-aspartate (NMDA) receptors in cerebral ischemia (Bialik and Kimchi, 2004; Tu et al., 2010). Tu et al. (2010) have found that DAPK1 physically and functionally interacts with the NMDA receptor NR2B subunit and this interaction leads to severe stroke neuronal damage.

NMDA NR2B receptor subunit constitutes a major subtype of glutamate receptor at extrasynaptic sites that evokes multiple intracellular catabolic processes responsible for neuronal death (Mathur et al., 2009; Tu et al., 2010). Knockdown of DAPK1 or blocking DAPK1- NMDA NR2B interaction protected mice against cerebral ischemic

damage and is considered to be a possible treatment for stroke (Tu et al., 2010). Along with its role in neuronal death, accumulating evidence implicates the importance of NMDA NR2B subunit-containing receptors in facilitating nociception (Boyce et al., 1999; Taniguchi et al., 1997) and suggested to be responsible for the development of chronic pain (Nagy et al., 2004). NR2B subunit has a relatively restricted distribution in nociceptive transmission and pain regulatory pathways such as in the forebrain (Laurie et al., 1997) and in the superficial dorsal horn of the spinal cord (Boyce et al., 1999; Nagy et al., 2004).

1.2 Statement of the Problem

All previous studies with regards to DAPK1 as a novel protein were carried out in the CNS. The presence of DAPK1 in the PNS and its role in peripheral nerve regeneration remains completely unexplored. Following an initial pilot study carried out in our lab at UAEU DAPK1 was detected in normal DRG neurons. It is, therefore, hypothesized that DAPK1 (i) along with other pro-apoptotic factors might be involved in the injury response mechanism leading up to successful peripheral nerve regeneration (ii) might have a significant contribution to intrinsic regulation of neuropathic pain after peripheral nerve injury through its relation with NMDA NR2B in DRG neurons.

1.3 Relevant Literature

1.3.1 Cellular and Molecular Changes after Peripheral Nerve Injury

Peripheral nervous system (PNS) injuries are common around the world. They affect more than one million people annually worldwide and account for incalculable health care costs. There are wide varieties of peripheral nerve injuries, which may result from neurological diseases, lacerations, simple nerve compression, crush, ischemia, traction and vibration. The outcome of a nerve injury may vary according to the etiology of the trauma. PNS injuries may lead to partial or total loss of motor, sensory and autonomic functions and have a marked impact on the quality of life of patients and economic relevance to society, with no easily available formula for successful treatment (Zochodne, 2008).

Research on treatment of nervous system injuries has been a long-standing goal of the neuroscience and medical community. Howell and Huber in 1893 carried the first controlled study of experimental injury and subsequent repair using dogs. This was followed by a similar study by Sherren in 1908 (Zachary and Holmes, 1946). Over the past century, numerous investigations have been carried out on peripheral nerve injury to reveal intracellular signaling processes and its regeneration response. However, a comprehensive understanding of the nature of the PNS injury response is yet to be attained. While neurons in the CNS have a limited capacity for regrowth after injury, neurons in the PNS can readily regenerate following injury. It has become clear that ability of the nerve to regenerate has a molecular basis (Makwana and Raivich, 2005).

Injury to the PNS initiates a cascade of degenerative cellular and molecular changes at the site of injury. Zochodne in his book, *Neurobiology of Peripheral Nerve Regeneration* in 2008, described the molecular details of how repair is achieved through degeneration and regeneration of severed axons followed by re-innervation of target tissues. After injury and degeneration, nerve fibers regrow from the proximal stump to the distal. Only if all these molecular steps are satisfactorily completed, will the injured nerve be able to regain their lost functionality. To achieve successful re-innervation, several key processes need to be precisely regulated and coordinated. It is worth knowing that the intrinsic growth capacity of an injured neuron is influenced by the external environment (Zochodne, 2008).

In this section, we will address the current neurobiology of the molecular mechanisms involved in both nerve degeneration and regeneration, which may help in achieving successful nerve regrowth and functional recovery. We will emphasize the current understanding of early molecular changes associated with nerve injury, degeneration nerve processes, non-neuronal cell roles, the subsequent activation of transcription factors, cytokines, neuropeptides, neurotrophic factors and other induced molecules that have been reported to play an important role after nerve injury.

1.3.1.1 PNS Anatomy

The nervous system comprises both the CNS and the PNS. The CNS originally develops from the neural tube, whereas the PNS develops from parts of the neural tube and neural crest cells during neurogenesis. Anatomically, the human and mammalian PNS consists of 12 cranial nerves and 31 pairs of spinal nerves linking the brain and the spinal cord to

the peripheral tissues. Ten out of 12 cranial nerves originate from the brain stem and mainly control the function of the anatomic structure of the head. The spinal nerves arise from the spinal cord and control the functions of the rest of the body (Hanani, 2005; Price, 1985, Lawson, 1992; Zochodne, 2008).

In the PNS, multiple axons are bound together by sheaths of connective tissue to form a nerve. Schwann cells enclose the axons either with a multi-layer myelin sheath, or with a simpler ensheathing arrangement. Less than half of the nerves are enclosed within myelin sheaths. The myelin sheath is discontinuous longitudinally by 1 micron (μm) length gaps called nodes of Ranvier at which the axon membrane conduct electrical potentials that are transmitted through the axon (Berta et al., 2008; Renganathan et al., 2001). Dendrites are numerous short processes around the soma that carry the action potential. Neurons can communicate with other neurons or muscle cells or glands across a junction between cells called a synapse.

PNS neurons can be classified, according to their shapes, into pseudounipolar, bipolar, or multipolar. In terms of function, PNS neurons are divided into a somatic and a visceral part. The somatic part consists of the nerves that innervate the skin, muscles, and joints. While the visceral part, also known as the autonomic nervous system, contains neurons that innervate the internal organs, glands and blood vessels. A typical peripheral nerve is composed of efferent motor axons originating from spinal motor neurons and afferent sensory axons originating from dorsal root ganglion (DRG) neurons.

The DRG conveys sensory information from the periphery to the CNS. DRG has a special location in the nervous system, as it is located in between the dorsal root and the spinal nerve. DRG neurons have two branches originating from a pseudounipolar neuron. They consist of a peripheral branch that innervates sensory targets and has the ability to regenerate when injured, and a central branch that extends into the spinal cord and relays information to the CNS but lacks the ability to regenerate following injury. Therefore, an injury to the peripheral nerve will affect both motor and sensory functions and induce retrograde reactions in both spinal cord and DRGs neuron. The PNS is not protected by bone or by the blood-brain barrier as the case in CNS, leaving it exposed to toxins and mechanical injuries (Hanani, 2005; Price, 1985; Lawson, 1992; Zochodne, 2008).

The basic subdivisions of DRG cells are large light (LL) A-cells and small dark (SD) B-cells intermixed within the ganglion (Duce and Keen, 1977; Kalina and Bubis, 1968; Kalina and Wolman, 1970; Lawson, 1979; Lawson et al., 1974; Novikoff et al., 1971; Spater et al., 1978) based on size of the perikarya or the morphology and distribution of Nissl bodies (Clark, 1926; Andres, 1961) or the Golgi apparatus (Duce and Keen, 1977). Rambourg et al. (1983) examined the lumbar dorsal root ganglia of rats by electron microscopy and identified three types of ganglion cells according to their size (A, B, C) from large to medium respectively. These were then subdivided further into six subtypes (A1, A2, A3, B1, B2 and C) based on the arrangement and three-dimensional organization of both Nissl bodies and Golgi apparatus in the perikarya (Figure 1).

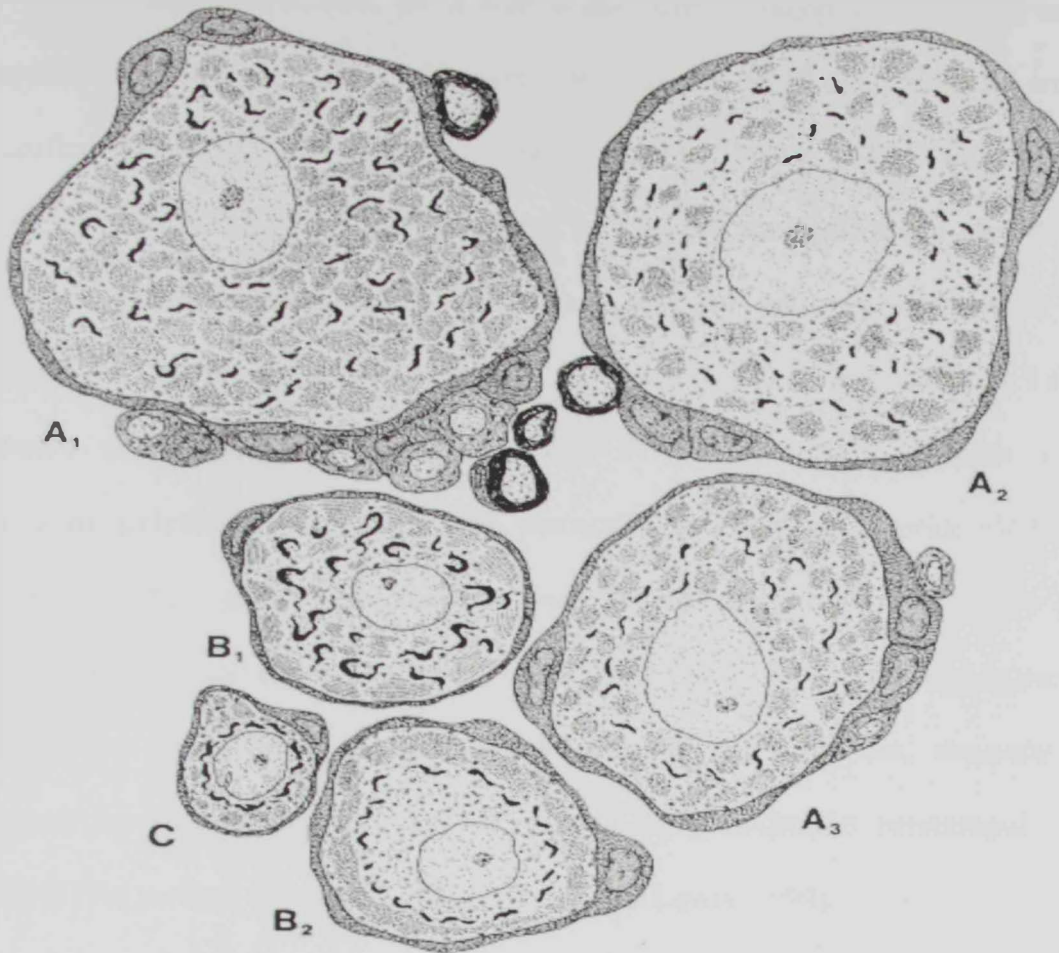


Figure 1: Illustrating the characteristic features of six types of neurons in rat lumbar DRG

A 1, A2 are large neuron with Nissl bodies evenly distributed throughout the cytoplasm and separated from each other by narrow spaces, wide strands of neuroplasm. A3 smaller than A1, A2 and displayed Nissl bodies widely dispersed throughout the perikaryon and a well-developed Golgi apparatus. B1, Small neuron with curved Golgi bodies arranged in a perinuclear ring and Nissl bodies located mainly in an outer cytoplasmic zone B2: Small neuron showing Golgi apparatus and Nissl bodies evenly distributed throughout the perikaryon, the Golgi apparatus forms a ring separated from the nucleus by mitochondria and smooth ER. C: smallest of all the ganglion cells with poor present of Nissl Bodies and a few flattened ER cisternae arranged in a parallel manner (Rambourg et al., 1983).

The large light neuron, the A type is also further classified as A α , A β , and A δ (myelinated, fast conducting). Moreover, the axons of small dark neurons are also classified as C fibers (non-myelinated, slow conducting) (Millan, 1999; Tandrup, 1993a) (Figure 2 a-c). Many of the small diameter neurons are nociceptive and they function mainly in thermo- and mechanoreception. They are either peptidergic or non-peptidergic. Peptidergic small dark neurons express peptides such as endothelin 1 (ET1), galanin, nociceptin, somatostatin, substance P (SP), pituitary adenylate cyclase activating polypeptide (PACAP), and vasoactive intestinal polypeptide (VIP). The majority of these peptidergic small neurons are identified to be isolectin B4 (IB4)-negative neurons and depend on nerve growth factor (NGF) for survival during postnatal development (Molliver et al., 1997; Hokfelt et al 1994) whereas, non-peptidergic neurons are IB4-positive neurons and depend on glial-derived neurotrophic factor (GDNF) for survival (Fang et al., 2005; Stucky and Lewin, 1999).

On the other hand, LL neurons serve as mechanoreceptors and proprioceptors (Lawson, 1992) and can be identified based on the expression of the heavy chain neurofilament, NF200. They also express trkA, trkC and p75 neurotrophin receptor (Devor et al., 1984; Kai-Kai, 1989; Kovalsky et al., 2009; Maher et al., 2009; Wickenden et al., 2009) (Figure 2 a-c). Currently, the DRG neuron has become the main focus to understand the mysteries that lead to successful neuronal regeneration and the control of neuropathic pain. Neuropathic pain can be defined as a chronic pain caused by an injury in the somatosensory system. After PNS injury, numerous changes have been detected in primary sensory neurons thought to be the main source for both regeneration and neuropathic pain.

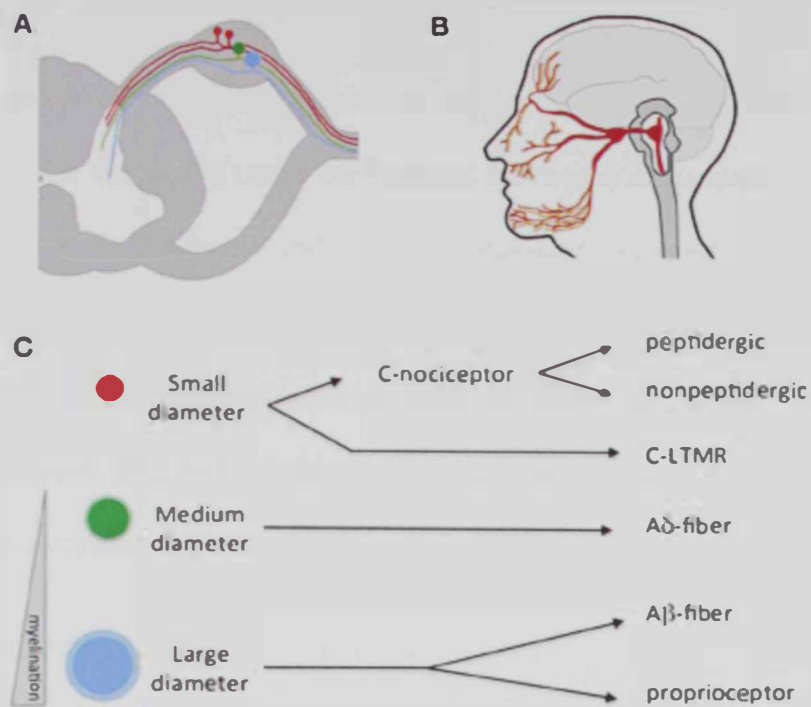


Figure 2: Anatomy of the somatosensory system, neuron cell bodies in the DRG.

(A) DRG location outside the spinal cord. (B) Trigeminal Somatosensory neurons with both central and peripheral targets. (C) Classification of Somatosensory neurons into small, medium and large diameter neurons based on their cell bodies size and myelination degree (Le Pichon et al., 2009).

1.3.1.2 Classification of Nerve Injuries

A peripheral nerve injury occurs when a nerve is compressed, crushed or severed. The severity of nerve injuries varies from minor, such as digital nerve injury to major, such as brachial plexus injury. The cellular changes after injury and their outcomes are determined by the amount of nerve damage and the degree of connective-tissue sheaths, the site of the lesion, the health and age of the patient (Hall, 2005). While nerve repair after injury in children is usually associated with full functional recovery, but that in adults and the elderly may experience slow or absent functional recovery. It was reported that more than half of the individuals over the age of 50 were not able to achieve any functional recovery after nerve repair following injury (Verdú et al., 2000).

Peripheral nerve injury is common and often causes long lasting disability, which may lead to severe consequences. It may cause pain, paralyze muscles or lead of adequate sensory feedback from nerve receptors in the target organs such as skin, joints and muscles (Lundborg and Rosén, 2007). It may lead to loss of touch perception, impaired or disturbed temperature perception and cold sensitivity.

Classification of nerve injury was described by Seddon in 1943 and by Sunderland in 1951 (Seddon, 1950; Sunderland, 1978). Seddon classified the severity of nerve injury into three major groups ranging from least to most severe: neuropraxia, axonotmesis and neurotmesis (Seddon, 1950). In the mild injury “neuropraxia”, the nerve remains intact but the signalling ability is damaged, where there is a physiological block within the axons. In “neuropraxia” there is no histological change in the axons or myelin sheath and full recovery is expected within a few weeks.

Axonotmesis is a moderate injury and it refers to the disruption of several axons and myelin sheath but without significant damage to the connective tissue layers (Seddon et al., 1943). It is an anatomical interruption of the axons with preservation of the perineurium and epineurium and it may be the result of a crush injury or neuroma formation. In this type of injury, recovery is expected within 4-6 weeks (Seddon, 1954). The most severe type of injury is neurotmesis, where axon, myelin sheath and the connective tissue elements are disturbed. Unfortunately, no functional recovery is possible without surgical intervention.

Sunderland classified the nerve injury in 1951 further into five different classes where first-degree injuries are equivalent to neuropraxia. 2nd, 3rd and 4th degree injuries correspond to axonotmesis and 5th degree injuries are equivalent to neurotmesis. Sunderland's classification requires histological analysis of the injured nerve (Birch, 2005; Sunderland, 1978).

Experimental sciatic nerve (SN) injury is a common approach that has been used to study the patterns of peripheral nerve regeneration. The three most popular methods are complete SN transection which is similar to neurotmesis, crush injury which is similar to axonotmesis and chronic constrictive injury (CCI) which is similar to neuropraxia (Chen et al., 2006).

1.3.1.3 Factors Influencing Neuronal Injury Response

CNS and PNS vary in their anatomy, physiology and regeneration ability after injury. Injured neurons in the mammalian PNS can regenerate and restore function but this requires several intrinsic axonal growth capabilities (Makwana and Raivich, 2005) and

extrinsic environmental support. In contrast to PNS damage, injuries to the CNS (brain and spinal cord) are almost always irreversible and would lead to permanent loss of function.

Injury to the peripheral nerve results in a sequence of molecular and cellular responses and a multitude of changes within an organism, including motor dysfunction, pain and associated cognitive and emotional comorbidities. When a peripheral nerve is axotomized, there is an immediate injury response in the area supplied by that nerve such as paralysis of the muscles and loss of sensation. PNS injury response could result in both neuronal survival and regeneration or neuronal cell death (Lundborg, 2000). Neuronal cell death might be an outcome of alterations in electrical activity, release of neurotoxic inflammatory products and loss of target derived neurotrophic support (Ambron and Walters, 1996; Fu and Gordon, 1997).

Adult peripheral neuron regeneration has been demonstrated in numerous studies both *in vivo* and *in vitro* (André et al., 2003; Jacob and McQuarrie, 1993; Lankford et al., 1998; Smith and Skene, 1997; Tanaka et al., 1992). On the other hand, many experimental studies have also shown the permanent loss of primary sensory neurons after injury (Schmalbruch, 1987; Ygge, 1989). It has been proven that proximal injuries produce more marked neuronal loss than distal injuries. Experimentally, cutting the nerve in the proximal thigh resulted in an average neuronal loss of 27% whereas cutting it at mid-thigh level resulted in a 16-23% loss (Arvidsson et al., 1986; Schmalbruch, 1987; Ygge, 1989) and cutting the proximal calf caused just a 7% drop in numbers (Ygge, 1989).

In the last two decades, observation of DNA fragmentation *in vivo* confirmed the above results (Groves et al., 1997; McKay Hart et al., 2002; Oliveira et al., 1997) and the extent of the loss ranges from 7 to 51% (Groves et al., 1997; Jivan et al., 2006; Liss et al., 1994, 1996; Ma et al., 2003; Rich et al., 1989; Tandrup, 1993b). Neuronal cell death rate depends on many factors such as proximity of axotomy to the DRG (Ygge, 1989), the age of the animal (Bahadori et al., 2001; Kerezoudi et al., 1995), axon diameter, length of the distal segment and the species used (Kline et al., 1964a, 1964b). All these factors have been shown to influence neuronal response, which may lead to neuronal death or high regenerative capacity.

Injury responses vary in different experimental model; for example loss of the axoplasmic cytoskeleton in small laboratory rodents begins one to two days after injury while it occurs within seven days in humans, these variations being approximately directly proportional to the size of the animal (Glass et al., 2002). Therefore, it is important to consider which animal model to use when analysing and integrating data as timing of injury response differs between species.

1.3.1.4 PNS Cellular Changes after Injury

PNS injury leads to a series of changes initiated both proximally and distally to the lesion site. Sensory neurons switch from a transmitting mode to a regrowth mode. Chromatolysis, where the cell body undergoes a sequence of changes characterized by swelling of the soma, nucleolar enlargement, displacement of the nucleus to the periphery and dissolution of Nissl bodies have been seen as a consequence of neuron injury (Lieberman, 1971; Kreutzberg, 1995).

Other changes including increases in cytoplasmic acid phosphatase and smooth endoplasmic reticulum with hypertrophy of Golgi apparatus. Neuron cell body swelling is due to tissue edema and exudation of acid mucopolysaccharides; it can be noted within one hour after injury and this soma enlargement may persist for 10 to 20 days after injury. Within 24 to 48 hours after injury, axon thickening is evident histologically (Brattgård and Thulin, 1965; Ducker et al., 1969).

Biochemical changes in the neurons of the DRG following injury are also associated with a strong increase in cellular metabolism and protein synthesis, changes in gene transcription, changes in translation, stability, or subcellular localization of translated proteins and post-translational modification of proteins (Bhave and Gereau, 2004; Caroni, 1998; Hall, 2001; Makwana and Raivich, 2005). It has been reported that local presynaptic processes may detach from the neuron soma and its dendritic tree. Interestingly, at the proximal stump axon sprouts can be noticed within hours of injury, and if the regenerative phenotype is successfully initiated, axon regrowth will start in days (Hall, 2005).

Generally, after nerve injury, the distal stump triggers major damage while the proximal stump is often only minimally damaged and can further regenerate towards the distal end. A distal growth cone can be seen that seeks out connections with the degenerated distal fiber. The proximal PNS injured nerve undergoes a sprouting process after injury (Bonilla et al., 2002) while the distal portion undergoes a stereotypical reaction, some simultaneous, others consecutive and are collectively called Wallerian degeneration (WD). Augustus Waller originally described WD in 1850 during his study

of the morphological consequence of cutting frogs' peripheral nerves. It is a degenerative cellular process initiated throughout the distal nerve portion and within a small reactive zone at the tip of the proximal stump of the axons after a traumatic injury (Koeppen, 2004; Pearce, 2000).

The type of nerve fiber affected (motor or sensory neurons, myelinated or unmyelinated fibers) and the type and severity of injury (from a slight crush to complete transection), leads to cascades of axonal degeneration events that differ in time and damage severity (Coleman, 2005) or demyelination (Nave and Trapp, 2008; De Vos et al., 2008). WD proceeds after complete nerve transection and leads to connective tissue sheath damage, while this damage is absent following nerve crush injuries (Rotshenker, 2011).

Axon degeneration is a final common pathway identified not only after a traumatic nerve injury, but also in many neurodegenerative diseases such as Parkinson's and Alzheimer's diseases and in demyelinating diseases like multiple sclerosis (Coleman, 2005; Coleman and Freeman, 2010). Intrinsic degenerative pathways that are triggered during WD are not correlated to cellular apoptosis (Finn et al., 2000). Wallerian Degeneration process begins within hours of injury with fragmentation of cytoskeleton proteins (microtubules and neurofilaments) and degradation of the axoplasm and axolemma in the distal nerve segment due to activation of ubiquitin-proteasome system (UPS) and calcium-dependent proteases, respectively (Vargas and Barres, 2007). Within hours following injury, the ends of the severed axons are sealed, followed by myelin and axonal break up to form ellipsoids.

The nerve injury interferes with the retrograde flow of signals from the normal innervation target. By 48 to 96 hours post injury, axonal continuity is lost and conduction of impulses no longer occurs. This may lead to the emergence of a negative, denervation signal, following the disconnection. This injury-induced discharge of axonal potentials, the interruption of target-derived factors and retrograde injury signals transported from the site of injury to the soma are believed to enable the neuron to respond to the trauma (Vargas and Barres, 2007; Finn et al., 2000).

WD in the PNS has always been associated with the induction of a strong inflammatory reaction which is highly regulated by immune cells (monocytes / macrophages, T and B lymphocytes, dendritic cells, neutrophils) and triggers a neuro-inflammatory response by resident non-neuronal cells where satellite cells (SCs) are believed to play an important role (Hawthorne and Popovich, 2011; Sanders and Jones, 2006).

1.3.1.4 .1 Satellite Cells

Satellite cells (SCs) are among the first to respond to nerve damage because of direct contact with the nerve. Satellite cells play an important role in nerve regeneration, as it has been shown that satellite cells proliferate, and increase in number around damaged DRG neurons (Hanani et al., 2002; Shinder et al., 1999). They also take part in phagocytosis of neuronal debris and up-regulate expression of a number of growth and neurotrophic factors (Pannese, 1978).

In addition, a significant increase in NGF and neurotrophin-3 (NT3) synthesis in satellite cells surrounding neurons in injured DRG were seen in animal models as early as 48 hours after nerve injury using *in situ* hybridization and immunohistochemical techniques (Zhou et al., 1999). More details about satellite cells role in nerve injury will appear in section 4 of this chapter.

1.3.1.4 .2 Schwann Cells

Myelinating and non-myelinating Schwann cells maintain axons and are vital for healthy neurons and neuronal survival after injury. Schwann cells form a multilayered lipid myelin sheath around large axons while small axons lack this feature as Schwann cells ensheath them and provides support without forming myelin. Following the breakdown of axons and myelin, Schwann cells in the distal stump undergo structural changes within 12 to 24 hours after the injury and profound disintegration 2 to 3 days after the injury.

Within 48 hours of injury, these Schwann cells stop producing myelin proteins by down-regulating steady-state mRNA levels for myelin components (LeBlanc and Poduslo, 1990), and up-regulating the regeneration-associated genes. These include GAP-43, neurotrophins NGF, BDNF and NT-4/5 trophic factors GDNF, LIF, pro-inflammatory cytokines TNF, IL-1 α , and IL-1 β , IL-6, neuregulin and its receptors, and growth factors such as fibroblast growth factor (FGF), interleukin-like growth factor (IGF), ciliary neurotrophic factor (CNTF) and vascular endothelial growth factor (VEGF) (Ide, 1996; Ishii et al., 1994; Mirsky and Jessen, 1999; Perrin et al., 2005; Shamash et al., 2002).

Schwann cells fragment their own myelin sheaths and have a role in the removal of the debris of WD by sequestration of small coils of myelin debris for phagocytosis. Phagocytosis of myelin debris is achieved partly through the action of the Schwann cells themselves, but also through haematogenous macrophages that infiltrate the injured area. Whole immune system plays a considerable role in nerve injury, the primary function is known to be mediated by resident cells (such as Schwann cells, resident macrophages and fibroblasts) because blood-borne monocytes infiltrate the injured nerve only 2 to 3 days after injury (Mueller et al., 2003). Together macrophages and Schwann cells begin phagocytosis of myelin debris and its clearance within 3 to 4 days after the injury, and the clearance is completed after 12 to 14 days. Myelin debris clearance is an important step to successful regeneration, because myelin associated proteins such as MAG, OMgp, Nogo are known to impede and inhibit successful regeneration (Chen et al., 2006; Schwab, 2004; Xie and Zheng, 2008).

Due to the loss of axonal contact, both myelinating and ensheathing Schwann cells divide, reaching peak proliferation around 4 days post-injury. The myelinating Schwann cells dedifferentiate into an immature phenotype, start proliferating longitudinally, and line up within each basal lamina tube to form long cellular chains known as the band of Büngner and degeneration of myelin by serving as a guiding structure for the regenerating axons (Frostick et al., 1998; Hall, 2001; Skene, 1984; Terenghi, 1999). It also produces molecules that help to provide a temporarily permissive microenvironment for axon regeneration (Hall, 2005). This microenvironment is usually maintained until proper target re-innervation is achieved successfully (Grafstein, 1975).

Approximately 2 to 20 days post injury, axoplasmic regeneration may start. This event is associated with the increased biosynthetic capabilities of the neuron at cell body. Extension of Schwann cells and connective tissue elements provides a pathway for migration of axoplasm filaments into the tubules of the distal stump. Synthesis of new axoplasm is mediated by migration along the remaining viable axon to the site of the nerve injury. Schwann cell migration and proliferation attempt to connect the proximal stump with the Schwann cell elements of the distal nerve stump.

The Schwann cells grow concurrently with the axoplasm and provide a framework for axonal growth (Assmus, 1978; Ducker et al., 1969; Swaim, 1972). Schwann cells develop protein complexes that serve as physical conduits to guide axons to their targets. The rate of axon regrowth is limited by the extension of these Schwann cell processes rather than by axonal growth (Son and Thompson, 1995). At this stage, Schwann cells have switched to a regenerative phenotype, converted their function from myelination of electrically active axons to growth support for regenerating nerve fibers (Fu and Gordon, 1997).

Denervated Schwann cells may secrete various molecules (Carey and Bunge, 1981; Skene and Shooter, 1983), some of which have been shown to be important for stimulating neurite growth *in vitro* (Richardson and Ebendal, 1982; Varon et al., 1983). They also actively secrete neurotrophic factors, and as they are proliferating, their numbers seem to be increased 4–17 times the original number seen in a normal nerve (Funakoshi et al., 1993; Mosahebi et al., 2001). Regeneration requires remyelination of the regenerated axons and the presence of myelin sheaths is evident 6 to 7 days after

regeneration has occurred in any zone (Ducker et al., 1969; Swaim, 1972). The two main endogenous modulators of Schwann cell remyelination are cell adhesion molecules (CAMs) and neurotrophic factors (Frostick et al., 1998; Lundborg, 2000; Lundborg and Rosén, 2007).

The molecular program that controls axon remyelination of denervated Schwann cells is similar to that for differentiation of immature Schwann cells in developing nerves, but the molecular mechanism that controls Schwann cell proliferation is different between regeneration and development. Nodes of Ranvier appear after day 14 in the regenerative axon. Apposition of myelin occurs for as long as one year following the repair process. The peripheral nerve's ability to regenerate might last approximately 12 months post injury (Peacock and VanWinkle, 1972).

1.3.1.4 .3 Macrophages

Mast cells accumulate in the endoneurium of injured nerves and secrete mediators that contribute to the recruitment of macrophages and neutrophils. Apolipoprotein-E and Galectin-3/MAC-2, which may help drive monocyte differentiation towards the M2 phenotype tissue macrophage are produced before and during monocyte recruitment (Moalem and Tracey, 2006; Zuo et al., 2003). The production of MCP-1 and MIP-1 α , which reaches a maximum at 1 day after injury, induces the recruitment of macrophages to the injured nerves (Perrin et al., 2005; Toews et al., 1998).

Blood-borne monocytes, spread over the entire nerve and start nerve infiltration from 2 to 3 days post injury. Both recruitment of monocytes and macrophage

accumulation peaks are observed approximately 7 days after injury (Bendszus and Stoll, 2003; Mueller et al., 2003; Perry et al., 1987; Taskinen and R oytt , 1997). This inflammatory response is turned off two to three weeks after injury and macrophages are rapidly eliminated (Mueller et al., 2003; Omura et al., 2005; Taskinen and R oytt , 1997).

1.3.1.4.4 PNS Versus CNS Cellular Injury Responses

Waller's description of WD was based on studies with transected peripheral nerves, but the main features of WD are observed after many types of injuries (crush, transection, chemical and/or toxic) in both the CNS and in the PNS, and are also present in the course of neurodegenerative and demyelinating diseases, suggesting a common triggering mechanism (Coleman and Perry, 2002). So far, we have seen the morphological changes in a nerve following peripheral injury, but a different scenario is observed in CNS WD. In contrast to PNS WD, CNS WD shows no signs of repair.

The CNS undergoes a phase of abortive sprouting around the injury site whereby the newly formed axons often appeared hypertrophied, irregularly branched and ending in clubs, points or rings as confirmed histologically by Cajal in 1928. Furthermore, morphological and metabolic changes of the neuronal cell body after injury (chromatolysis) and beneficial inflammatory mechanisms that occur following PNS injury are diminished or absent following an equivalent injury to the CNS (Lieberman, 1971).

The role that Schwann cells play in PNS injury is almost absent in oligodendrocytes in CNS although they also form myelin in the nerve fiber in the CNS. Oligodendrocytes undergo apoptosis and are not involved in myelin debris phagocytosis

and signalling. Therefore, CNS WD is significantly slower in the CNS and is incomplete (Vargas and Barres, 2007). Delayed macrophage recruitment, exposure of myelin inhibitory proteins, formation of an astroglial scar and secretion of inhibitory molecules such as chondroitin-sulphate proteoglycans all result in a hostile microenvironment for axon regrowth (George and Griffin, 1994) and establish irreversible loss of function of the target organs.

1.3.1.5 PNS Molecular Changes After Injury

Injury to peripheral neurons results in a series of molecular and cellular responses that are associated with, and that may play an important role in, the mounting of a successful nerve regenerative response, and the ensuing recovery of function. Nerve regeneration has been studied intensively worldwide via histological methods (Fitzgerald et al., 1967; Orgel et al., 1972; Waris, 1978; Diamond and Jackson, 1980). Most studies on regeneration have focused primarily on how to induce axon regrowth in the CNS. Although the molecular details of PNS regeneration are gradually growing more in the last decade, the current view is far from complete.

The most favorable neuronal population that serves as an ideal model to study the interaction between peripheral regenerative mechanisms and CNS growth are the dorsal root ganglia sensory neurons due to their unique anatomical conformation. Neurons in the CNS lack the ability to regenerate, due in part to an unfavorable environment for regrowth (Filbin, 2003; McGee and Strittmatter, 2003; Schwab and Bartholdi, 1996), but also because of the irreversible reduction in the intrinsic regenerative capacity of mature neurons (Goldberg et al., 2002).

On the other hand, injury to a peripheral nerve triggers the initiation of complex sequences of molecular responses that incorporate a sequence of biochemical and morphologic alterations. These include distal axon degeneration, host cell migration and proliferation, debris clearance, axon guidance and maturation, as well as re-innervation of target end organs. All of these events play an important role in the successful regenerative response and the eventual recovery of function.

There are some limitations to PNS regeneration. Although the injured nerve may undergo repair and regrowth but the diameter of regenerated axons, their conduction velocity and excitability remain below normal levels for a long time (Fields and Ellisman, 1986a, 1986b). As a consequence, recovery of re-innervated organs is incomplete and often motor and sensory functions are inadequate.

The “conditioning” nerve lesion studies of Woolf and colleagues (Chong et al., 1999; Neumann and Woolf, 1999) showed that the peripheral nerve injury, but not the central process, generates an enhanced axonal growth state. For decades, numerous studies have been carried out to unravel the essential elements of the PNS regeneration program. Protein purification, unbiased genetic methods, and assessment of candidate genes and proteins have all been attempted, resulting in the identification of large number of injury regulated genes and proteins. Axonal regeneration is thought to be induced by signals initiated from the injury, including calcium spikes and the retrograde transport and nuclear import of regeneration factors (Rossi et al., 2007). The first signs of axon regrowth in the distal segment may take place as early as 24 hours after injury, or may be delayed for weeks in more severe injury.

Successful functional axonal regeneration depends on both a permissive environment and neuronal intrinsic growth capacity. Until today, our knowledge about the different mechanisms that activate the intrinsic growth capacity of neurons after injury remains very limited. Each component of the PNS injured nerve reacts in an individual manner to the injury process. The sequence begins at the proximal segment of the cell body, proceeding to the distal segment, the injury site itself, and end up at the target organ.

Primary sensory neurons alter their phenotype both with regard to messengers, receptors and function after injury, so that DRG neurons adapt to the new situation by inhibiting excitatory transmitters, enhancing inhibition and promoting survival and regenerative mechanisms. The PNS injured neuron contributes rapidly to the arrival of injury-induced signals by the induction of transcription factors, adhesion molecules, growth-associated proteins and the structural components needed for axonal elongation. The functional goal of regeneration is to replace the distal nerve segment degenerated after injury, to re-innervate the target organs and to recover their function.

Gene microarray studies have identified a series of injury-related and regeneration-associated genes such as regulatory receptors and transcription factor genes whose expression is altered after injury (Costigan et al., 1998; Méchaly et al., 2006; Xiao et al., 2002). Here we will investigate the changes of transcription factors, cytokines, neurotrophic factors and neuropeptides after PNS injury.

1.3.1.5.1 Neuropeptides

Histochemical examination of lumbar DRG has characteristically revealed that 30% and 50% of neurons contain substance P (SP) and calcitonin gene related peptide (CGRP) respectively; and 96% of the SP-positive cells also showed CGRP immunoreactivity (Ju et al 1987a, 1987b). After PNS injury, a marked decrease in SP expression in the dorsal horn was reported primarily by Jessell et al. in 1979. Later, Shehab and Atkinson (1986) and McGregor et al. (1984) showed that sciatic nerve injury triggered down-regulation of SP, cholecystokinin, somatostatin and fluoride resistant acid phosphatase (FRAP) and up-regulation of vasoactive intestinal polypeptide (VIP) in the dorsal horn of the spinal cord. Importantly, Shehab and his colleagues were first to show VIP upregulation in injured neurons in the DRG whose axons have been injured following sciatic nerve transection (Shehab and Atkinson, 1986; Shehab et al., 1986).

Subsequently, CGRP down-regulation (Dumoulin et al., 1991; Noguchi et al., 1990; Zhang et al., 1995b) was also documented. Furthermore, other studies have also shown an up-regulation of neuropeptide Y (NPY) (Shehab et al., 2003, 2004; Wakisaka et al., 1991; Zhang et al., 1995b), galanin (Hökfelt et al., 1987; Villar et al., 1989) and neurokinin-1 receptor (NK1r) (Goff et al., 1998) following peripheral nerve injury.

1.3.1.5.2 Transcription Factors

Identification of early clusters of transcription factors involved in a transcription-dependent program that reactivates the neuron's intrinsic axonal growth capacity (Smith and Skene, 1997) is particularly important because early activated transcription factors

may control subsequent events in transcriptional pathways and is likely to be one of the first steps required to mount a cell-autonomous regenerative response.

Various genes are activated primarily to produce vast amounts of lipids and proteins for axonal outgrowth in DRG cell bodies (Robinson, 2000). Several transcription factors such as c-Jun (Broude et al., 1997; Jenkins and Hunt, 1991), cAMP responsive element binding protein (CREB) (White et al., 2000), STAT3 (O'Brien and Nathanson, 2007) and ATF3 (Tsujino et al., 2000) are up-regulated and activated.

Nerve injury triggers the transcription factor ATF3 as well as c-Jun activation (Herdegen et al., 1991; Jenkins and Hunt, 1991; Lindwall and Kanje, 2005; Makwana and Raivich, 2005; Pearson et al., 2003; Raivich et al., 2004; Seijffers et al., 2007; Tsujino et al., 2000). ATF3 is rapidly induced in all axotomized sensory DRG neurons, but also peripheral and not central axonal injury induces early ATF3 activation (Tsujino et al., 2000). Moreover, it is well characterized that up-regulation of c-Jun is consistently accompanied by a successful regeneration response in different injury models (Herdegen et al., 1991; Jenkins and Hunt, 1991; Lindwall and Kanje, 2005). Gap-43, p21/Waf1's expression is also up-regulated after peripheral nerve injury (Hoffman, 1989; Bonilla et al., 2002; Mason et al., 2002).

A growth cone is a motile structure formed at the tip of the growing axon during both neuronal development and axonal regrowth after nerve injury. Skene and colleagues have shown that GAP-43 which is considered as an important regulator of growth cone motility during nervous system development, is highly induced after peripheral axotomy and when overexpressed with CAP-23 *in vitro*, they activate an

intrinsic growth capacity in DRG neurons similar to that induced by a conditioning lesion (Bomze et al., 2001; Skene and Willard, 1981).

Many genes have been shown to peak in the distal stump 7 days after sciatic injury in mice (Araki et al., 2001). However, Akt and S6 phosphorylation were detected as early as 15 minutes after ligation (Abe et al., 2010). Several sciatic nerve studies have shown that several molecules such as the expression of neurotrophic factor (NGF, BDNF), development (SOX11), neuropeptides (NPY and GAL), cytokines (IL-6, CNTF), cell death (GADD45, annexin-1 (ANXA1), HSP27, cytochrome P450 1b11 (CYP1B1), calcium signalling (calcium channel alpha-2-delta subunit), cytoskeletal proteins (GAP43 and SPRR1A) and other secreted molecules are involved in cell-to-cell communication. All of these molecules may participate in the activation of neighboring non-neuronal cells around the cell body of the injured neuron and in the distal nerve fibers (Bomze et al., 2001; Skene and Willard, 1981). Many of these identified transcription factors and secreted molecules control the expression of neuropeptides, ion channel expression and their own expression.

1.3.1.5.3 Cytokines

Axonal injury activates several transcription factors through the local release of cytokines. These include, for example, the gp130 cytokines, leukemia inhibitory factor (LIF), interleukin-6 (IL-6) and ciliary neurotrophic factor (CNTF). Cytokines are classical injury-induced mediators that play significant roles in the regulation of immune responses, hemopoiesis and inflammation. Inflammation is required for successful axonal regeneration (Barrette et al., 2008; Boivin et al., 2007). Within hours after

neuronal injury, endoneurial levels of the early inflammatory cytokines, TNF- α and interleukin (IL)-1 α , secreted mostly by Schwann cells, start to increase in the distal nerve stump (Hall, 2005).

Almost all the cytokines of the IL-6 family (except CNTF) are strongly up regulated after PNS injury, and expression levels change in non-neuronal cells in the distal part, as well as in glial cells and even in neurons in DRGs and motor pools. Effective IL-10 levels are reached 7 days after injury. It is then; that IL-10 gradually down-regulates the production of cytokines reaching the lowest level about 2 to 3 weeks after injury, thereafter, degenerated myelin is cleared (Barrette et al., 2008; Boivin et al., 2007; Hall, 2005).

1.3.1.5.4 Neurotrophic Factors

It is well known that neurotrophic factors modulate the development, maintenance and function of the vertebrate nervous system. They also enhance functional regeneration, by supporting axonal growth after injury. Expression of neurotrophic factor changes within both the proximal and distal portions. Neurotrophins such as NGF and BDNF are up-regulated after peripheral nerve injury, but their role in peripheral axon regeneration is not clear.

Neurotrophin 4/5 mRNA, as well as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF), are up-regulated in the distal stump but their roles in peripheral axon regeneration are still not fully understood (Makwana and Raivich, 2005; Markus et al., 2002; Snider et al., 2002). Some of these neurotrophins such as NGF, which is secreted by SC, is increased early

after nerve injury but at 3 weeks its level decreases. Once remyelination is complete, the expression of neurotrophins and their receptors returns to normal (Funakoshi et al., 1993; Taniuchi et al., 1986).

1.3.1.6 Active Molecular Pathways after PNS Injury

It is now well known that injured axons initiate local activation and retrograde transport of several mitogen-activated protein kinases MAPKs family, including Erk1/Erk2 (Hanz et al., 2003; Perlson et al., 2005), the c-Jun N terminal kinase (JNK) (Cavalli et al., 2005) and the protein kinase G (Sung et al., 2006).

Both *in vitro* and *in vivo* studies helped to identify molecular pathways that follow nerve damage. Several signalling pathways have been intensively studied and considered to be important for promoting axonal nerve regrowth or neuronal cell death. Studies *in vitro* have demonstrated that activation of PKA, Ras/PI-3K/Akt (PI-3K/Akt), or Ras/Raf/ MAPK/ERK (MAPK/ERK) signalling pathways by neurotrophins and GDNF could promote PNS neuronal survival and enhance neurite outgrowth (Chierzi et al., 2005; Hetman et al., 1999; Liot et al., 2004; Soler et al., 1999).

1.3.1.6.1 p52

The discovery of p53 dates back to when it was identified as a target protein of SV40 large T antigen (Lane and Crawford, 1979; Linzer and Levine, 1979). Surprisingly, in the same year its high level was noted in different cancer cells by many research groups (Jenkins et al., 1984; Kress et al., 1979; Lane and Crawford, 1979; Linzer and Levine, 1979). p53 is a transcription factor famous for its role in immortalized cells (May and

May, 1999) and it plays a very important role in the cell development from the progression of the cell cycle to differentiation (Lohrum and Vousden, 1999).

p53 also plays a pivotal role in the determination of the cell fate through DNA repair and genome stability (May and May, 1999; Rotter et al., 1980; Vogelstein et al., 2000; Vousden and Lu, 2002). In addition p53 is involved in the cellular response to stress in developing and homeostatic tissues (Mendrysa et al., 2011). A wide variety of studies have established that the main function of p53 is the pro-apoptotic activity through its ability to direct the cell towards programmed cell death by the activation of the key factors of apoptosis (Brodsky et al., 2004; Liu et al., 2004; Schmitt et al., 2002; Schumacher et al., 2001; Symonds et al., 1994) as shown in (Figure 3).

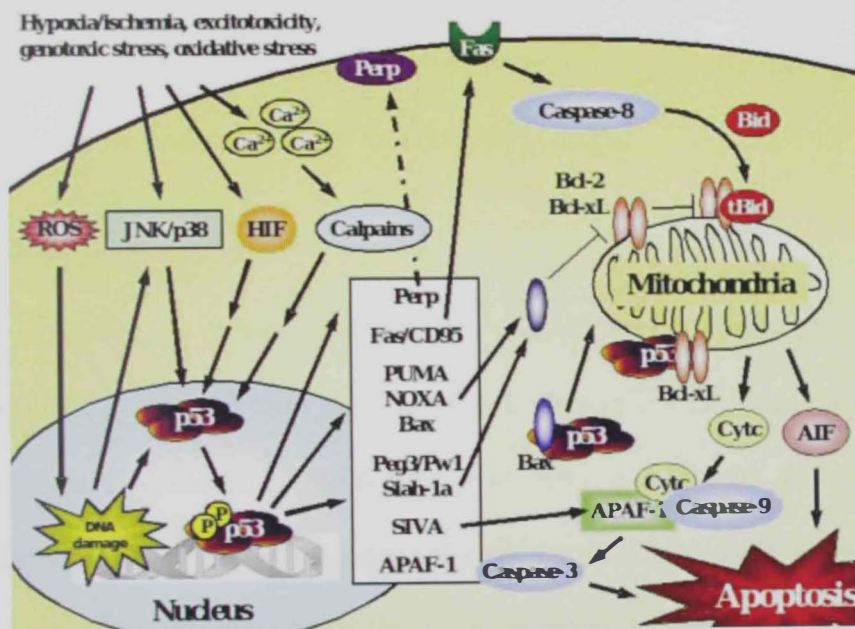


Figure 3: p53-dependent cell death in neurons.

Accumulation and transcriptional activation of p53 occurs rapidly in response to a wide variety of insults including DNA damage, oxidative stress, metabolic compromise or excitotoxicity (Culmsee et al., 2005).

It has been suggested that, in order to execute its apoptotic function, p53 operates via both intrinsic and the extrinsic pathways of apoptosis (Yu and Zhang, 2005). It should be noted that, through regulating extrinsic apoptotic pathway, p53 plays a major role in inducing apoptosis by chemotherapeutic agents (Liu et al., 2004; Sheikh et al., 1998) and the main target of p53 in this pathway are the cell membrane proteins such as Fas, DR4, and DR5 (Bouvard et al., 2000; Müller et al., 1998).

Even though, p53 mainly operates via the intrinsic apoptotic pathway in response to a variety of cellular stressors (Willis and Adams, 2005). The cornerstone of this pathway is the mitochondria due to the presence of the proapoptotic Bcl-2 family member on its membrane (Symonds et al., 1994). The role of p53 in this pathway depends on its ability to activate the key pro-apoptotic factor Bax which in turn infiltrates the mitochondria. This liberates apoptogenic factors, including cytochrome *c* and DIABLO (also known as Smac), which activates a group of aspartate-specific proteases known as caspases (Youle and Strasser, 2008).

1.3.1.6.2 Bax

Bax is a pro-apoptotic protein that belongs to Bcl2 family found sequestered in the cytoplasm in its tetrameric form upon receiving a stress stimuli that ultimately leads to its migration to the mitochondria (Wolter et al., 1997), it triggers a cascade activation leading the cell to undergo programmed cell death (Gavathiotis et al., 2008). Bax molecules were found attached to the outer membrane of the mitochondria and the endoplasmic reticulum (Griffiths et al., 1999; Wolter et al., 1997).

Based on the upstream signal that leads to the activation of Bax, two models namely a direct and indirect model have been distinguished. The direct model results from direct attachment of Bax to apoptotic messengers such as Bid, Bim, and Puma, while the indirect model is produced after Bax displacement from pro-survival proteins (Chipuk and Green, 2008; Dewson and Kluck, 2009; Leber et al., 2010).

It is believed that the activation of BCL2-associated X protein (Bax) has no return point in the cell destiny that ends up in death. Such activation requires three indispensable steps which start with conformation change, followed by mitochondrial translocation and finally oligomerisation that inevitably causes mitochondrial dysfunction and apoptosis (Annis et al., 2005; Goping et al., 1998; Gross et al., 1998).

Briefly, once activated Bax and another pro-apoptotic protein Bak, translocate to the mitochondria and form pore like complexes in the mitochondrial outer membrane lipid bilayer that serve eventually as channels for apoptotic effectors such as cytochrome *c* to migrate towards the cytoplasm. Once cytochrome *c* reaches the cytoplasm, it binds to Apaf-1 and leads to the activation of a series of specific apoptotic factors such as caspase-9, caspase-3, caspase-6 and caspase-7 leading inevitably to cell death (Dewson and Kluck, 2009, 2009).

The pro-apoptotic molecule Bax plays a major role in cell death and axonal regeneration. Libby et al. (2005) showed partial or complete deletion of the Bax gene in mice protected from glaucoma. In addition, the deletion of the Bax gene did not prevent the cell death from NMDA-induced excitotoxicity in retinal ganglia. However, it did prevent cell death after optic nerve crush. Multiple studies have confirmed that the

deletion of such a gene significantly reduce cell death induced following axotomy in neonatal mice (Deckwerth et al., 1996; Kinugasa et al., 2002; White et al., 1998).

In this section, we have summarized the events known to occur after an injury in the peripheral nervous system. Research to understand the mysteries behind the neurobiology of peripheral nerve regeneration is still ongoing. Many cell death-related proteins have been intensively studied in the CNS but their functional roles in PNS have not yet been explored. One of these proteins is the Death associated protein kinase 1 (DAPK1). DAPK1 signal transduction pathways and its role in the nervous system will be addressed in details in the next section.

1.3.2 DAPK1 Signal Transduction Pathways and Its Role in the Nervous System

Death associated protein kinase 1 (DAPK1), a calcium/calmodulin ($\text{Ca}^{2+}/\text{CaM}$) regulated serine/threonine kinase, was originally identified by Deiss et al. (1995) in a genetic screen in which an antisense library was used to detect genes necessary for interferon ($\text{IFN-}\gamma$) -induced death in HeLa cells (Bialik et al., 2004; Tu et al., 2010). DAPK1 positively mediates cell death in a wide array of cell systems (Cohen et al., 1997, 1999; Deiss et al., 1995; Inbal et al., 1997; Jang et al., 2002; Pelled et al., 2002; Raveh et al., 2001; Yamamoto et al., 2002; Lin et al., 2010).

However, several studies have shown that the activity of DAPK1 is not only restricted to cell death as pro-survival functions of the enzyme have also been documented showing conflicting activities by DAPK1. This paved the way for further investigations into the role of this protein (Inbal et al., 1997; Zalckvar et al., 2009). The DAPK1 gene is well conserved through evolution in different invertebrates such as C.

C. elegans and chordates and mammals (Tong et al., 2009). This actin microfilament-associated Ca^{2+} /(CaM)-dependent kinase has a unique multidomain structure, including modules that mediate protein–protein interactions such as ankyrin repeats and the death domain (Cohen et al., 1997).

DAPK1 is abundant in the brain (Bialik et al., 2004) and has been linked to neuronal injury associated diseases. Its expression needs to be elucidated as it could serve as a target for therapeutic intervention in the treatment of neurodegeneration. This section will cover a short overview of DAPK1 cellular signal transduction and its role in the nervous system.

1.3.2.1 DAPK1 Structure and Family Members

DAPK1 was the first member of a family of serine/threonine kinases described, that includes at least four other kinases that share a significant homology in the catalytic domain with DAPK1 (Inbal et al., 2000; Kawai et al., 1999). ZIP(Dlk)-kinase, a serine/threonine kinase with a C-terminal leucine zipper domain, and DAPK1-related protein-1 (DRP-1) are both closely related to DAPK1, and their catalytic domains show identity with that of DAPK1 (Inbal et al., 2000; Kögel et al., 1998).

Figure 4 illustrates the multi-domain structure of DAPK1. A close examination of DAPK1 structure shows very little difference from other kinases. DAPK1 kinase domain has a classical 12 subdomain composition typical of all serine/ threonine kinases, followed by a region that shares high homology with the calmodulin regulatory domains of other kinases. DAPK1 has two P-loop motif structures with beta-sheets in the N-terminal and alpha helices in the C-terminal. Adjacent to the latter, a catalytic

domain for ATP binding and eight ankyrin repeats have been identified (Deiss et al., 1995; Mosavi et al., 2004; Tereshko et al., 2001; Velentza et al., 2001).

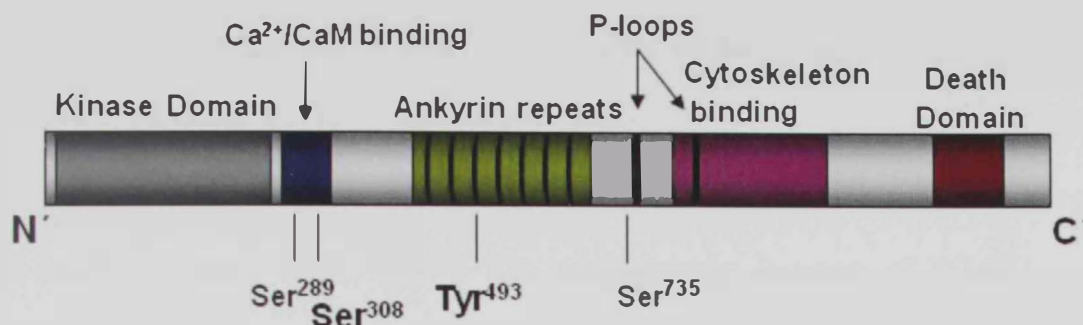


Figure 4: DAPK1 Multi-domain structure.

The ankyrin repeat is a 33aa motif that mediates protein-protein interactions (Mosavi et al., 2004). The P-loop is a nucleotide ATP/GTP binding motif found in many proteins (Saraste et al., 1990). Inbal et al., (2000) and Tereshko et al., (2001) have reported that the crystal structure of the DAPK1 catalytic domain at high resolution reveals an unusual highly basic structured loop in the N-terminal domain. The location of the loop in the DAPK1 catalytic structure near the peptide recognition region raises the possibility that this could be a site of interaction with a regulatory protein or a site of modification in DAPK1 that would regulate its kinase activity. The cytoskeletal binding domain mediates the interaction between proteins and the cytoskeleton (Hoeflich et al., 2004).

The death domain of DAPK1 is a conserved stretch of around 80aa which is commonly found in death receptor proteins, and acts as a protein interaction domain and a platform for the death-inducing signalling complex (DISC) formation (Chaigne-Delalande et al., 2008). The serine rich C-terminal tail of DAPK1 plays a negative feedback role to the putative function of the death domain (Feinstein et al., 1995).

The kinase domain of DAPK1 is required for its cell killing activity (Cohen et al., 1997). However, the death association domain is important for fulfilling the function. The cytoskeleton and ankyrin repeats are thought to be involved in subcellular targeting of DAPK1 (Cohen et al., 1999). The multi-domain structure of DAPK1 and its participation in various apoptotic systems imply that the protein may interact with a wide range of intracellular components to exert its action.

1.3.2.2 DAPK1 Induced Pathways

There has been a debate for almost twenty years whether DAPK1 is a pro- or anti-apoptotic kinase. Several studies have shown the involvement of DAPK1 in regulation or execution of cell death in response to various stimuli such as death receptor activation, cytokines, matrix detachment, ceramide3 and others (Cohen et al., 1997, 1999; Deiss et al., 1995; Inbal et al., 1997; Jang et al., 2002; Pelled et al., 2002; Raveh et al., 2001; Yamamoto et al., 2002; Bajbouj et al., 2009).

DAPK1 is linked to both type I apoptotic and type II autophagic cell death in both caspase-dependent and caspase-independent manners (Cohen et al., 1997). In addition, genetic engineering techniques to overexpress wild type or mutant forms of DAPK1 in mouse demonstrated the pro-apoptotic function of DAPK1 (Cohen et al.,

1997; Deiss et al., 1995; Shohat et al., 2001). However, many other studies showed that the over-expression of DAPK1 does not induce a caspase-dependent apoptosis under normal growth conditions (Inbal et al., 1997; Jin et al., 2001). It has been suggested that, depending on the particular cell type, the response to specific apoptosis inducers can vary and DAPK1 can either promote (Chen et al., 2005; Cohen et al., 1999; Pelled et al., 2002) or antagonize apoptosis (Jin et al., 2001, 2002).

1.3.2.2.1 Anti-Apoptosis

Schumacher et al. (2002) were the first research group to discover the new pro-survival activity of DAPK1 in cells using two very established models of cell death. They found a significant increase in DAPK1 activity in the ischemic rat hippocampus during the recovery phase. They also showed an increase in the expression of DAPK1 mRNA and protein level in nerve growth factor (NGF)-treated PC12 cells, which raised the possibility of DAPK1 as a player in cell survival and regeneration (Schumacher et al., 2002).

Northern blot and *in situ* hybridization analyses have showing high expression of DAPK1 in the lungs and brain. It is important to note that such high expression was found in neurogenesis specific areas of the brain such as post mitotic regions within cerebral cortex, hippocampus, and cerebellar Purkinje cells (Schumacher et al., 2002; Yamamoto et al., 1999). These other studies by Inbal et al., (1997) and Jin et al., (2002) have documented that DAPK1 does not induce apoptosis under normal growth conditions, DAPK1 has also been speculated to exert a cyto-protective role in cellular homeostasis (Jin and Gallagher, 2003).

Activation of the Ras-MAPK pathway negatively regulates the apoptotic function of DAPK1 as the phosphorylation of DAPK1 upon the activation of RAS-ERK pathway has been correlated with the suppression of the apoptotic activity of DAPK1 (Ballif and Blenis, 2001) (Figures 5 and Figure 6). Moreover, P90 ribosomal S6 kinase (RSK) 1 and 2 which are downstream effectors of ERK 1/2 have recently been demonstrated to be novel targets of DAPK1 (Roux and Blenis, 2004; Shimamura et al., 2000) and Ser-289 has been identified (using mass spectrometry) as a novel phosphorylation site in DAPK1, which is regulated by RSK (Anjum et al., 2005) (Figure 5 and Figure 6).

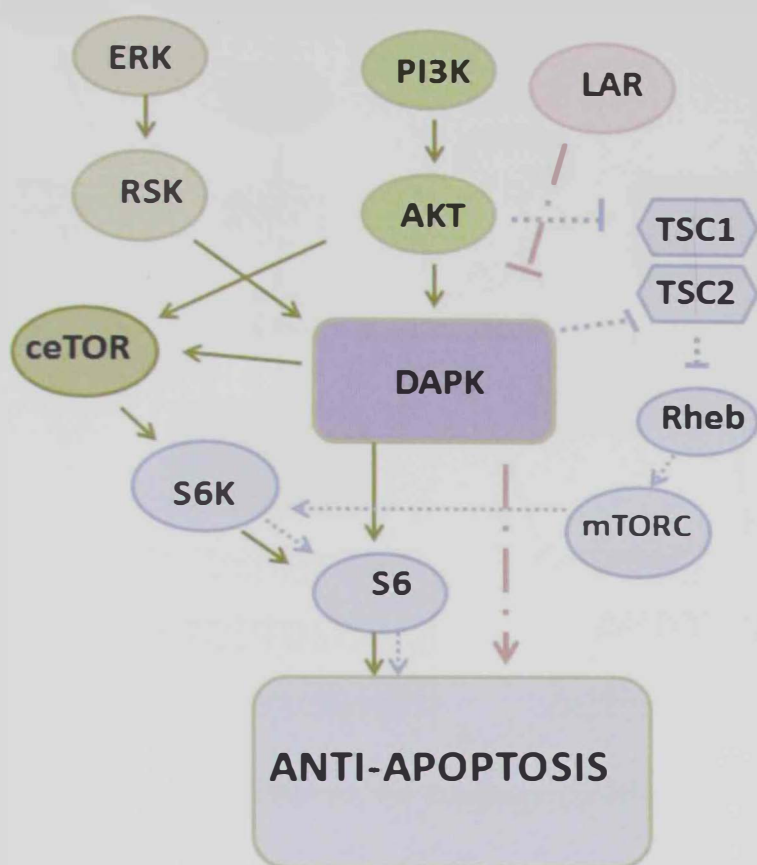


Figure 5: DAPK1 anti-apoptosis signals transduction pathways

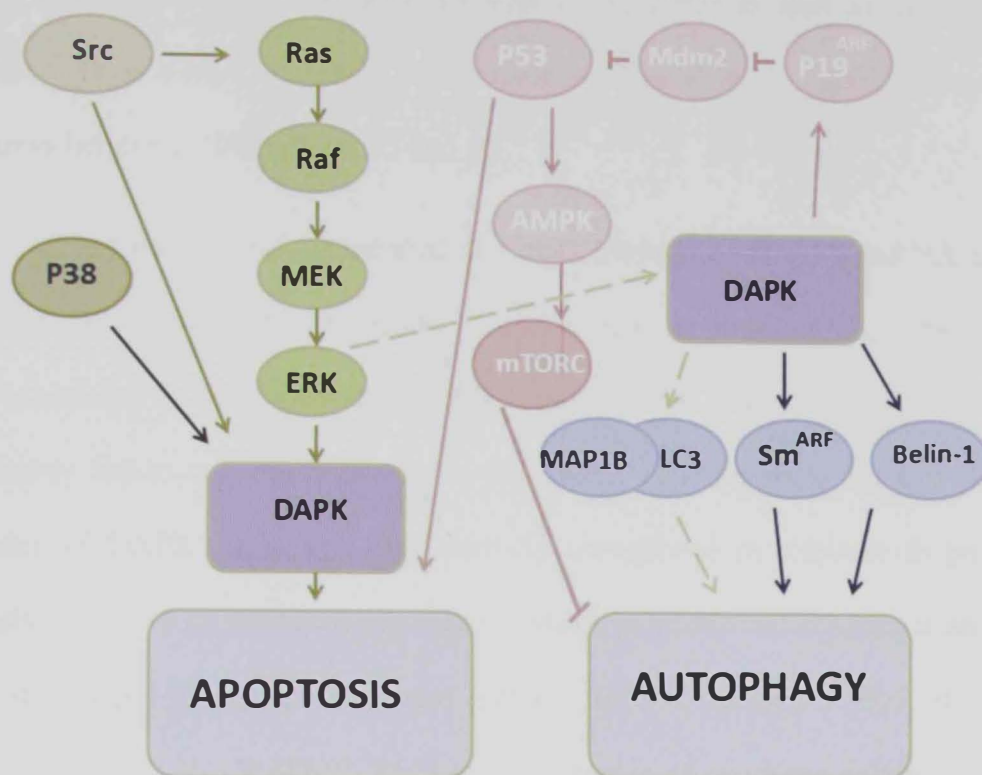


Figure 6: DAPK1 apoptosis and autophagy signals transduction pathways

1.3.2.2.2 Apoptosis

It has been proven that DAPK1 is involved in multiple cell death signalling in response to IFN- γ , TNF- α , Fas, and TGF- β (Cohen et al., 1997, 1999; Deiss et al., 1995; Inbal et al., 1997; Jang et al., 2002; Pelled et al., 2002; Raveh et al., 2001; Yamamoto et al., 2002; Bajbouj et al., 2009). The death-promoting effects of DAPK1 depend on multiple factors like catalytic activity, the correct intracellular localization and the presence of the death domain (Cohen et al., 1997, 1999). It is worthy noting that the death domain of

DAPK1 has the ability to interact with a group of intracellular cell death mediators such as Src (Chen et al., 2005), PDCD6 (Lee et al., 2005), FADD (Henshall et al., 2003), TNFR1 (Cohen et al., 1999), RSK (Anjum et al., 2005) as well as many oncogenic factors such as c-Myc, p53, E2F and ceramide 3 (Inbal et al., 2002; Lin et al., 2010; Schumacher et al., 2002) (Figure 5 and 6).

It has been also demonstrated that the inhibition of DAPK1 mRNA translation reduced IFN- γ induced cell death in HeLa cells (Cohen et al., 1997). DAPK1 overexpression in the neonatal fibroblast induced an ample activation of p53 and ultimately led to cell death (Raveh et al., 2001). Another study showed that in the absence of DAPK1, p53 was only partially unregulated in response to proliferative signals generated by oncogene expression, which indicates that DAPK1 is an upstream activator of p53 along this particular pathway (Martoriati et al., 2005; Raveh et al., 2001) (Figure 6). P-p38 MAPK has been identified as an upstream regulator of DAPK1 during TNF α induced apoptosis in colorectal cancer cells (Bajbouj et al., 2009).

A significant increase in DAPK1 expression pattern has been seen 1 hour after the exposure of hippocampal neurons to neurotoxin ceramide. Interestingly, six hours later, huge cell death has been observed among the neuronal population (Pelled et al., 2002). Chen et al. (2005) showed that activation of ERK in DAPK1 expressing human erythroblast (D2) cells promotes cell death. Furthermore, bidirectional signals transduced by DAPK1-ERK interaction promote the apoptotic effect of DAPK1 has been reported (Feinstein et al., 1995). TSC2 is directly phosphorylated by Akt and by ERK, resulting in functional inactivation of the TSC1-TSC2 complex and mTORC1 activation (Proud,

2007). TSC2 negatively regulates DAPK1 by promoting its lysosome-dependent degradation. Recently, Kang et al. (2010) demonstrated that DAPK1 mediated cell death through interaction with DANGER (Nikolaidis et al., 2007; van Rossum et al., 2006) (Figures 5 and 6).

1.3.2.2.3 Autophagy

The involvement of DAPK1 in Type II cell death (autophagy) may settle all contradictory studies that have questioned the role of DAPK1, whether as a friend or a foe to the cell. Bialik and Kimchi (2010) and Jin et al. (2001) suggested that the dependence of investigators on assays, for activation of caspase and caspase-dependent apoptotic events, like DNA fragmentation would have missed the autophagic component of DAPK1 expression. Expression of activated forms of DAPK1 and DRP-1 trigger autophagy and cell death independent of caspase activity has been reported in carcinoma cells with nonfunctional p53 (Pelled et al., 2002).

Several recent studies have mapped DAPK1 function to distinct stages in autophagy signalling. These include the Beclin-1/phosphatidylinositol 3-kinase (PI(3)K) complex which is necessary for autophagosome formation, and an interaction with the LC3 binding protein, MAP1B, which may regulate vesicle trafficking (Bialik and Kimchi, 2010). Beclin 1, an essential autophagic protein, is a BH3-only protein that binds Bcl-2 anti-apoptotic family members. The dissociation of Beclin 1 from Bcl-2 inhibitors is essential for its autophagic activity (Cao and Klionsky, 2007), and therefore is tightly controlled. Zalckvar et al. (2009) have found that DAPK1 phosphorylates

Beclin 1 on T119, a critical residue within its BH3 domain, and thus promotes Beclin 1 dissociation from Bcl-XL and result in autophagy induction (Figure 6).

Another autophagic pathway that DAPK1 induces is mammalian target of rapamycin (mTOR) pathway. mTOR, which negatively regulates autophagy is activated by Rheb (small GTP binding protein) that is negatively controlled by TSC1/2. It has been shown that phosphorylation of TSC2 via various modulators including AKT/PKB, ERK, and RSK, led to inactivation of TSC1/2 and AMP kinase. This stimulation to TSC1/2 will activate or inhibit mTOR, respectively, (Chan, 2009) (Figure 6).

MAP1B, a microtubule lattice binding protein plays a major role in autophagy (Halpain and Dehmelt, 2006; Wang et al., 2007) and Harrison et al., (2008) provided evidence for DAPK1 interaction with MAP1B and their co-localization in microtubules. Expression of MAP1B synergized with DAPK1 to suppress growth and cell viability, yet conversely, partial depletion of MAP1B attenuated the growth suppression activity of over expressed DAPK1 suggesting the ability of DAPK1 to induce autophagosomes and membrane blebbing that can be affected by DAPK1/MAP1B synergy (Harrison et al., 2008) (Figures 5 and 6).

1.3.2.3 DAPK1 and Diseases

Emerging evidence has revealed the role of DAPK1 in tumor suppression (Inbal et al., 1997; Raveh et al., 2001). DAPK1 expression is frequently lost in tumors due to hypermethylation of the DAPK1 gene (Cohen and Kimchi, 2001). It is triggered by different cell death-regulators and dysregulated in several cancer types, including non-small cell lung, head, neck, and pancreatic adenocarcinoma (Dansranjavin et al., 2006;

Kim et al., 2001; Michie et al., 2010; Sanchez-Cespedes et al., 2000). In addition, it has been shown that DAPK1 expression provides a unique mechanism that links suppression of apoptosis to metastasis (Inbal et al., 1997).

The involvement of DAPK1 in induction of renal tubular cell death in chronic obstructive uropathy (COU) following unilateral ureteral ligation has been considered (Yukawa et al., 2005). In another study, DAPK1 participation in tubular cell apoptosis following renal ischemia reperfusion injury has been characterized (Kishino et al., 2004). It has been shown that the inactivation of DAPK1 gene transcription via methylation may boost severity of inflammation in ulcerative colitis (Gandesiri et al., 2012). These data suggested that DAPK1 might be an important target employed to suppress inflammation and disease progression in UC.

Another disease in which DAPK1 has been involved in is atherosclerosis. DAPK1 mRNA and protein is significantly increased in atherosclerotic plaques, but the mechanism by which this occurs is not yet clear (Martinet et al., 2002). Furthermore, DAPK1 has been implicated in the molecular response to ischemic brain injury and apoptosis-mediated cell death in neurons (Pelled et al., 2002; Yamamoto et al., 1999, 2002). It is considered as a strong positive regulator of neuronal apoptosis *in vitro* and *in vivo* (Pelled et al., 2002; Schori et al., 2002; Yamamoto et al., 2002).

1.3.2.4 DAPK1 in Nervous System

1.3.2.4.1 DAPK1 in CNS

DAPK1 mRNA has been shown to be principally abundant in the adult brain (Bialik and Kimchi, 2004). DAPK1 expression decreased remarkably in the brain immediately after birth, limiting it to restricted mature neuronal populations such as olfactory bulb, hippocampal formation and cerebellar Purkinje and granule cells (Tian et al., 2003a; Sakagami and Kondo, 1997; Yukawa et al., 2004). This was based on the analysis of patterns of DAPK1 expression observed from the 13th embryonic day (E13) and was, thereafter, detected throughout the entire embryonic period (Yamamoto et al., 1999). DAPK1 mRNA has been detected in the mantle and ventricular zones of the entire neuraxis in the early stages of brain formation. This differentially regulated expression of DAPK1 during development and its restricted expression in mature neuronal population indicate that DAPK1 might be involved in some other neuronal functions besides executing developmental neuronal programmed cell death (Sakagami and Kondo, 1997).

DAPK1 has been suggested to have other functional roles such as regulating exocytosis of neurotransmitter release by phosphorylation of syntaxin-1 (Tian et al., 2003a) and protecting neurons during development or recovery from hypoxic-ischemic injury (Schumacher et al., 2002). Moreover, DAPK1 has been shown to function as a mediator of multi-types of stress signals induced by deprivation of neuronal cells from Netrin-1, and stimulation of NMDA receptors in cerebral ischemia (Bialik et al., 2004; Tu et al., 2010).

Wu et al. (2011) have shown that DAPK1 inhibits microtubule (MT) assembly by activating MARK/PAR-1 family kinases MARK1/2, which destabilize MT by phosphorylating tau and related MAP2/4. This relation with DAPK1 has demonstrated DAPK1 function in modulating MT assembly and neuronal differentiation. It also provides a molecular link between DAPK1 and tau phosphorylation, an event associated with AD pathology (Wu et al., 2011; Kim et al., 2014). DAPK1 was recently shown to have apoptosis-independent effects on the cytoskeleton (Kuo et al., 2006) and its association with stress fibers to induce inhibition of integrins by the cytoskeletal-binding domain of DAPK1 has been reported (Bialik et al., 2004). DAPK1 has also been connected with neurite outgrowth regulation in mice brain (Fujita et al., 2008).

DAPK1 interacts with neogenin, a receptor for repulsive guidance molecule (RGM) that has diverse functions in the developing central nervous system (Fujita et al., 2008). The same study showed that cytochrome c release from mitochondria induced by neogenin is dependent on DAPK1. It also demonstrated that DAPK1 is required for neogenin-induced cell death in the developing chick neural tube. Furthermore, neogenin induced cell death is abolished in the presence of RGM which blocks DAPK1 activity. Moreover, following NGF-induced differentiation of rat PC12 cells, DAPK1 catalytic activity and protein levels increased drastically. These and other neuronal recovery results indicate that DAPK1 may have a previously unexplored role in neuronal development or regeneration following injury (Schumacher et al., 2002).

It has been shown that DAPK1 is critical for the induction of neuronal cell apoptosis in response to various stimuli such as seizure, ischemia and ceramide in both

cultured cells and animal models experiments (Li et al., 2006; Liu et al., 2012; Mitoma et al., 1998; Pelled et al., 2002; Yukawa et al., 2006). Recently, DAPK1 activities have been implicated in the molecular pathways activated during seizure-induced neuronal death (Araki et al., 2004; Henshall et al., 2003).

DAPK1 expression was induced in a rat seizure model in regions of the brain that were not undergoing apoptosis (Henshall et al., 2003). Augustinack et al. (2002) showed DAPK1 protein expression was elevated in temporal lobe epilepsy. It has been published from different distinct studies that the induction of seizures following kainic acid administration was accompanied by significant increase in DAPK1 in the CA3 region of the rat hippocampus and more interestingly the same trends were seen concerning p53, which suggest the interaction between the two proteins and their involvement in cell death in this models of neurodegeneration (Araki et al., 2004; Henshall et al., 2003).

Middle cerebral artery occlusion (MCAO) is a model used to study neuronal death. It has been shown in line with previous studies that the induction of ischemia was associated with an elevation in DAPK1 expression (Schumacher et al., 2002; Shamloo et al., 2005). DAPK1-deficient mice showed a decrease in cell death following administration of glutamate or after knockout of DAPK1 that has been shown to protect retinal ganglion cells from glutamate toxicity (Schori et al., 2002).

It is well known that A β peptide is a neurotoxin and it is broadly used to mimic Alzheimer's disease (AD) to explore the mechanisms behind neuronal death in this neurodegenerative diseases. In an *in vitro* study, Cheung et al. (2011) showed that the

exposure of cultured neurons to A β results in cell death which is accompanied by activation of DAPK1. AD brain cells express a number of apoptosis related proteins such as ZIP (Zipper Interacting Protein) kinase and Bim/BOD (Bcl-2 interacting mediator of cell death/Bcl-2 related ovarian death gene). This increase has been suggested to be regulated by DAPK1 expression (Engidawork et al., 2001).

Interestingly, aberrant methylation of DAPK1 has been implicated in tumorigenesis of neurofibromas or neurofibrosarcomas (Alonso et al., 2003; Gonzalez-Gomez et al., 2003a, 2003b). In addition, another *in vitro* study revealed that the induction of cell death in ceramide-3 treated hippocampal neurons was mediated by DAPK1 (Pelled et al., 2002). The study showed that DAPK1-deficient mice neurons treated by acyl ceramide were less sensitive to apoptosis, implying that DAPK1 played a central role in ceramide-induced neuronal cell death in neurons (Pelled et al., 2002). Furthermore, the role of DAPK1 in neural cells has been shown in several vertebrate species.

DAPK1 has been implicated in p53-mediated apoptosis, a pathway activated following epileptic brain injury, where expression of p53 increased within the ipsilateral hippocampus (Araki et al., 2004) (Figures 5 and 6). DAPK1 has also been studied in the human brain and suggested as a molecular regulator of neuronal death in epilepsy (Henshall et al., 2004). DAPK1 has also been shown to function as a mediator of multi types of stress signals induced by deprivation of neuronal cells from Netrin-1, and stimulation of NMDA (Bialik et al., 2004; Tu et al., 2010). Tu et al., (2010) have found that DAPK1 has a physical and functional interaction with the NMDA NR2B subunit

receptors in cerebral ischemia and this interaction leads to severe stroke neuronal damage (for more details see section 3).

Taken together, these studies suggest that DAPK1 is widely involved in neuronal cell death response to different forms of acute neuronal damage as well as multiple neurological disorders. The current body of knowledge raises the possibility of DAPK1 as a therapeutic target for diseases characterized by rapid neurodegeneration, such as stroke or traumatic brain injury. DAPK1 has an appreciated role in neuronal development or recovery from injury that potentiates the future application of therapies targeting DAPK1.

1.3.2.4.2 DAPK1 in PNS

Although extensive studies have focused on DAPK1 expression pattern in the CNS and its role in neuronal death or survival, its presence in PNS and its role in the peripheral nerve regeneration remain completely unexplored.

This section summarized DAPK1 signalling pathways in autophagy, apoptosis, and stressed the important role it might play in the nervous system. The next section will explore the DAPK1 relationship with NMDA NR2B subunits and the involvement of NMDA NR2B proteins in neuronal death and the development and maintenance of neuropathic pain.

1.3.3 The Role of NMDA Receptors in Neuronal Death and Neuropathic Pain

The neurotransmitter receptors in the mammalian CNS have been classified into ligand-gated ion channel (ionotropic) and G-protein-coupled (metabotropic) receptors (Mayer

and Miller, 1990; Schoepp and Conn, 1993; Sommer and Seeburg, 1992). Ionotropic receptors are subdivided into kainite, NMDA (N methyl-D-aspartate) and AMPA (RS-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors (Barnard and Henley, 1990; Mayer and Miller, 1990; Sommer and Seeburg, 1992). It is well known that, GABA is the main inhibitory whereas NMDA is the primary excitatory neurotransmitter that mediates fast excitatory synaptic transmission in the CNS (Ryan et al., 2008; Traynelis et al., 2010).

NMDA receptors are different from AMPA and kainate receptors in various aspects like: high Ca^{2+} permeability, Mg^{2+} blockade in a voltage-dependent manner at resting membrane potential and requires the co-agonist, glycine, for activation (Ryan et al., 2008). NMDA receptor subunits are classified into three families: NR1 (GluN1), NR2 A-D (GluN2A–D) and NR3 A-B (GluN3A, B) (Gonda, 2012; Laube et al., 1997). Functional NMDA receptors are composed of the two obligatory NR1 and one or more modulatory NR2A-D subunits (Mori and Mishina, 1995). Beside the studies done on NR3 subunits, its functional role is still not completely understood (Meguro et al., 1992; Paoletti and Neyton, 2007). The pharmacological and functional properties of the NMDA receptor such as single channel conductance, Mg^{2+} blockade and channel deactivation time depend on the composition of the NMDA subunit complex. Different combinations of NMDA subunits produce unique receptors with distinctive pharmacological and biochemical properties (Tovar and Westbrook, 1999; Traynelis et al., 2010).

Multiple studies have shown that NMDA receptors are activated by both

glutamate and its co-agonist glycine depending on the location of its binding sites in NR2 and NR1 subunits respectively (Anson et al., 1998; Hirai et al., 1996; Kew et al., 2000; Laube et al., 1997; Wafford et al., 1995). NR2 subunits show 40-70 percent homology and are differentially expressed throughout the brain during development (Monyer et al., 1994; Watanabe et al., 1993).

1.3.3.1 The Role of NMDA Receptors in Neuropathic pain

Neuropathic pain is a major health issue generally defined as a chronic pain state caused by peripheral nerve injury (Bennett and Xie, 1988; Decosterd and Woolf, 2000; Kim and Chung, 1992; Seltzer et al., 1990) or central nerve injury, or both (Campbell and Meyer, 2006; Ossipov et al., 2002).

Although, numerous studies have attempted to understand the cellular mechanism of neuropathic pain (Campbell and Meyer, 2006; Costigan et al., 2009; Saadé and Jabbur, 2008; Sandkühler, 2009; Devor, 2006), its etiology remains a clinical challenge (Asano et al., 2012; Longstreth, 2005). Pain hypersensitivity manifests itself in many forms including hyperalgesia which implies an exaggerated nociceptive response to originally noxious stimulation, allodynia which indicates a nociceptive responses to non-noxious stimulation and finally, spontaneous pain (Al-Chaer et al., 2000; Li et al., 2011a, b; Zhuo, 2007). Hyperalgesia is usually linked with activity dependent changes in the spinal cord (Dubner and Ruda, 1992), a process that may share common features of synaptic plasticity in other neural systems (Ali and Salter, 2001; Malenka and Nicoll, 1999; Woolf and Salter, 2000). It has been speculated that synaptic plasticity contributes to pain perception, fear and memory (Li et al., 2010). Synaptic plasticity can be defined

as the ability of connections or synapses between two neurons to change in strength and that has been recognized as the key mechanism for chronic pain (Li et al., 2010).

Recently, Miwa et al. (2008) have reported that NMDA NR2B receptors accumulate on synaptic sites and are responsible for the unique properties of synaptic function and plasticity (Miwa et al., 2008). In addition, long-term potentiation (LTP), long-term depression (LTD) and spatial learning, which require activation of NMDA receptors in the hippocampus, are well-characterized forms of synaptic plasticity (Ji et al., 2003a; Li et al., 2010; Wu and Zhuo, 2009). Over the last two decades, a large body of literature has documented the involvement of NMDA receptors in mediation of nociception and development of spinal hyper-excitability (Bleakman et al., 2006; Haley et al., 1990; Ren et al., 1992; Woolf and Thompson, 1991; for review see Wu and Zhuo, 2009).

Compelling evidence has accumulated over the past years, demonstrating that the activation of NMDA receptors in the spinal dorsal horn is essential for triggering the development and maintenance of central and peripheral sensitization (Bleakman et al., 2006; Davar et al., 1991; Dougherty et al., 1992; Gaudreau and Plourde, 2004; Gaunitz et al., 2002; Mao et al., 1992, 1993; Ren and Dubner, 1993; Seltzer et al., 1990; Tal and Bennett, 1993; Ultenius et al., 2006; Urch et al., 2001; Willert et al., 2004; Woolf and Thompson, 1991). Spinal NMDA receptors, particularly the NR2B subunit are responsible for the development of central sensitization (Hu et al., 2009; Pedersen and Gjerstad, 2008; Renno, 1998).

It is well established that the nociceptive transmission pathway starts from peripheral fibers of the DRG, which then conveys to the dorsal horn of spinal cord and, finally, to supraspinal structures such as brain stem, thalamus, somatosensory cortex, insular cortex, and cingulate cortex (for review see Wu and Zhuo, 2009). Not surprisingly, NMDA receptors are present in pain pathways from peripheral DRG to the brain. Sato et al. (1993a) reported that NR1 and NR2 subunits of NMDA receptor are both expressed in DRG neurons. It has been shown that NMDA receptors are synthesized in DRG neuronal somata and transported to both central terminals in spinal cord, brainstem, trigeminal nucleus and to peripheral terminals in the skin and muscles (Coggeshall and Carlton, 1998a; Liu et al., 1997). Morphological studies have also demonstrated NMDARs expression on myelinated and unmyelinated axons in peripheral somatic tissues (Carlton et al., 1995; Coggeshall and Carlton, 1998a).

Various immunohistochemical localization studies of glutamate receptors have revealed that NMDARs are predominantly expressed on small and medium diameter neurons (Lee et al., 2001; Sato et al., 1993b; Willcockson and Valtschanoff, 2008). In a marked observation, a large proportion of unmyelinated peripheral axons (47%) have been found to be immunopositive for NMDA NR1 receptors in the normal digital nerve (Du et al., 2006). Importantly, following hind-paw inflammation, a significant increase in the proportion of positively labelled axons was seen at the 2nd and 7th day, but not on the 14th day (Du et al., 2006). In support to that, NR2B, NR2C, and NR2D subunits, but not NR2A, were found to be present in peripheral nociceptive fibers of primary afferents terminals (Chen et al., 2010).

It has been also reported that NR1/NR2B containing NMDA receptors are expressed in both A-fibers and C-fibers (Marvizón et al., 2002). NMDARs also have been found to be expressed centrally in second order nociceptive neurons in the spinal cord and medullary dorsal horn causing pain hypersensitivity after tissue injury (Ji et al., 2003b). Normally, NMDA NR2B has a limited distribution in the superficial dorsal horn (Boyce et al., 1999; Nagy et al., 2004). Remarkably, after partial chronic constriction injury to the sciatic nerve NR2B subunits containing NMDA receptor expression was increased while NR1 was decreased in the superficial dorsal horn, suggesting the involvement of such receptor (NMDA NR2) in the neuropathic process (Wilson et al., 2005).

1.3.3.1.1 Are NMDA Receptors involved in Pro-Nociception or Anti-Nociception?

Although, convincing evidence from several studies anticipated NR2B as the major subunit involved in mediating the development and maintenance of neuropathic pain (Abe et al., 2005; Geng et al., 2010; Iwata et al., 2007; Li et al., 2011a), considerable controversies have also been reported regarding NMDAR's role in nociception in the peripheral and central terminals of primary afferent neurons (Bardoni et al., 2004; Coggeshall and Carlton, 1998b; Du et al., 2003; Liu et al., 1997). It has been found in multiple studies that the blockade of NMDA receptors reduces pain transmission after noxious stimuli in animals and during chronic pain in humans (Castroman and Ness, 2002; Peles et al., 2004).

Moreover, in a behavioural study, activating NMDA receptors in normal skin led to mechanical allodynia and hyperalgesia pain which was diminished after co-injecting

with the NMDA antagonist MK-801 (Zhou et al., 1996). In line with that, Finch et al. (2009) have demonstrated that the topical injection of the NMDAR antagonist, ketamine, was able to reduce allodynia in patients with complex regional pain syndrome. Likewise, in separate studies Davies and Lodge (1987) and Dickenson and Sullivan (1987) have reported that NMDA antagonists inhibit the hyper-excitability of spinal cord nociceptive neurons induced by C-fiber stimulation.

On the other hand, other studies found that NMDAR antagonists, MK-801 or ketamine, in peripheral application had essentially no effect in decreasing inflammatory and neuropathic pain (Aley and Levine, 2002; Sawynok and Reid, 2002). A number of studies have clearly shown that peripheral administration of NMDAR antagonists or genetic deletion of NR1 subunit in primary sensory neurons exert anti-hyperalgesic action in rodents (Du et al., 2003; McRoberts et al., 2011). In another study, the deletion of NR1 gene in peripherin-expressing DRG neurons resulted in about 75% reduction of NR1 expression as determined by western blot analysis (McRoberts et al., 2011).

In marked contrast, in a recent study carried by Pagadala et al. (2013), in which NR1 was selectively deleted from all DRG neurons, but not from central spinal cord, using Advillin-Cre driver. NR1 knock-out mice (NR1-cKO), displayed mechanical and thermal hypersensitivity, higher excitability and enhanced excitatory synaptic transmission when compared to the wild-type. Based on these results, the authors suggested the possible anti-nociceptive effects of NMDAR activation in primary sensory neurons (Pagadala et al., 2013). The studies addressed above clearly state the conflicting

roles of NMDARs in primary sensory neurons and hence it remains unsettled whether NMDARs function as pro-nociceptive or anti-nociceptive receptors.

1.3.3.2 The role of NMDA Receptors in Neuronal Death

NMDA receptors have been documented to be involved in various physiological function such as: brain development, learning and memory formation, excitatory synaptic transmission and plasticity, mood control, long-term potentiation (LTP) and long-term depression (LTD) (Banerjee et al., 2009; Collingridge et al., 2010; Fitzgerald, 2012; Ikonomidou et al., 1999; Lee et al., 1999; Traynelis et al., 2010; Yoshimura et al., 2003). However, under pathological conditions, NMDARs can cause neuronal death by 'excitotoxicity' (Lee et al., 1999; Rothman and Olney, 1995; Simon et al., 1984).

Changes in glutamatergic transmission and NMDAR activation have been considered as a common pathological event in various diseases such as stroke (Choi and Rothman, 1990), Parkinson's disease (Turski et al., 1991), epilepsy (Sloviter, 1991), Huntington's disease (Schwarcz et al., 1983) and Alzheimer's disease (Ulas et al., 1992). Extensive studies have shown that regulation of NMDA receptors may take part in psychoses and some neurodegenerative disorders (Carlsson and Carlsson, 1990; Choi, 1988). In addition, NMDAR blockers protect neurons from ischemic neuronal injuries both *in vitro* (Lipton and Rosenberg, 1994; Liu et al., 2007; Rothman and Olney, 1995) and *in vivo* (Chen et al., 2008; Lipton and Rosenberg, 1994; Liu et al., 2007; Rothman and Olney, 1995; Simon et al., 1984).

Forrest et al. (1994) found that NR1 receptor subunit disruption in transgenic mice led to an early postnatal death. Besides, Tang et al., (1999) found that NR2B

receptor subunit overexpression in mice caused an enhanced hippocampal LTP, sustained NMDA receptor currents and improved memory. Furthermore a series of old and more recent studies have emphasized the role of the NMDA NR2B receptor in various types of neuronal death (Chen et al., 2008; Choi and Rothman, 1990; DeRidder et al., 2006; Lee et al., 1999; Liu et al., 1997; Rothman and Olney, 1986, 1995; Simon et al., 1984; Terasaki et al., 2010; Zhou and Baudry, 2006). It has been reported that NR2B receptors are the main activator for most death signalling transduction pathways (Lai and Wang, 2010; Martin and Wang, 2010).

NR2B is mainly present in extra-synaptic locations (Mathur et al., 2009), whereas NR2A occur mainly in neuronal synapses. This different distribution of NMDA subunits lead to two opposing events, neuronal death and neuronal survival respectively (Tovar and Westbrook, 1999; Traynelis et al., 2010).

1.3.3.3 The Relationship between NMDA NR2B Receptor and DAPK1

Recently, several proteins that contribute and interact with NMDA NR2B neuronal death in extra-synaptic sites have been characterized (Lai and Wang, 2010; Martin and Wang, 2010; Tu et al., 2010). DAPK1 has been considered as a novel protein that interacts physically and functionally with the NMDA NR2B receptor in ischemic neurons in the brain (Tu et al., 2010).

Two decades ago, Choi and Rothman (1990), and Rothman and Olney (1986) reported that ischemia led to an excessive amount of glutamate release in the extracellular space and the induced NMDA receptor hyper-activation that led to Ca^{2+} influx and neuronal cell death. Later, Tu et al (2010), found that activation of NMDA

NR2B during stroke caused Ca^{2+} influx that activated the Ca^{2+} /Cam regulated protein kinase DAPK1. Using co-immunoprecipitation with NR2B-specific antibodies and mass spectrometry, Tu et al revealed that cerebral ischemia recruits DAPK1 into the NMDA NR2B receptor protein complex in the cortex of adult mice. A constitutively active DAPK1 phosphorylates NR2B subunit at Ser-1303, which in turn enhances the NR1/NR2B receptor channel conductance. DAPK1-NMDA NR2B interaction acts as a central mediator for stroke damage.

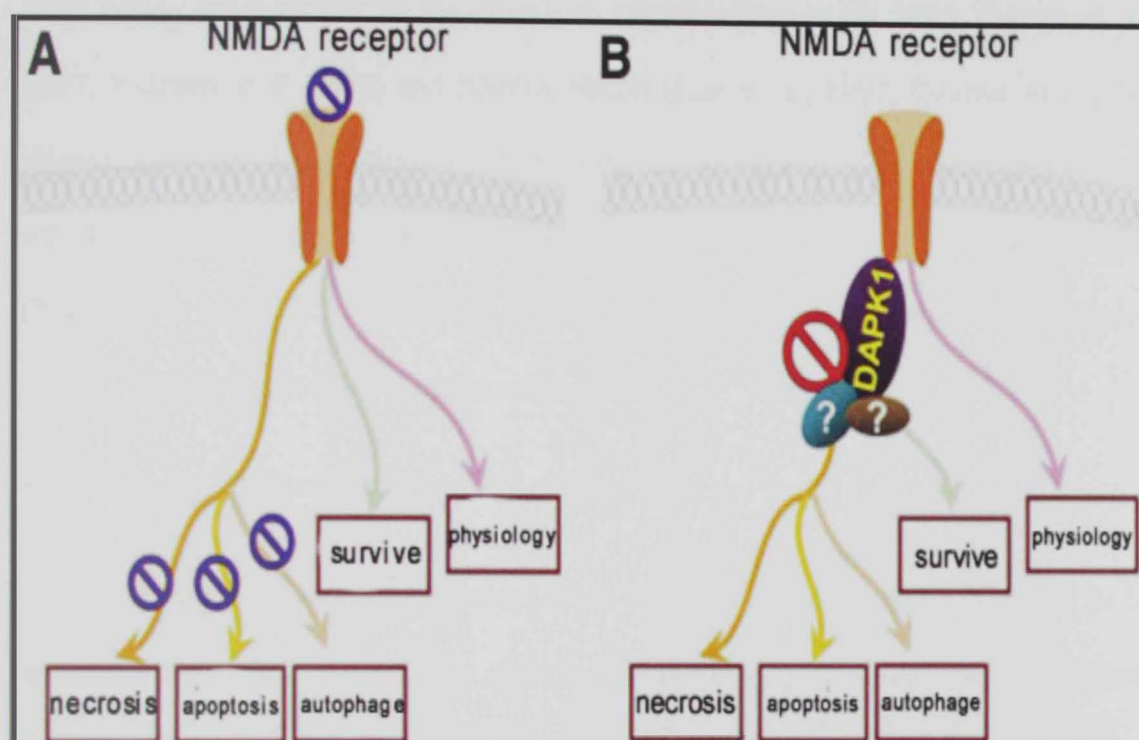


Figure 7: Proposed diagram illustrating the differences between blockade of NMDA receptor and NMDA NR2B-DAPK1 in stroke

NMDA NR2B-DAPK1 downstream signaling causes various neuronal events in stroke. (A) Blockade of NMDA receptor lead to inhibition of necrosis, apoptosis and autophagy, disturbing physiological synaptic transmission and survival signaling. (B) Blockade of NMDA NR2B-DAPK1 downstream signaling prevents various neuronal cell death including necrosis, apoptosis and autophagy, inhibition of cell survival probably with no effect on physiological synaptic transmission (From Shu et al. 2014).

Notably, disruption of this protein-protein interaction in DAPK1-NMDA NR2B complex by designing an interference peptide NR2B_{CT} which was able to block both DAPK1-NMDA NR2B interaction and potentiation of NMDAR function or by DAPK1 knockdown mechanism, was able to protect neuronal cells in mice against cerebral ischemic damage. This mechanism has been considered as a possible treatment for stroke (Tu et al., 2010; Shu et al. 2014) (Figure 7).

Markedly, DAPK1 and NMDA NR2B are both distributed ubiquitously in the CNS during development. In the adult both DAPK1 (Tian et al., 2003; Sakagami et al., 1997, Yukawa et al. 2006) and NMDA NR2B (Luo et al., 1997; Charton et al., 1999) showed a restricted expression predominantly in the hippocampus, cerebral cortex and olfactory bulbs. Many investigators have shown NMDA NR2B expression in the PNS (Pagadala et al., 2013; Liu et al., 1997; Coggeshall and Carlton, 1998). However, DAPK1 expression in PNS remains unidentified.

1. 3.4 Satellite Cells and Neuropathic Pain

It has been almost two centuries since these cells were discovered by Valentin in 1839 and were given the name satellite cells by the famous Spanish neuroscientist Santiago Ramón y Cajal in 1909 (Stephenson and Byers, 1995). The shape of satellite cells has been the core of a long debate, whether these cells are astrocyte-like or not, but it is now well established that they have a laminar shape and lack true processes (Hanani, 2005).

Histological examination of sensory ganglia revealed that there exists tight connections between satellite cells and also between them and neurons (Byers and Costello, 1988; Pannese, 1974). Satellite glial cells (SGC) outnumber neurons in the

PNS and play a crucial protective role by forming an envelope around the cell body (Pannese, 1981; Pannese et al., 2003). However, in contrast to its supportive and protective functions, more recently, emerging evidence has revealed that such cells might contribute to pain following nerve injury in different neuropathic pain models (Huang et al., 2010; Jasmin et al., 2010; Warwick and Hanani, 2013).

Close examination of the literature shows a great focus on the role of neurons in the processing of neuropathic pain but not many studies have been conducted on the glial cells and their contribution to such processes that might be an outlet for winning the clinical battle against neuropathic pain. Moreover apparent changes in the satellite cells after nerve injury spotlights directly on such cells regarding involvement in neuropathic pain. Indeed, several studies have also shown the incrimination of glial cells in the exacerbation of neuropathic pain.

1.3.4.1 Activation of SGC after PNS Injury

GFAP, a marker of satellite cells in the PNS and astrocytes in the CNS, had been found to be increased noticeably by the third day after nerve transection and remained at high levels 6 weeks later in L4 and L5 DRG (Woodham et al., 1989a). A similar observation has been made in the satellite cells of the trigeminal ganglion (TG) in response to dental injury (Stephenson and Byers, 1995). Also in line with such observations, many groups were able to duplicate them in different PNS injury models such as chronic constriction injury (CCI) (Dubový et al., 2010; Ohara et al., 2008), partial nerve ligation (Xu et al., 2008) and L4 spinal nerve ligation (SNL) (Xie et al., 2009b).

In support of the concept of the activation of satellite cells following PNS injury, Arkhipova et al. (2010) recently demonstrated a significant increase in the number of satellite cells using different sciatic nerve injury technique for example, crushing, transection and ligation. In addition they showed an increase in the thickness of SC and area of contacts with neurons in a rat's dorsal root ganglia.

1.3.4.2 Role of SGC in Neuropathic Pain

A study that showed the involvement of satellite glial cells (SGCs) in neuropathic pain in an animal pain model showed a 6.5 fold increase in the gap junction between these cells after the injury. Inhibition of formation of such gap junctions led to significant reduction of pain intensity and opened the door to the development of gap junction blockers as potential alternatives in pain therapy (Huang et al., 2010; Warwick et al., 2014).

The relation between neurons and satellite cells after axotomy of the infraorbital nerve was examined by Miyagi et al.(2006). They showed an increase in the gap junction between satellite cells and neurons during development of spontaneous pain behavior. Furthermore, in support of that, another group succeeded in reversing such developments by knocking out the Cx43 gene, a key protein in the formation gap junction (Vit et al., 2006). Further studies of the relation between the two cells in the peripheral nervous system by Cherkas et al. (2004) showed an increase in coupling between neurons and satellite cells that coincided with a significant neuronal excitability after infraorbital nerve transection. Moreover, in line with the relation between neurons and satellite cells, it has been shown that a great amount of ATP secreted from neurons was up-taken by satellite cells and it is believed that following nerve transection,

neurons used the ATP as a mediator to activate satellite cells (Dubový et al., 2006; Takeda et al., 2007).

Vit et al. (2009) in a recent study attempted to reduce the pain using viral gene therapy by effectively using an increase in GABA transmission as an inhibitory strategy to slow down the excitability seen during neuropathic pain. The delivery of the adenoviral vector that contained the glutamic acid decarboxylase (GAD) gene directly into trigeminal ganglia induced significant increase of GABA synthesis mainly in satellite glial cells that persisted even after six days. Such increase in the level of inhibitory neurotransmitters coincided with a significant decrease in pain behavior in the orofacial formalin models. In line with the previous study, Naik et al. (2008) provided evidence that direct injection of a GABA receptor agonist into the dorsal root ganglion was antinociceptive in a model of peripheral nerve injury. Taken together, data from these studies demonstrate the therapeutic potential of directly increasing the amount of GABA in the ganglion that functions to suppress neuronal activity that occurs in response to nerve damage and the development of peripheral and central sensitization.

There is ample evidence that satellite glial cells are involved in the development and exacerbation of neuropathic pain. In models of neuropathy after sciatic nerve section and peripheral inflammation, Lu and Richardson (1991) showed an increase in the number of glial cells in the superficial dorsal horn and lateral spinal nucleus of the spinal cord in IFN- γ knockout mice. Lee et al., (1998) and Xie et al., (2009a) demonstrated that a significant depletion in the concentration of NGF- β , plays a major role in the activity and survival of neurons and enhances the pain responses of neurons in the PNS, occurs

in the DRG following sciatic nerve injury. In order to compensate for such shortage of NGF- β , Lindholm et al., 1987 and Matsuoka et al., (1991) found that the SGCs begin to synthesize NGF- β , and such changes were maintained for up to 2 months (Lee et al., 1998; Zhou et al., 1999).

It has been demonstrated that in order to treat back pain and sciatica, a medical procedure called autologous application of nucleus pulposus is used to alleviate the pain. However, such treatments are found to induce mechanical hyperalgesia that lasts for more than one month. Otsu et al., (2010) found that such hyperalgesia was accompanied by the activation of satellite cells in the DRG and also the activated glial cells showed a prolonged increase in GDNF expression. Such phenomena were associated with recovery of neuronal function and a reduction of pain. In addition, Hammarberg et al. (1996) demonstrated an elevation of GDNF mRNA in satellite cells and Schwann cells of L4/L5 DRGs following chronic injury to the adult rat sciatic nerve which remained high for up to five months.

The studies cited above might draw the conclusion that satellite cells play a vital part in the pathophysiology of pain following nerve injury and interpreting the mechanisms underlying their involvement in such phenomena might help in the understanding of neuropathic pain.

CHAPTER 2: MATERIALS AND METHOD

2.1 Experimental Procedures

All the experimental procedures were approved by the Animals Ethics Committee of the College of Medicine and Health Sciences at UAEU, as well as performed in accordance with the UK Animals (Scientific Procedures) Act, 1986.

2.2 Surgical Procedures and Sciatic Nerve Injury

Adult male Wistar rats (190–220 g) obtained from the College of Medicine and Health Sciences animal facility (Al Ain, UAE) were anaesthetized with a mixture of ketamine (100 mg/kg) and xylazine (20 mg/kg) delivered intraperitoneally. The skin over the right thigh region was shaved, cleansed with 70% alcohol and then an incision was made in the skin. The sciatic nerve was exposed and cut at the mid-thigh level proximal to its major divisions into tibial, common peroneal and sural nerves. The skin was then sutured using 4.0 silk sutures. The left (contralateral) sciatic nerve was left intact to serve as an internal control. At the end of surgery, rats were transferred back to their cages and allowed to recover for 2 hours, 7 days or 14 days.

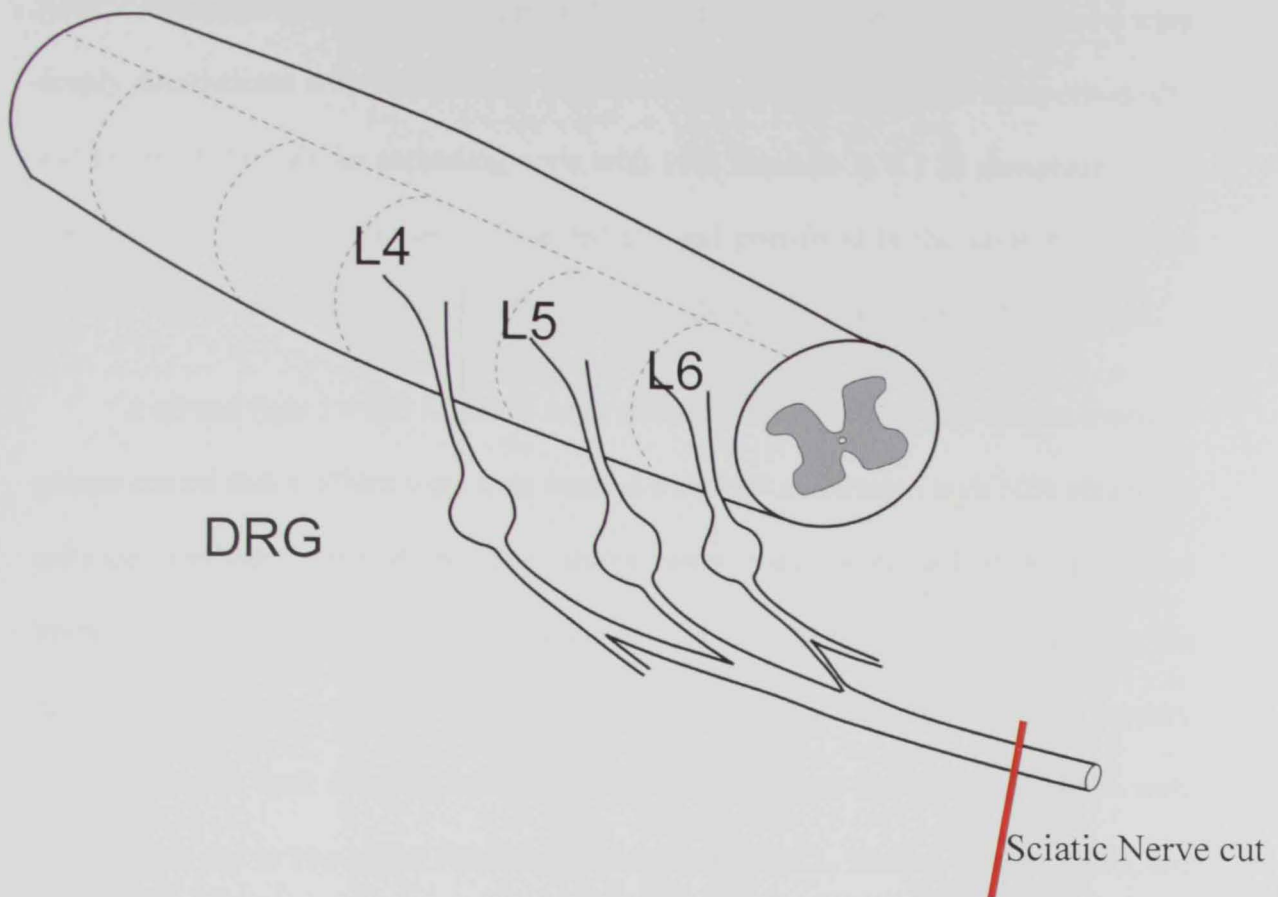


Figure 8: A schematic diagram showing sciatic nerve injury as peripheral nerve injury model

The peripheral nerve injury model has been used in this study. It is also showing that sciatic nerve is formed mainly by L4 and L5 nerves with a minor contribution from L6 (Shehab et al., 1986 and Shehab, 2009).

2.3 Immunohistochemistry

After 2 hours (n=4), 7 days (n=4) and 14 days (n=4) of sciatic nerve injury, the rats were deeply anesthetized with an overdose of urethane (2 ml, 25%) injected intraperitoneally and perfused through the ascending aorta with 10% formalin in 0.1 M phosphate buffer (pH 7.4). L4 and L5 DRG were dissected out and post-fixed in the same fixative for another 1 hour and then stored in 30% sucrose in phosphate buffer (pH 7.4) overnight.

Left and right L4 and L5 DRG were all cut in a cryostat (15 μ m) and mounted on gelatin coated slides. Slides were then washed with PBS and treated with 50% ethanol to enhance antibody penetration. The slides were then processed with either an immunofluorescent or peroxidase method of immunohistochemistry according to the protocols described previously (Shehab et al., 2003, 2004, 2008). Details of the primary antibodies used, their sources, and concentrations are shown in Table 1. Sections were stained with one or two of the following antibodies (DAPK1, NMDA NR2B, ATF3, and GFAP) diluted in PBS containing 0.3% Triton and incubated overnight at room temperature (RT).

For double immunofluorescent staining, on the following day the sections were rinsed and were incubated for 1 hour with a mixture of Rhodamine Red-X-AffiniPure Donkey Anti- Mouse IgG (1:100; from Jackson ImmunoResearch, West Grove, PA, USA) and Alexa Fluor 488-AffiniPure Donkey Anti-Rabbit IgG (1:200; from Jackson ImmunoResearch).

Because both NMDA NR2B and ATF3 antibodies were produced in rabbits, the sections were incubated with anti-NMDA NR2B on the first day followed by Alexa

Fluor 488-AffiniPure Donkey Anti-Rabbit IgG (1:200; from Jackson ImmunoResearch) as a secondary antibody. Then after rinsing the sections 3 times with PBS, they were incubated with anti-ATF3 overnight at RT followed by Rhodamine Red-X-AffiniPure Donkey Anti- Rabbit IgG (1:100; from Jackson ImmunoResearch) for 1 hour at RT.

For triple immunofluorescent staining, sections (10 μ m) were incubated with a mixture of the following antibody (DAPK1, CGRP and IB4) overnight at RT. To reveal IB4 binding, sections were initially incubated for 1 hour in a solution of IB4 (Vector Laboratories; 1 mg/ml) and then incubated with goat anti-IB4 overnight. After rinsing, the sections were incubated for 1 h with a mixture of species-specific secondary antibodies (Rhodamine Red-X-AffiniPure Donkey Anti-Mouse IgG (1:100), Alexa Fluor 488-AffiniPure Donkey Anti-Rabbit IgG (1:200) and donkey anti-goat IgG conjugated to Alexa 488 (1:100), all of which were obtained from Jackson ImmunoResearch, USA). After another rinse, sections were mounted with a glycerol-saline medium. All the antibodies were diluted in PBS (pH 7.4) containing 0.3% Triton. We have used negative control staining in which primary antibodies were omitted and sections were incubated with only a mixture of the above-mentioned secondary antibodies.

For peroxidase staining, sections were incubated overnight in either mouse anti-DAPK1 (1:4,000) or rabbit anti-NMDA NR2B (1:8,000). After rinsing with PBS, the sections were incubated with biotinylated anti-mouse or anti-rabbit secondary antibody (1:500, Jackson ImmunoResearch) for one hour, followed by Extravidin peroxidase conjugate for another one hour (1:1,000, Sigma-Aldrich, St Louis, MO, USA). Finally, after rinsing 3 times with PBS for 5 minutes each, the slides were incubated for 5

minutes in a solution of 3, 3'-diaminobenzidine (DAB) (25 mg/50 ml of phosphate buffer, pH 7.4 with 7.5 µl hydrogen peroxidase (30%) and 1 ml nickel chloride (3%) added to it). Slides were then washed, dehydrated in graded alcohol, cleared in xylene, cover slipped and examined under a light microscope.

Antibody	Species raised in	Dilution (Fluorescent - peroxidase)	Source
DAPK1	Mouse	1:250- 1:4000	BD, Biosciences, San Diego, CA, USA
ATF3	Rabbit	1:300	SantaCruz Biotechnology, Santa Cruz, CA, USA
NMDA NR2B	Rabbit	1:500- 1:8000	Millipore, Billerica, MA, USA)
GFAP	Mouse	1:400	Vector Laboratories, Inc., CA, USA)
IB4	Goat	1:5000	Vector Laboratories, Inc., CA, USA)
CGRP	Rabbit	1:2000	Peninsula Laboratories, Bachem Group, USA

Table 1: List of primary antibodies used in this study.

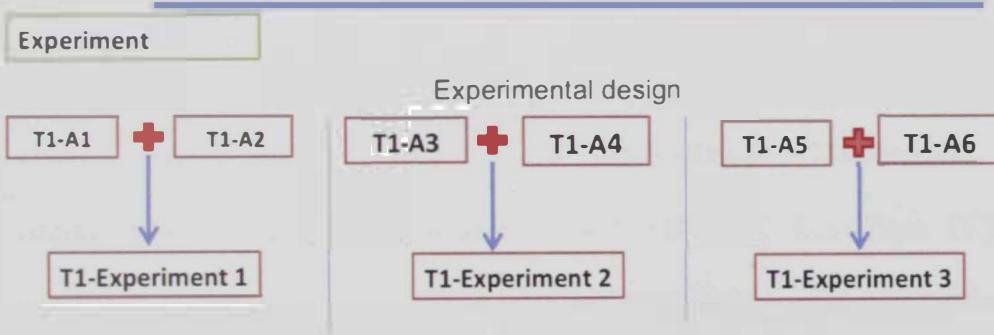
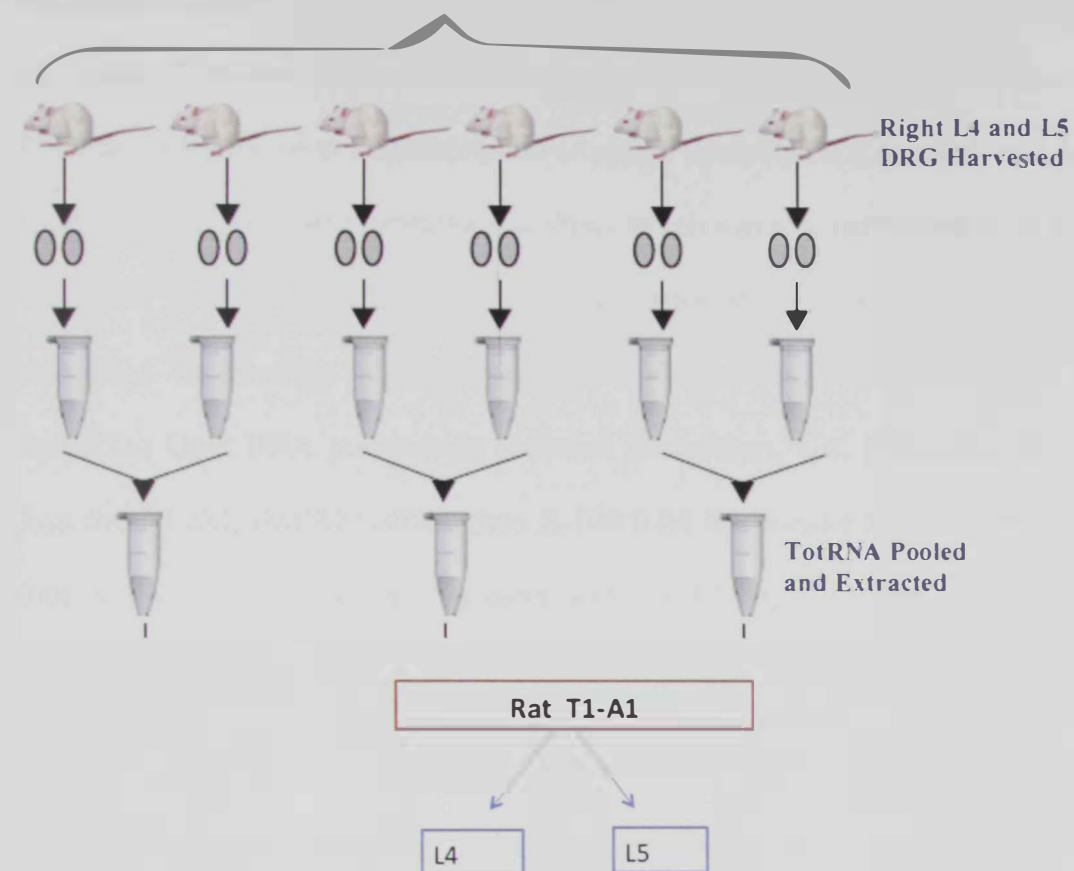
Representative digital images were captured using a Zeiss AxioCamHRc Digital camera with AxioVision 3.0 software (Carl Zeiss, Germany). Some sections were also examined with a Nikon C1 laser scanning confocal microscope.

2.4 Quantitative Real Time PCR

After 2 hours (n=6), 7 days (n=6) and 14 days (n=6) of sciatic nerve transection the rats were decapitated, L4 and L5 DRG were dissected and immediately left in RNA later[®],

then stored at -80°C for a later measurement of the relative gene expression of DAPK1, NMDA NR2B (Grin2b), p38 (MAPK14), p53, Bax and Akt, ERK5 (MAPK7).

Right Sciatic Nerve Transected



T= Time Point **A**= Animal Note: 6 Animal for each treatment and each two will represent one Experiment and the rest is replicate

Figure 9: Design of gene expression analysis experiments

Total RNA was extracted using TRI Reagent® Solution (Life Technologies Corporation, NY, USA) according to the manufacturer protocol. Quality and quantity of the extracted RNA was estimated using NanoDrop instrument (Thermo Fisher Scientific, DE, USA). First-strand cDNAs were prepared in duplicates from 1 µg of the extracted RNA using MuLV reverse transcriptase (Applied Biosystems, CA, USA) and served as template for relative gene expression analysis which was also performed in duplicates on ABI-7500 Real Time PCR System (Applied Biosystems, CA, USA) using TaqMan chemistry. The reaction mixture consisted of 50 ng cDNA, PCR Gold buffer, 0.375 U AmpliTaq Gold DNA polymerase (Applied Biosystems, CA, USA), MgCl₂ 6.0 mM, Rox dye 50 nM, DMSO 0.4%, Triton X-100 0.04 %, Nonidet P-40 0.04 %, Tween-20 0.04 %, forward and reverse primers and FAM-BHQ-1 labelled probe (Biosearch Technologies, Inc., CA, USA) or (Life Technologies Corporation, NY, USA) Table. 2.

Bax, p53 and 60S acidic ribosomal protein P0 (RPLP0) primers and probes were designed using the online RealTimeDesign™ software (Biosearch Technologies, CA, USA) in a way that at least one of the primers was spanning an exon-intron junction within their respective gene. DAPK1, NMDA NR2B, ERK5, Akt and p38 primers and probes were acquired from (Life Technologies Corporation, NY, USA). Immunofluorescence data generated by the ABI-7500 Real-Time PCR System was subjected to analysis using the Real-time PCR Miner analysis software (Zhao and Fernald, 2005). Calculated reaction efficiencies and CTs were then used to calculate the relative gene expression using the delta-delta CT methodology. Results for the gene expression were normalized with RPLP0 gene expression as a house-keeping gene.

Primer	Sequence or Reference	
RPLP0	Forwad Primer	5'-CAGAGGTGCTGGACATCACAG-3'
	Reverse Primer	5'-CACATTGCGGACACCCTCTAG-3'
p53	Forwad Primer	5'-CGAGATGTTCCGAGAGCTGAATG
	Reverse Primer	5'-GTCTTCGGGTAGCTGGAGTG
Bax	Forwad Primer	5'- ACCAGGGTGGCTGGGATG
	Reverse Primer	5'- GTGAGTGAGGCAGTGAGGAC
DAPK1	Rn01442011_m1	(Life Technologies Corporation, NY, USA)
NMDA NR2B	Rn00680474_m1	(Life Technologies Corporation, NY, USA)
p38	Rn00578842_m1	(Life Technologies Corporation, NY, USA)
ERK5	Rn01466573_g1	(Life Technologies Corporation, NY, USA)
Akt	Rn00583646_m1	(Life Technologies Corporation, NY, USA)

Table 2: References of genes used in this study

2.5 Morphological and Statistical Analysis

Photographs of DAPK1 and ATF3 stained sections were taken using an AxioCam HRc Digital camera with AxioVision 3.0 software to capture images (Carl Zeiss, Germany). The resulting files were used to generate the figures in Adobe PhotoShop CS6. AxioVision 3.0 software has been used to count number of neurons that express DAPK1, ATF3 and merged of both in L5 DRG neurons 7 days and 14 days after sciatic nerve transection.

AxioVision 3.0 software was also used to measure the surface area of DAPK1- and NMDA NR2B-labeled neurons in the DRG. Only neuronal profiles showing

obvious nuclei were included in the analysis. Neuron measurements were expressed as cross-sectional areas. Three sections per L5 ganglion from three rats were used. The number of neurons counted for each time points were in the range of 240 to 600 neurons from three independent animals in each group. The data were transferred to Excel program (Microsoft) spreadsheets for further statistical and graphical analyses.

A *t*-test for equality of means was used to find out whether DAPK1 significantly decreased in the injured neurons 14 days as compared with 7 days after the injury. An ANOVA followed by a Tukey post-test was used to analyze DAPK1 and NMDA NR2B surface size measurement results.

All data are reported as the mean \pm standard error of the mean. Gene expression results were analyzed using an ANOVA followed by a Tukey post-test. Statistical significance was accepted at $p < 0.05$ level. All analyses were done using Prism software (Prism v.6 GraphPad Software Inc., San Diego, CA).

CHAPTER 3: RESULTS

3.1 DAPK1 Gene Expression in the Dorsal Root Ganglion

The expression pattern of the genes of DAPK1, NMDA NR2B (Grin2b), p38 (MAPK14), p53, Bax, ERK5, and Akt was measured at 2 hours, 7 days and 14 days after sciatic nerve injury using TaqMan gene expression assay qRT PCR (n=18). The selection of genes was based on either their documented relation with DAPK1 or their role in peripheral nerve injury. Our goals in this experiment were: First, to investigate the expression of DAPK1 gene in the normal dorsal root ganglion (DRG) neurons. Secondly, to study the relative expression of DAPK1 gene in DRG neurons at different times following peripheral nerve axotomy. Thirdly, to study the relative expression of key pro- and anti-apoptotic genes of NMDA NR2B (Grin2b), p38 (MAPK14), p53, Bax, ERK5, and Akt in relation to DAPK1 expression at the designated time intervals.

The expression of each gene was normalised to the endogenous control gene 60S acidic ribosomal protein P0 (RPLP0) for that sample which gives an 'absolute expression' of the gene. The results showed that DAPK1 mRNA is expressed in normal uninjured DRG neurons (Figure 10A). At an early stage (2 hours) after injury, the level of DAPK1 was significantly increased in the injured DRG compared to uninjured DRG on the contralateral side ($p < 0.05$, 2.2 fold). However, at a later stage, a profound decrease in DAPK1 levels was observed at 7 days with a further reduction at 14 days ($p < 0.05$, 0.4 fold) postoperatively compared with the contralateral uninjured side.

Furthermore, we found that NMDA NR2B had a remarkable mRNA increase pattern during the course studied as its level increased at 7 days and continued to up-regulate dramatically at 14 days postoperatively ($p < 0.05$, 3.6 fold) (Figure 10B).



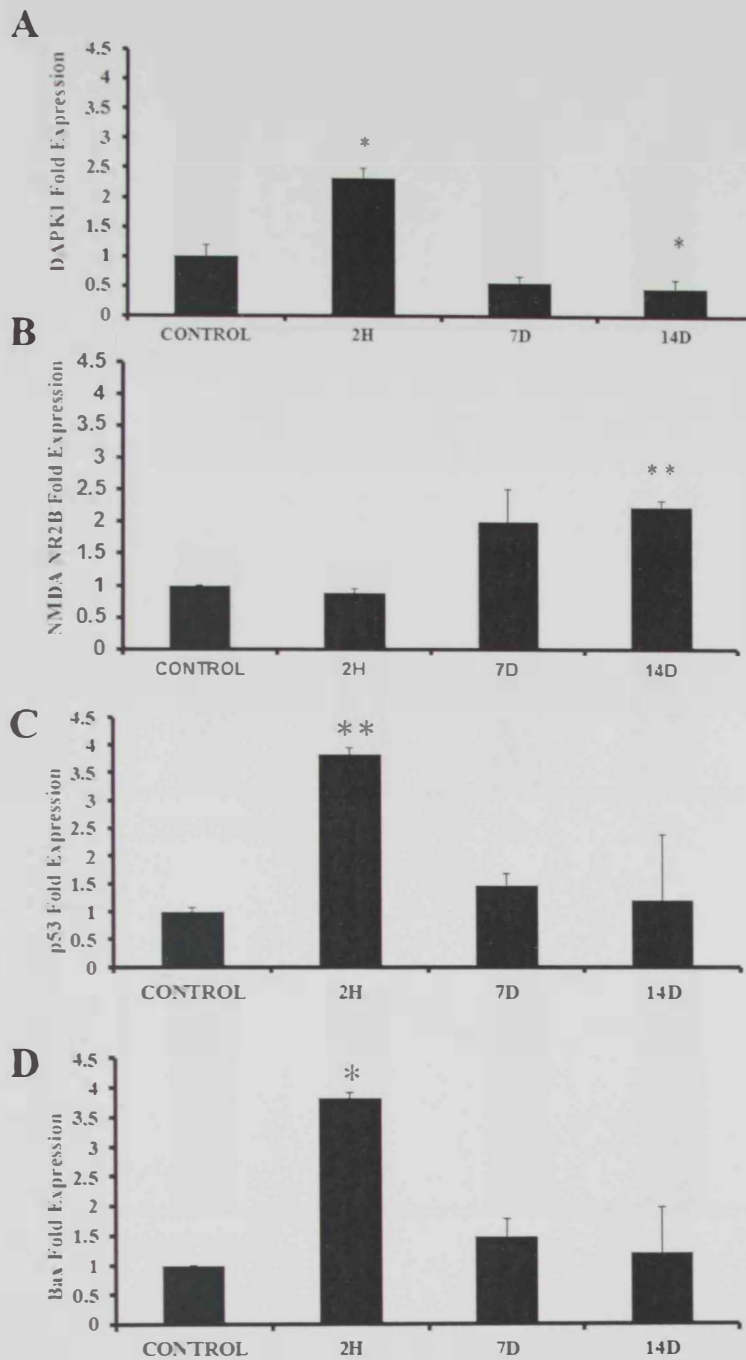


Figure 10: Real time qRT-PCR results of total DAPK1, NMDA NR2B, p53, and Bax mRNA levels following sciatic nerve injury

DAPK1 (A), NMDA NR2B (B), p53 (C) and Bax (D) mRNA levels in injured L4 and L5 DRG compared with the uninjured side after 2 hours, 7 and 14 days postoperatively. * $p < 0.05$. ** $p < 0.01$ and *** $p < 0.001$. Data are expressed as mean (\pm SEM).

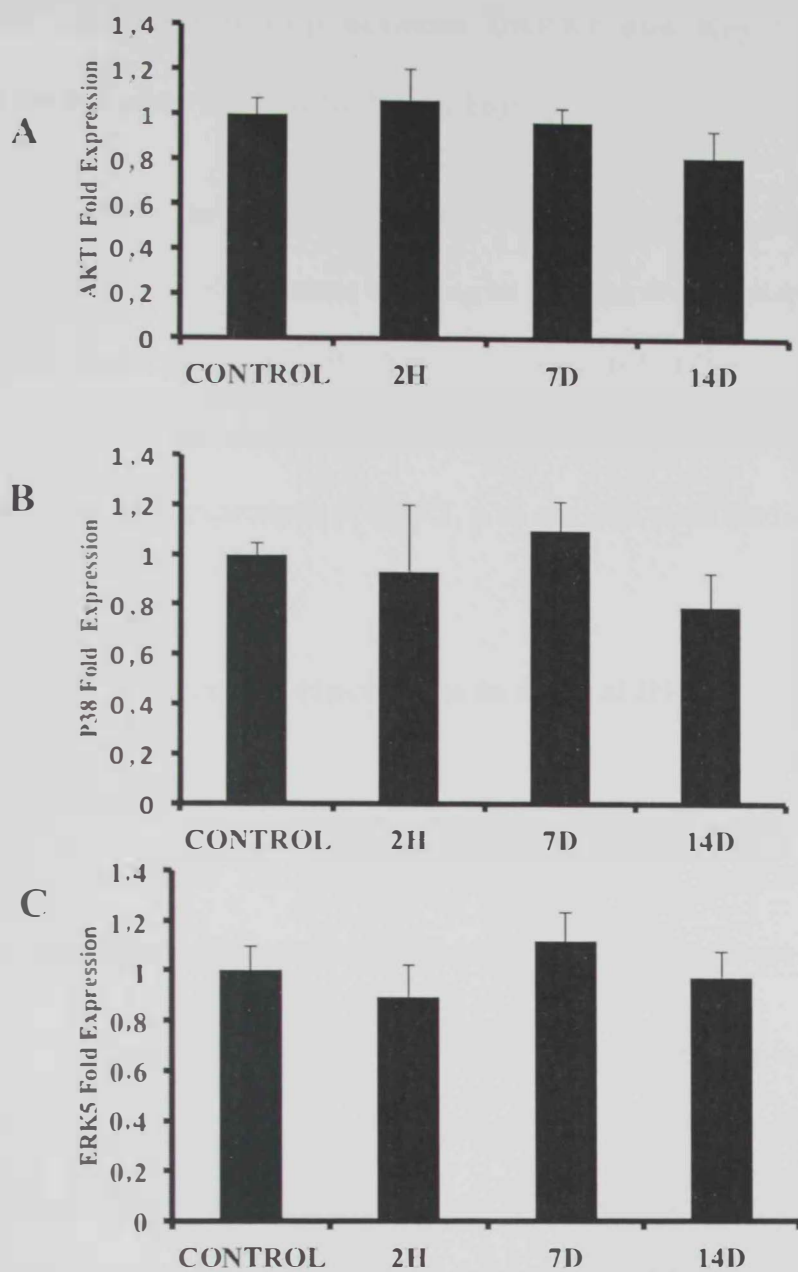


Figure 11: Real time qRT-PCR results of total Akt, p38, ERK5 mRNA levels following sciatic nerve injury

Akt (A), p38 (B), ERK5 (C) mRNA levels in the injured L4 and L5 dorsal root ganglia compared with the non-injured side at (2 hours, 7 and 14 days) after injury. * $p < 0.05$. ** $p < 0.01$ and *** $p < 0.001$. Data are expressed as mean (\pm SEM).

3.2 The Relationship between DAPK1 and Key Pro- and Anti-Apoptotic Genes Following Sciatic Nerve Injury

Interestingly, the expression patterns of both p53 and Bax go hand in hand with the level of DAPK1 in DRG neurons showing an increase at early stage (3.8 fold and 3.7 fold at 2 hours respectively, $p < 0.01$) then down-regulated at 7 and 14 days after injury (Figure 11-A, C, D). In comparison, the results revealed that injury of the sciatic nerve did not alter the gene expression of ERK5, p38 and Akt at all studied time intervals (Figure 11 A-C).

3.3 DAPK1 protein expressions in normal DRG

The aim of this experiment was to investigate the expression pattern of DAPK1 protein in normal intact DRG neurons. CGRP and IB4 were used as normal contents of unmyelinated and presumably thin myelinated DRG neurons (Shehab, 2011).

The results showed that DAPK1 immunoreactivity is normally present in normal intact L4 and L5 DRG and were discernible in small to medium-sized diameter neurons (Figure 12). Although both IB4- and CGRP-immunoreactivities were detected in small sized neurons, CGRP was also observed in medium- to large-sized neurons. Triple immunofluorescent labelling showed that the vast majority of DAPK1 positive neurons were IB4 positive (74.40%). Some DAPK1 immunofluorescent positively labelled neurons were also CGRP (45.78 %) and some of them expressed both CGRP and IB4 (29.76%) (Figure 12).

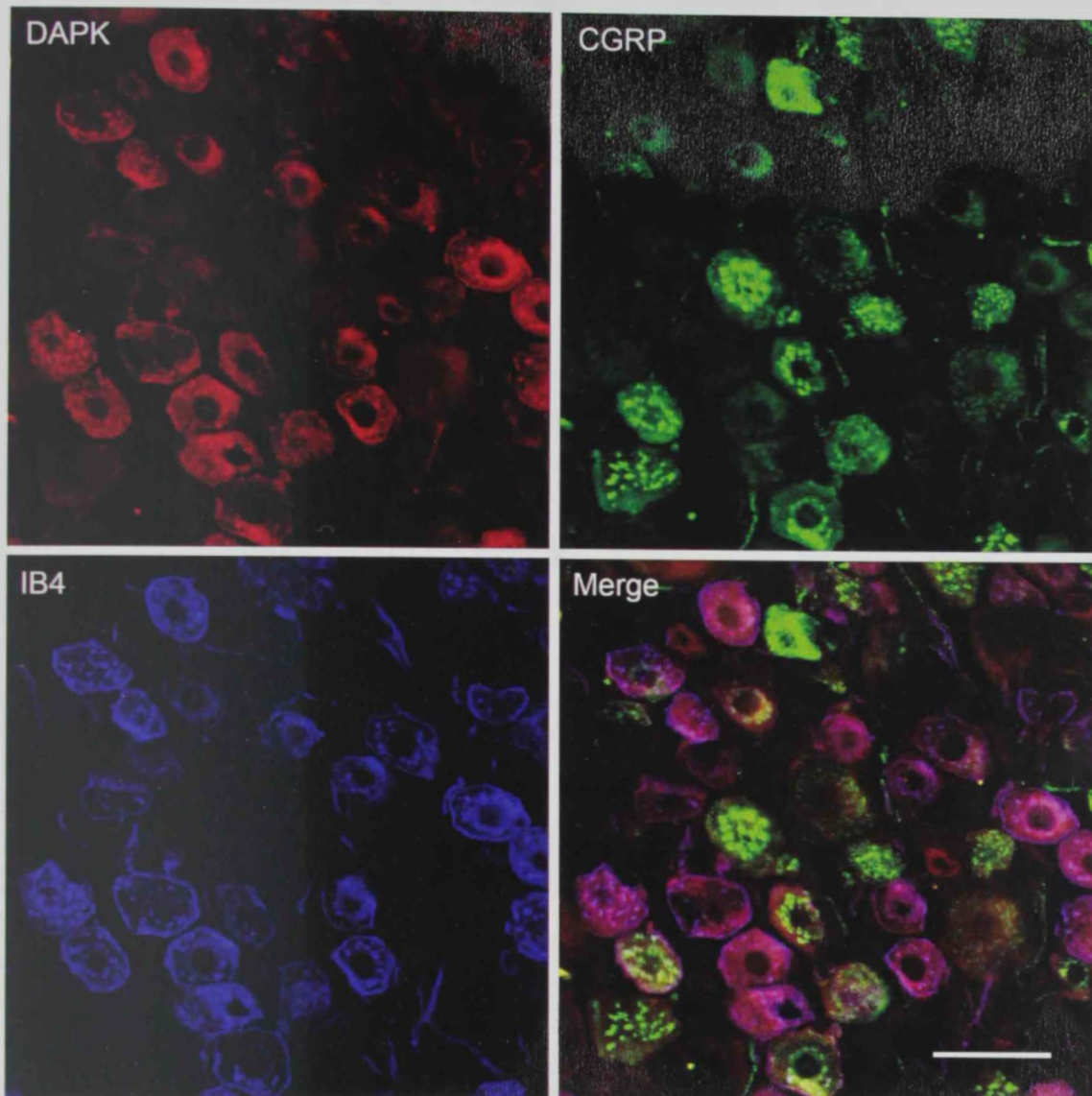


Figure 12: DAPK1, CGRP and IB4 triple immunostaining in normal intact L5 DRG neurons

DAPK1 (red), CGRP (green) and IB4 (blue) triple immunostaining in normal intact L5 DRG neurons. DAPK1 immunoreactivity in normal DRG neurons was discernible primarily in small to medium-sized diameter neurons. Triple immunofluorescent labelling showed that most of DAPK1 positive cells were IB4 positive. Moreover, some DAPK1 positive neurons co-stained both CGRP and IB4. Confocal image with Scale bar = 50 μ m.

3.4 DAPK1 and ATF3 Protein Expressions in DRG

The aim of this experiment was to investigate the expression pattern of DAPK1 protein in normal and injured L4 and L5 DRG neurons during various time intervals (2 hours, 7 days and 14 days) using the peroxidase method of immunohistochemistry and immunofluorescence staining. ATF3 was used as a marker for neuronal injury. The results showed that DAPK1 was expressed in normal uninjured L4 and L5 DRG neurons and was discernible primarily to small to medium sized diameter neurons (Figure 13D and Figure 14D). In line with our gene expression results, our immunofluorescence labelling showed strong immunoreactivity of DAPK1 in DRG neurons in L4 (Figure 13G) and L5 (Figure 14G) after 2 hours.

Similarly as observed with the qRT PCR technique, a decrease in immunostaining of DAPK1 was seen after 7 days and 14 days postoperatively in L4 (Figure 13 J.M) and L5 (Figure 14 JM) DRG after injury. The immunofluorescent double labelling showed that 76.11% (Figure 16) of DAPK1 positive cells were also ATF3 positive in the DRG of both L4 (Figure 13-J-L) and L5 (Figure 14-J-L). However, 14 days following axotomy, significantly less neurons were double labeled ($t = 4.831$ $df = 16$) (Figure 16, $p < 0.001$) as only 53.89% of DAPK1 positive cells were ATF3 positive in both L4 (Figure 13M-O) and L5 (Figure 14M-O) DRG. The individual mean values of DAPK1 immunoreactivity in injured ATF3 positive neurons for both 7 days and 14 days are provided (Table 3).

Number of days postoperatively	Mean \pm SEM	Difference between means:
7 days	76.11 \pm 3.510	-22.22 \pm 4.600
14 days	53.89 \pm 2.974	95% confidence interval: -31.97 to -12.47
R squared: 0.5932, t=4.831 df=16		

Table 3: Comparison between the number of DAPK1 +ve neurons in injured ATF3 +ve L5 DRG 7 days and 14 days after sciatic nerve transection.

In support of the above findings, peroxidase immunohistochemical staining produced similar results to DAPK1 immunoreactivity after 2 hours, 7 days and 14 days in injured L4 DRG (Figure 15 B-D) compared to the uninjured side (Figure 15 A).

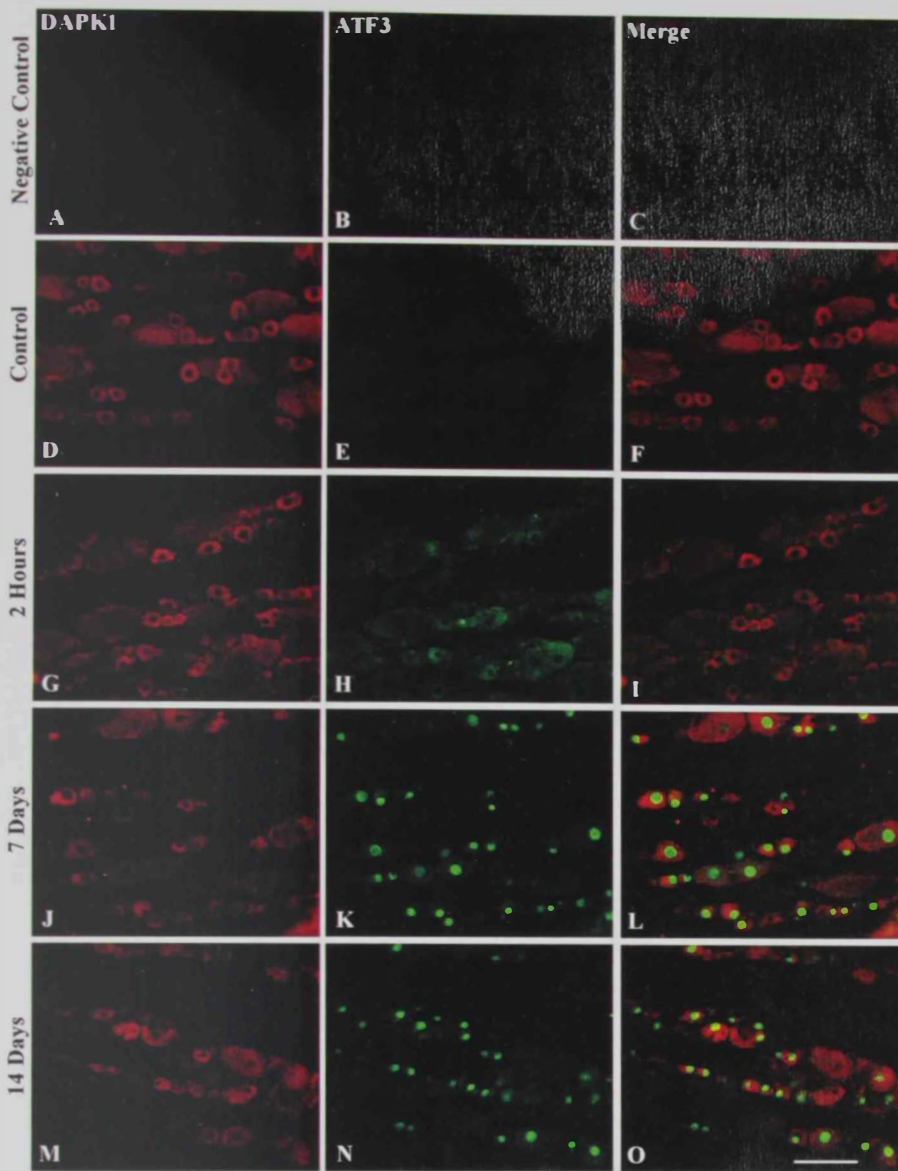


Figure 13: DAPK1 and ATF3 double immunostaining in uninjured and injured L4 DRG 2 hours, 7 days and 14 days after sciatic nerve transection.

DAPK1 (red) and ATF3 (green) double immunostaining in uninjured and injured L4 DRG 2 hours, 7 days and 14 days after sciatic nerve transection. DAPK1 immunoreactivity in uninjured (D) and injured (G) DRG 2 hours postoperatively is discernible primarily to small to medium-sized diameter neurons. The number of DAPK1 immunoreactive neurons decreased 7 days (J) and 14 days (M) postoperatively. Double immunofluorescent labelling showed that most of DAPK1 positive cells were injured neurons as they were also ATF3 positive (J-L) after 7 days of surgery. However, the number of DAPK1 +ve neurons which are also ATF3 +ve decreased after 14 days following axotomy in L4 (M-O). Panels A-C are images of negative control staining of DAPK1 and ATF3 respectively. Scale bar = 100µm.

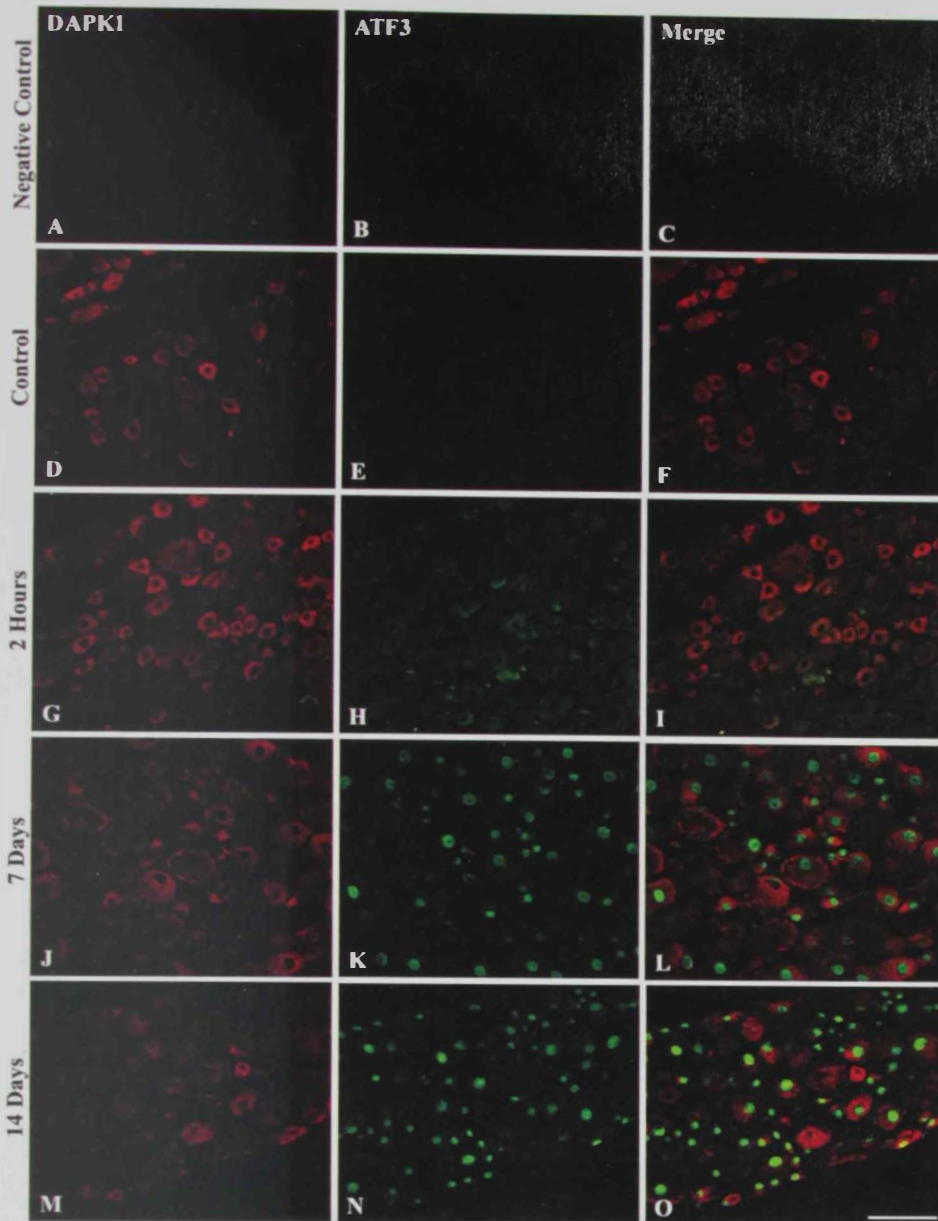


Figure 14: DAPK1 and ATF3 double immunostaining in uninjured and injured L5 DRG 2 hours, 7 days and 14 days after sciatic nerve transection

DAPK1 (red) and ATF3 (green) double immunostaining in uninjured and injured L5 DRG after 2 hours, 7 days and 14 days following sciatic nerve transection. DAPK1 immunoreactivity in uninjured (D) and injured (G) DRG 2 hours postoperatively is discernible primarily to the small to medium diameter neurons. The number of DAPK1 immunoreactive neurons decreased 7 days (J) and 14 days (M) postoperatively. Double immunofluorescent labelling showed that most of DAPK1 positive cells were injured neurons as they were also ATF3 positive (J- L) after 7 days of surgery. However, the number of DAPK1 +ve neurons which are also ATF3 +ve decreased after 14 days following axotomy in L5 (M-O). Panels A-C are images of negative control staining of DAPK1 and ATF3 respectively. Scale bar =100 μ m.

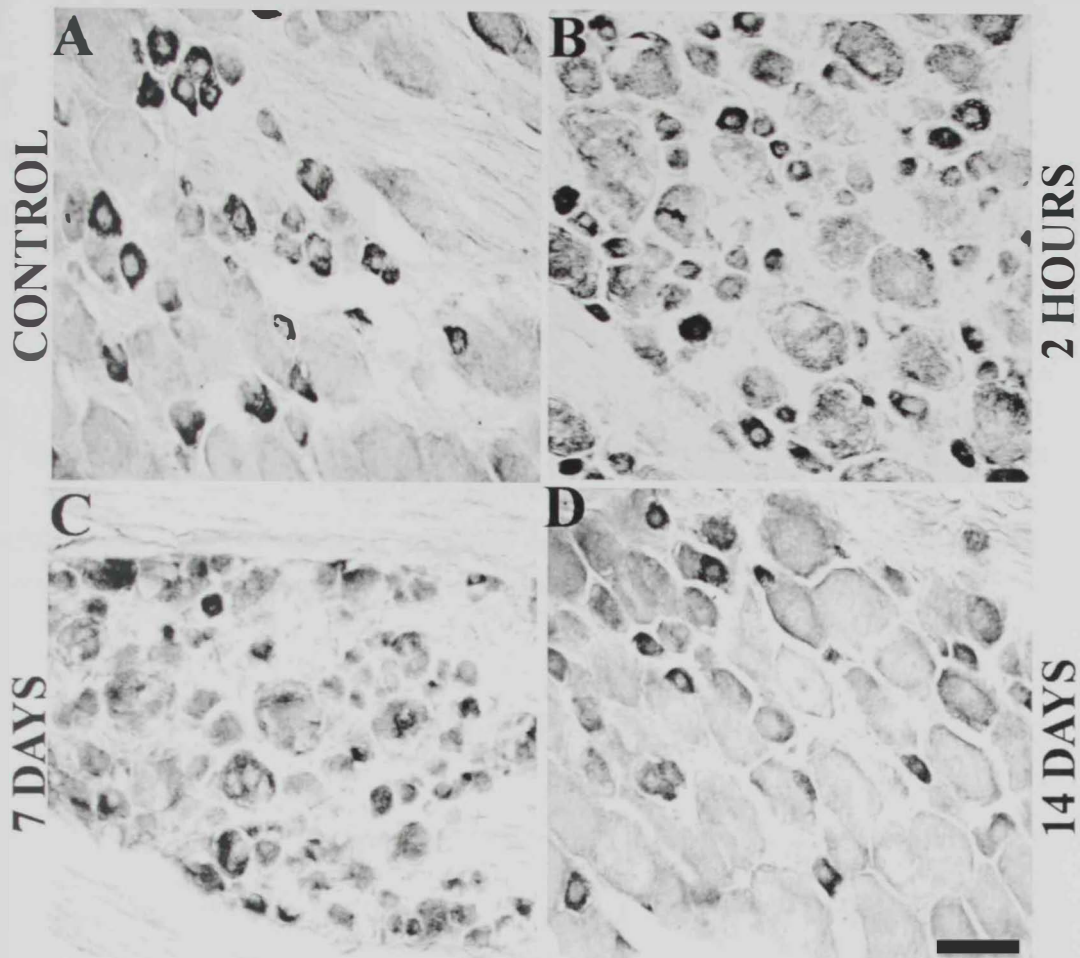


Figure 15: DAPK1 immunoreactivity in uninjured and injured L4 DRG 2 hours and 7 and 14 days after sciatic nerve transection as revealed by peroxidase method of immunohistochemistry.

DAPK1 immunoreactivity in uninjured (A) and injured (B) DRG 2 hours postoperatively is discernible primarily to small to medium-sized diameter neurons. The number of DAPK1 immunoreactive neurons decreased 7 (C) and 14 days (D) postoperatively. Scale bar = 100 μ m.

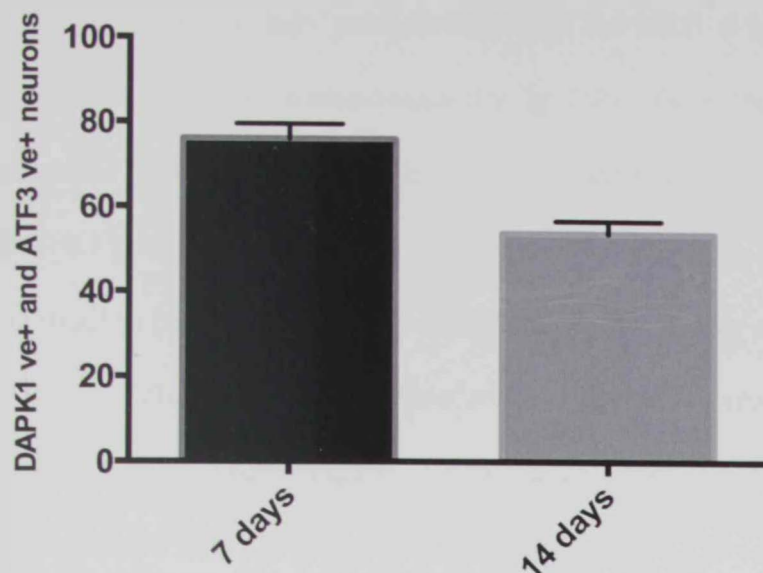


Figure 16: Statistical analysis of DAPK1 immunoreactivity in injured L5 DRG neurons 7 and 14 days after sciatic nerve transection

DAPK1 positive neurons showed a significant decrease ($p < 0.001$) in ATF3 positive injured neurons 14 days compared to 7 days postoperatively. Data are expressed as mean (\pm SEM).

3.5 DAPK1–NMDA NR2B relationship in the DRG

Since DAPK1 has been documented to play a role in neuronal death in the brain via its interaction with NMDA NR2B protein (Tu et al., 2010), we tested this relationship in DRG following peripheral nerve injury. Immunofluorescence staining was used to measure the level of protein expression of DAPK1 and NMDA NR2B in the DRG 2 hours, 7 days and 14 days after sciatic axotomy.

Our results showed that NMDA NR2B is present in normal small- and medium-sized diameter DRG neurons (Figure 17E). Our immunofluorescent and peroxidase immunohistochemistry staining also showed that a decrease occurred in the number of

NMDA NR2B labelled neurons in ipsilateral ganglia after 7 days with a further reduction after 14 days postoperatively in L4 DRG (Figure 17K,N and Figure 19B), compared with the immunoreactivity in DRG from the contralateral uninjured side (Figure 17E and Figure 19A). In addition, statistical analysis showed that 98.675% of all DAPK1 positive neurons were co-localized with NMDA NR2B positive neurons mainly of small to medium-size (<900 μm^2) but really in large neurons (>900 μm^2), using an ANOVA followed by a Tukey test as a multiple comparison as a post test in normal L4 and L5 DRG neurons (Figure 18 and Figure 17D-F). This co-localization has been also detected at 2 hours (Figure 17G-E), 7 days (Figure 17J-L) and 14 days (Figure 17M-O) where all DAPK1 positively labelled neurons were also NMDA NR2B positive in L4 DRG after nerve transection and vice versa.

We have noticed that DAPK1 and NMDA NR2B followed a similar protein immuno-labelling in the cytoplasm of small- to medium-sized DRG neurons (Figure 18) and they both showed a clear decline in their expression levels at 14 days (Figure 17E, J). The result of the NMDA NR2B immunostaining contradicts the NMDA NR2B gene expression analysis where its level went high at 7 and 14 days following surgery (Figure 10B). Interestingly, the NMDA NR2B- but not DAPK1-immunoreactivity was expressed in the DRG forming an envelope around their large-sized neurons both at 7 days (Figure 17J-L) and 14 days (Figure 17M-O) postoperatively. This expression of NMDA NR2B around large neurons was detected in both L4 and L5 DRGs (Figure 17B) (Figure 17M-O).

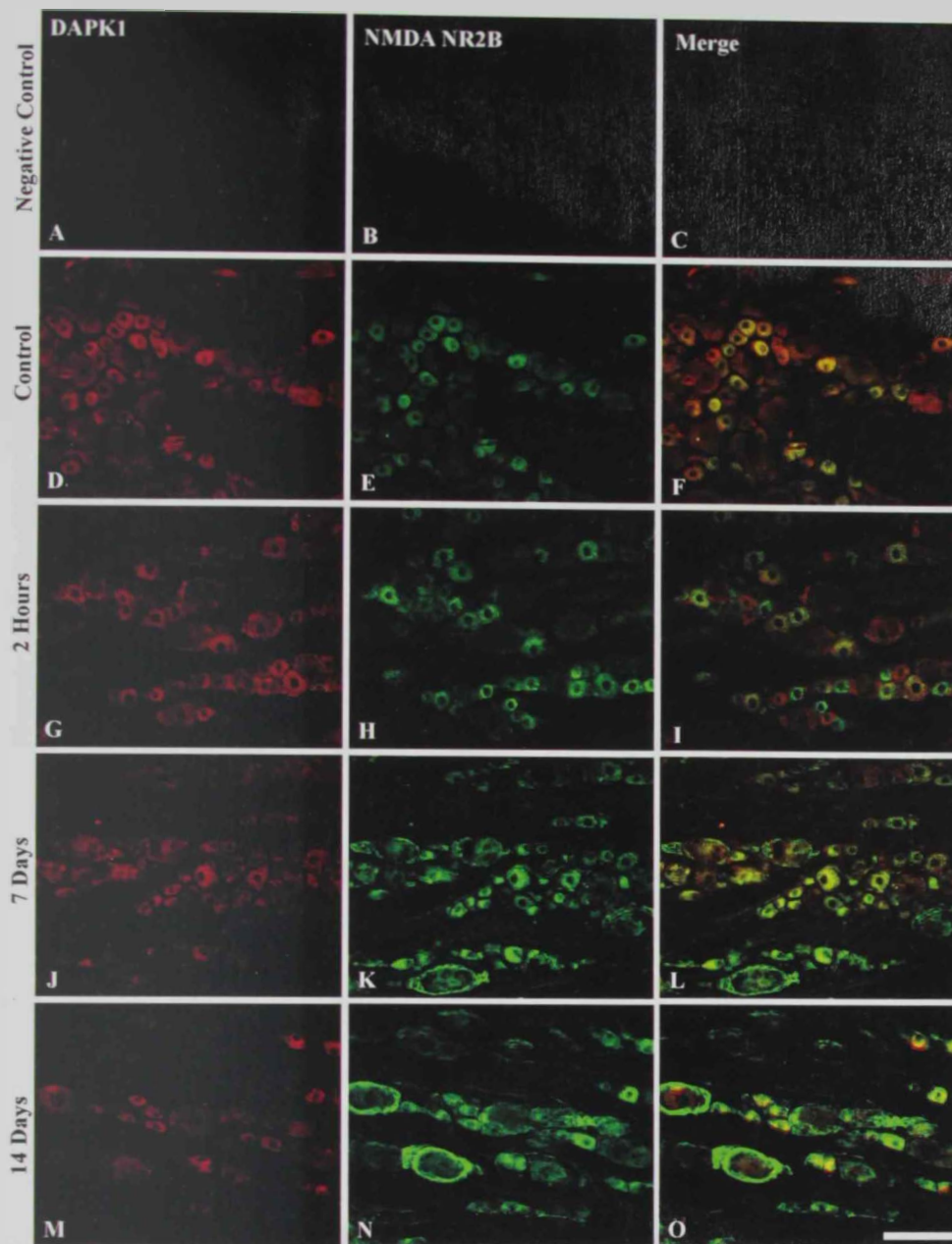


Figure 17: DAPK1 and NMDA NR2B double immunostaining in uninjured and injured L4 DRG 2 hours, 7 days and 14 days after sciatic nerve transection

DAPK1 (red) and NMDA NR2B (green) double immunostaining in uninjured and injured L4 DRG 2 hours, 7 days and 14 days after sciatic nerve transection. DAPK1 immunoreactivity in uninjured (D) and injured (G) DRG 2 hours postoperatively is discernible primarily in small to medium-sized diameter neurons. The number of DAPK1 immunoreactive neurons decreased after 7 days (J) and 14 days (M) postoperatively. Double immunofluorescent labelling showed that the majority of DAPK1 positive cells were also NMDA NR2B positive (J- L) 2 hours and 7-14 days after axotomy. Panels A-C are images of negative controls staining of DAPK1 and NMDA NR2B, respectively. Scale bar =100 μ m.

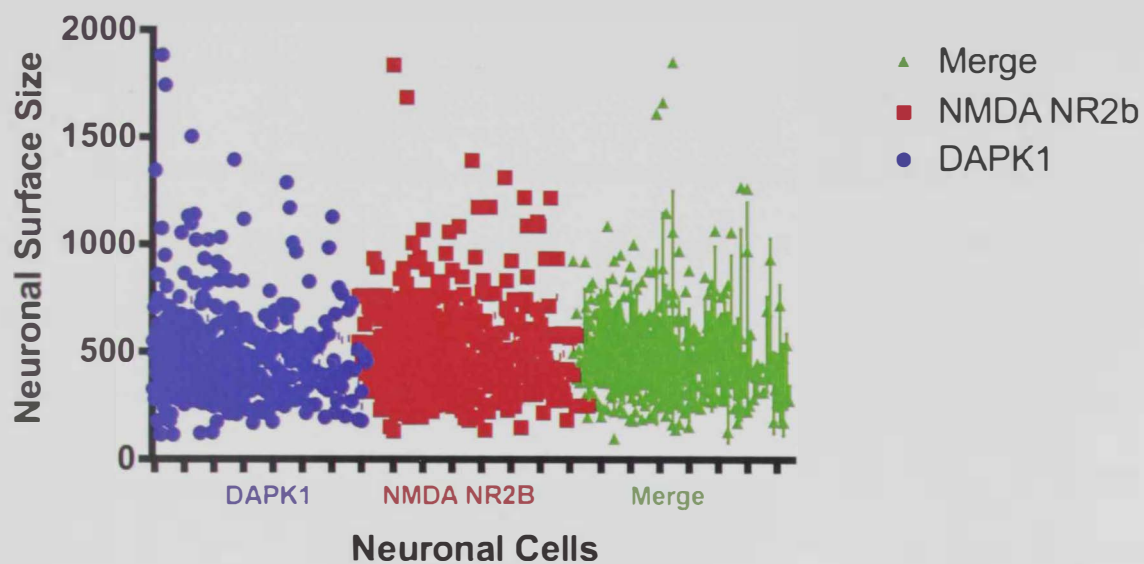


Figure 18: DAPK1 and NMDA NR2B neuronal surface size of normal DRGs neurons

DAPK1 (blue), NMDA NR2B (red) and merge (green) neuronal surface area of normal DRGs neurons. The graph shows that DAPK1 and NMDA NR2B are co-localized in normal L5 DRG neurons. DAPK1 and NMDA NR2B have almost the same disruption pattern and neuronal surface area. Both DAPK1 and NMDA NR2B are mainly expressed in small to medium-sized DRG neurons. Data represent ANOVA test using Prism version6.

3.6 NMDA NR2B in Satellite Cells of Large Injured Neurons

Having established an increase in the NMDA NR2B immunoreactivity at 7 days and 14 days after injury, the next experiment was designed to answer 3 questions: (i) Does NMDA NR2B express in satellite cells? (ii) If so, does NMDA NR2B express in satellite cells around small- or large-sized neurons in the DRG after nerve injury? (iii) Are these neurons, which are surrounded by NMDA NR2B immunoreactivity, injured or uninjured cells?

To answer these questions, double immunofluorescent staining of NMDA NR2B and GFAP (glial fibrillary acidic protein) as a marker for satellite cells (n=4) and NMDA NR2B with ATF3 as a marker for injury (n=4) was carried out. The co-localization of NMDA NR2B and GFAP immunoreactivities around large neurons indicates an increased level of NMDA NR2B in active satellite cells 7 days after sciatic nerve injury (Figure 20G-E). An intense immunoreactivity for co-localized GFAP and NMDA NR2B was noticed 14 days postoperatively (Figure 20J-L). Our immunofluorescent staining also showed that these large DRG neurons were injured cells as they were all ATF3 positive at 7 days (Figure 21G-E) and 14 days (Figure 21J-L) postoperatively.

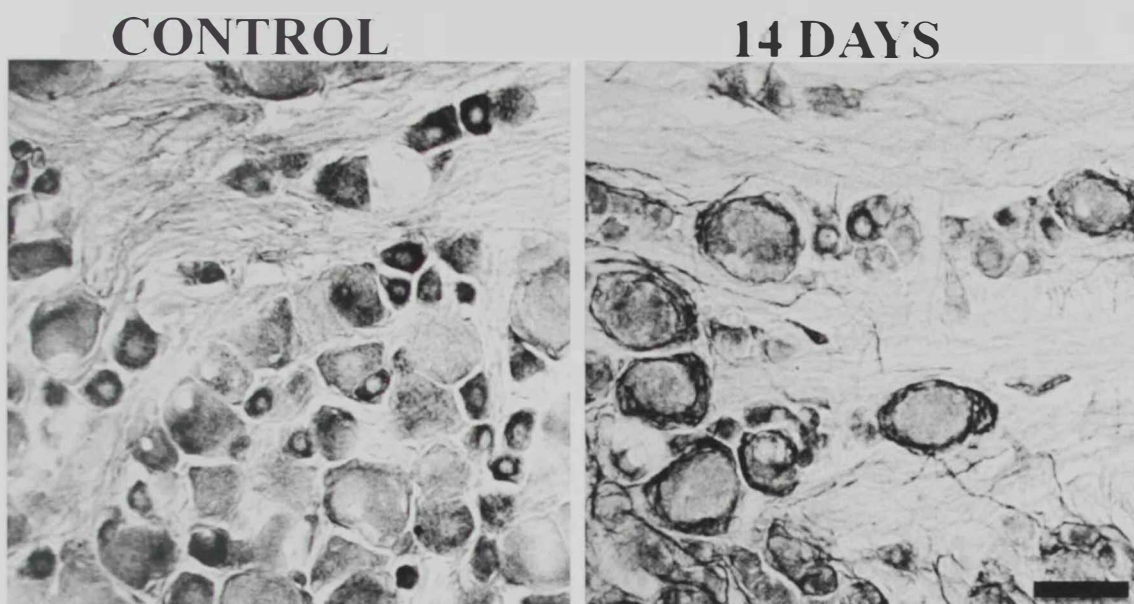


Figure 19: NMDA NR2B immunoreactivity 14 days after sciatic nerve injury in the ipsilateral L4 DRG compared with the control contralateral side revealed with peroxidase method of immunohistochemistry

NMDA NR2B immunoreactivity is highly expressed around large-sized neurons forming an envelope surrounding the cell bodies in injured neurons (B) which was not observed in the uninjured side (A). Scale bar =100 μ m.

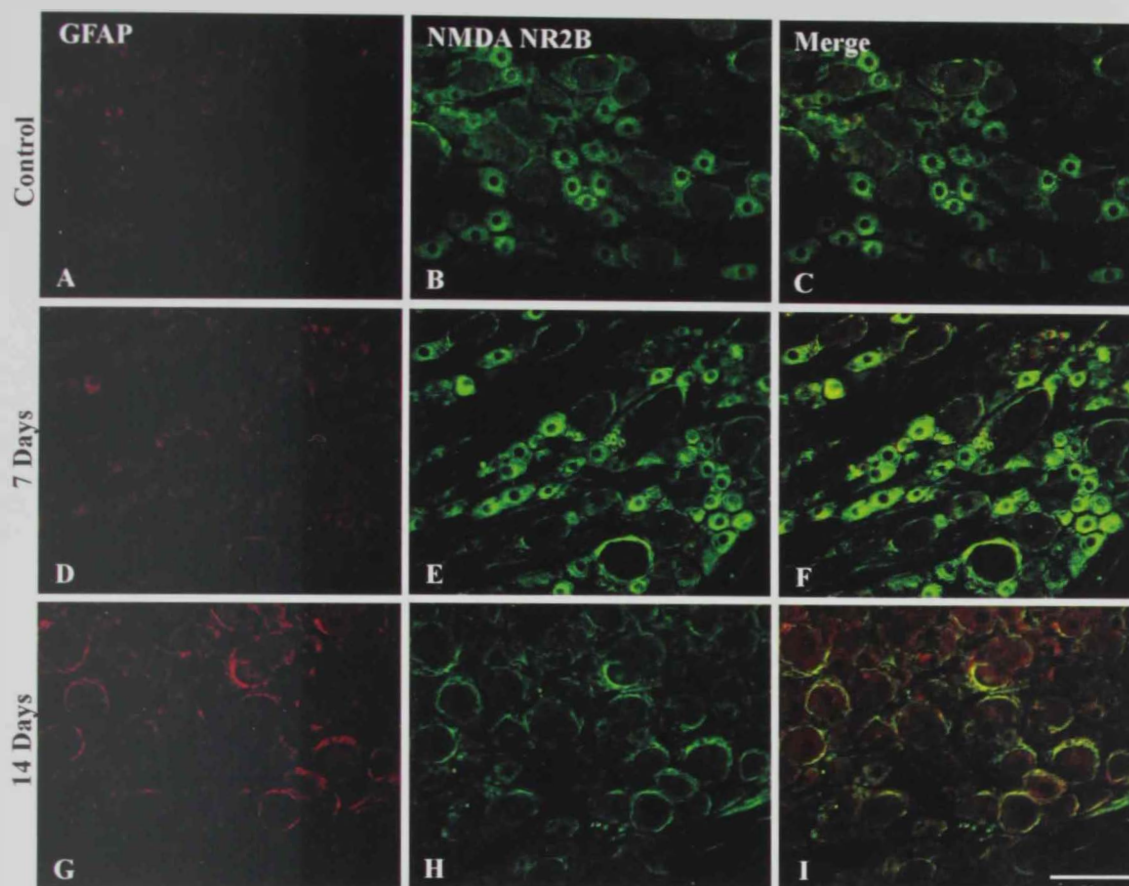


Figure 20: Effects of sciatic nerve transection after 7 and 14 days on the expression of GFAP and NMDA NR2B in the injured L4 DRG

Effects of sciatic nerve transection after 7 and 14 days on the expression of GFAP (**red**) and NMDA NR2B (**green**) in the injured L4 DRG. Note the down-regulation of NMDA NR2B immunoreactivity in the small-sized neurons and its up-regulation around the large-sized neurons (**H**) and the up-regulation of GFAP (**D, G**). The co-localization of NMDA NR2B and GFAP immunoreactivity (**F, I**) indicates its up-regulation in the satellite cells surrounding large neurons. Scale bar = 100 μ m.

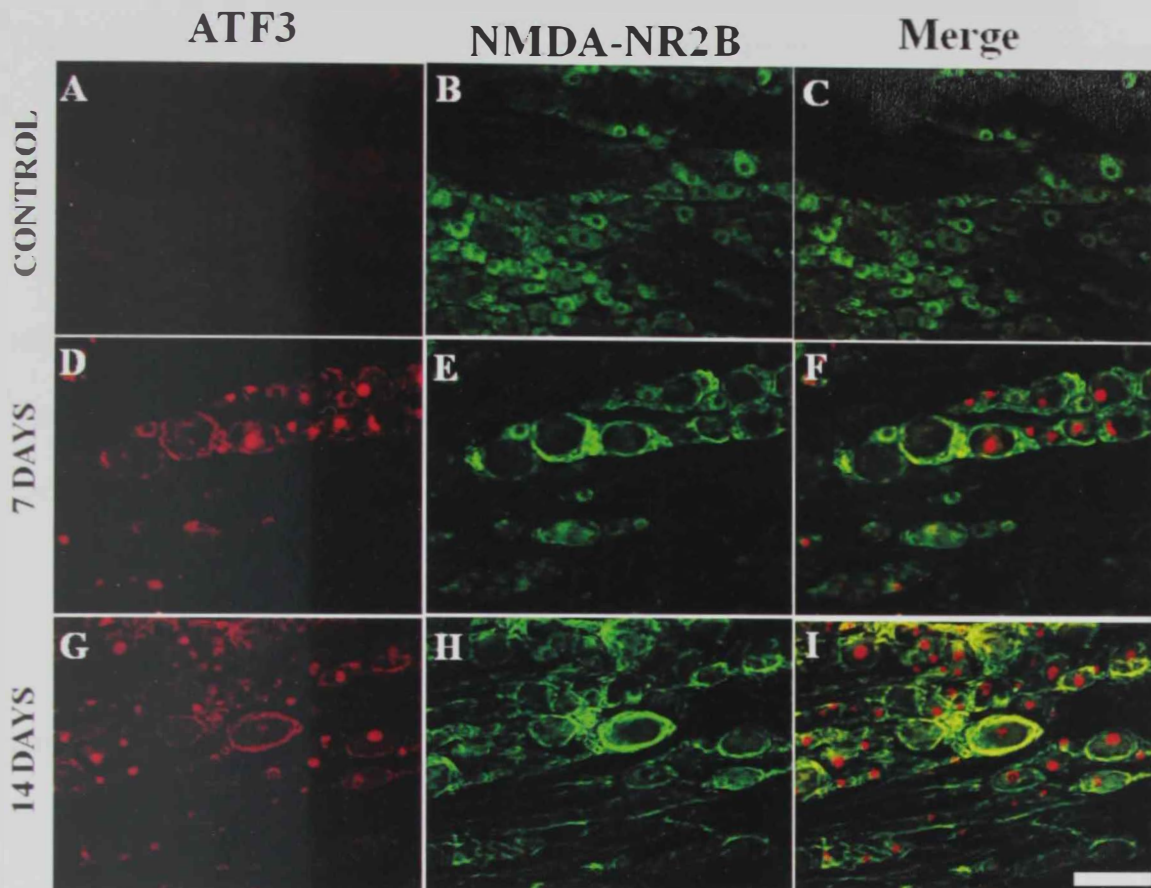


Figure 21: ATF3 and NMDA NR2B double immunostaining in uninjured and injured L4 DRG after 7 and 14 days following sciatic nerve transection

ATF3 (as a neuronal injury marker, **red**) and NMDA NR2B (**green**) double immunostaining in uninjured and injured L4 DRG after 7 and 14 days following sciatic nerve transection. NMDA NR2B immunoreactivity was reduced in the small neurons 7-14 days postoperatively (E, H) but was up-regulated in satellite cells surrounding injured large neurons (D-I). Scale bar =100 μ m.

CHAPTER 4: DISCUSSION

The main aims of this study were to investigate DAPK1 expression pattern along with key pro- and anti-apoptotic cell signaling molecules (p53, Bax, Akt, ERK5, p38 and NMDA NR2B) and to verify the possibilities of DAPK1-NMDA NR2B interaction in DRG neurons at 2 hours, 7 days and 14 days following sciatic nerve injury. The findings were: DAPK1 mRNA was expressed and translated to functional protein in normal DRG neurons. Importantly, after nerve injury a prominent increase in DAPK1 mRNA was detected at 2 hours. However, 7 and 14 days postoperatively, DAPK1 displayed a marked down-regulation in its mRNA and protein level. Regarding the relationship between DAPK1 and other pro and anti-apoptotic factors, we found that DAPK1, p53 and Bax exhibited almost the same expression pattern in axotomized L4 and L5 DRG.

Furthermore, our immunostaining was in line with our DAPK1 gene expression analysis observed an early increase in immunoreactivity followed by a reduction in the number of positively labeled cells in a late stage. In addition, NMDA NR2B gene expression analysis showed a continuous up-regulation at 7 days followed by further significant increases 14 days after injury. In contrast, our immunofluorescent stain results showed a decrease in the protein level of NMDA NR2B in DRG neurons during the same time period. Interestingly, a strong positive NMDA NR2B immunoreactivity appeared in the satellite cells that surrounded injured large-sized neurons in both L4 and L5 DRG.

Taken together these results suggest that DAPK1 might have an important role, along with other pro-apoptotic molecules and down-regulation in the NMDA NR2B in

injured neurons, in peripheral nerve regeneration. The results might also suggest the involvement of up-regulation of NMDA NR2B in satellite cells and not neurons in the DRG in the development of neuropathic pain 7-14 days after peripheral nerve injury.

4.1 DAPK1 Expression in Normal DRG

DAPK1 is an intracellular protein that mediates and transmits apoptotic cell death signals in various cells, including CNS neurons. DAPK1 expression is significantly elevated in the rat brain in acute models of injury such as ischemia and seizure (Pelled et al., 2002; Schumacher et al., 2002; Shamloo et al., 2005). A previous study showed that in DAPK1 knockout mice a dramatic reduction in infarct volume and enhanced neurological function following cerebral ischemia (Tu et al., 2010). In addition, the DAPK1 activity level was increased in PC12 cells upon exposing to apoptotic induction, and that inhibition of DAPK1 activity rescues the cells (Yamamoto et al., 2002).

In this study, we aimed to find out whether DAPK1 is expressed in the peripheral nervous system and what happens after peripheral nerve injury. Our results showed that DAPK1 mRNA is normally expressed and translated to functional protein in normal intact uninjured L4 and L5 DRG neurons and is found primarily in the small to medium diameter neurons. Some previous studies have shown that the over-expression of DAPK1 under normal growth conditions does not induce a caspase-dependent apoptosis (Inbal et al., 1997; Jin et al., 2001).

In normally developing neurons, DAPK1 not only plays the classical role of programmed cell death in post-mitotic neurons during development, but also other neuronal functions have been suggested. This was based on analysis of patterns of

DAPK1 expression where DAPK1 mRNA was initially observed in the mantle and ventricular zones of the entire neuraxis in the early stages of brain formation. This expression decreased remarkably in the brain after birth, limiting it to restricted mature neuronal populations including olfactory bulb, hippocampal formation, cerebellar Purkinje and granule cells. This differentially regulated expression of DAPK1 during development and its restricted expression in mature neuronal population indicate that DAPK1 might be involved in some neuronal functions besides executing the developmental neuronal-programmed cell death (Sakagami and Kondo, 1997). Accordingly, we suggest that the presence of DAPK1 in normal DRG neurons may associate with its normal physiological function.

In addition, our results showed that DAPK1 expression was mainly discernible in small to medium DRG neurons. Most of DRG nociceptive neurons are small diameter neurons (Basbaum et al., 2009). Therefore, DAPK1 may play a role in modulating nociception in PNS.

4.2 DAPK1 expression in injured PNS

4.2.1 DAPK1 Expression at Early Stage of Peripheral Nerve Injury

This study showed sciatic nerve axotomy caused a dramatic change in DAPK1 expression pattern. As a result of injury, DAPK1 gene expression is highly up-regulated at early stage (2 hours) in injured L4 and L5 DRG compared with contralateral uninjured neurons. Similarly, at this time point our immunohistochemistry results showed that the

numbers of DAPK1 positively labelled neurons were markedly increased, and localized primarily in the neuronal cytoplasm.

It is well documented that injured neurons switch the expression of regeneration-associated genes on and off according to the regeneration program (Raivich and Makwana, 2007; Sun and He, 2010). Raivich et al. (1991) found that sciatic nerve axonal injury interferes with the retrograde immunofluorescence of trophic signals, disinhibiting the normally suppressed regenerative process, within 12–24 hours following injury. Nerve injury activates intracellular cascades like the mitogen-activated protein kinase (MAPK) pathway and the phosphoinositide 3-kinase (PI3K) pathway in axotomized neurons (Hammarlund et al., 2009; Kiryu et al., 1995; Liu et al., 1997; Markus et al., 2002; Namikawa et al., 2000; Park et al., 2008; Zhou and Snider, 2005). Although, our knowledge about DAPK1 activities in PNS is limited and all the previously available data from CNS, *in vivo* and *in vitro* studies showed that an increase in DAPK1 expression levels is always associated with neuronal injury events (Henshall et al., 2003; Araki et al., 2004; Shamloo et al., 2005). Thus, we suggest that DAPK1 up-regulation at this early stage may interfere with the synthesis of immediate PNS injury response and may take part in various cell signaling pathways.

4.2.2 DAPK1 Expression at Late Stage of Peripheral Nerve Injury

Previous studies carried out in CNS, showed an increase in DAPK1 level after hippocampus damage (Schumacher et al., 2002; Velentza et al., 2001) and ischemic injury (Shamloo et al., 2005; Tu et al., 2010). In addition, DAPK1 activity level is increased in PC12 cells upon exposure to apoptotic induction and inhibition of DAPK1

activity rescues the cells (Yamamoto et al., 2002). The absence of DAPK1 has been shown to protect neurons from a wide variety of acute toxic insults (Fujita and Yamashita, 2014).

Recently, Kim et al (2014) have shown that DAPK1 inhibition significantly increased microtubule assembly and accelerated nerve growth factors-mediated neurite outgrowth. These findings were demonstrated in the CNS where axons fail to regenerate. However, our study was performed in PNS where injured axons have the ability to regenerate. Our data showed that a profound decrease in DAPK1 level 7 days after peripheral nerve axotomy, with further reduction at 14 days postoperatively. This down-regulation of DAPK1 in this late stage might be a part of the regenerative process and may indicate that the decrease in this cytoskeletal-associated protein kinase is an important component to allow regeneration, neurite outgrowth and may help in recovery of injured DRG neurons.

We have used ATF3 as an injury marker in the nervous system (Nakagomi et al., 2003; Ohba et al., 2003; Tsujino et al., 2000). Our results showed an increase in ATF3 expression in the cytoplasm but not the nucleus of the peripherally transected nerve at 2 hours. The increase in the cytoplasm is indicative of an increase in the synthesis of ATF3. Once synthesized, it is then translocated to the nucleus where we observed it to be abundantly expressed at 7 days and remain so 14 days post injury. The increase in ATF3 expression and nuclear localization is in agreement with previously reported data demonstrating that ATF3 increase robustly in all axotomized sensory DRG neurons

indicating that the induction of ATF3 is part of the overall neuronal response to injury (Averill et al., 2004; Tsujino et al., 2000).

4.3 Relationship of DAPK1 with Key Pro- and Anti-Apoptotic Genes in PNS Injury

Compelling evidence now shows the functional outcome of DAPK1 signaling is mostly dependent on the input signal (Lin et al., 2010). We have found that the expression of both p53 mRNA and Bax was down-regulated 7 days after injury. This pattern was directly proportional to the decrease in the expression of DAPK1 in injured DRG neurons suggesting that DAPK1, p53 and Bax might be regulated through a common molecular pathway following peripheral nerve injury. Martoriati et al. (2005) have demonstrated DAPK1 as a transcriptional target of p53 as both showed a signaling feedback loop in which DAPK1 and p53 activated each other.

Recently, it has been shown that DAPK1 death domain (DAPK1DD) directly bind to DNA binding motif of p53 (p53DM) and phosphorylates p53 at serine-23 (p S 23) which activates Bax and mediates ischemic neuronal death in brain (Araki et al., 2004, Pei et al., 2014). The disrupting of DAPK1DD and p53DM interaction has been suggested to be therapeutically effective against ischemic insults (Pei et al., 2014). Taken together, our results suggest DAPK1 might be an upstream activator of p53, which may activate Bax in response to sciatic nerve injury. These results are demonstrating that DAPK1 down-regulation along with other pro-apoptotic molecules may be a significant contributor to intrinsic regulation of PNS neuronal regeneration.

4.4 DAPK1 and NMDA NR2B Relationship

The present study has also investigated the expression pattern of NMDA NR2B receptor before and after sciatic nerve injury. Tu et al. (2010) recently reported that DAPK1 constitutes a specific cell death-signalling molecule that is linked directly to glutamate receptor channels in the brain. They also demonstrated that DAPK1 physically and functionally interacts with the NMDA NR2B receptor at extra-synaptic sites where it acts as a central mediator for stroke damage. They further showed that DAPK1 was recruited into the NMDA NR2B receptor protein complex in the cortex of adult mice after cerebral ischemia (Tu et al., 2010).

In this study, the immunohistochemical staining showed that there was an interesting relationship between the expression of DAPK1 and NMDA NR2B receptor in the DRG neuron. The results showed that NMDA NR2B mRNA expression increased at 7 days after sciatic nerve injury and continued to up-regulate significantly 14 days postoperatively. In comparison, although DAPK1 was significantly increased after 2 hours, it began to decrease 7-14 days in ipsilateral DRG. The dramatic down regulation of DAPK1 mRNA was shown after 14 days of sciatic nerve transection. These results have been further investigated immunohistochemically.

The expression of NMDA receptors has been already established in previous studies, which demonstrated that they are expressed in the cell bodies of primary afferent neurons (Ma and Hargreaves, 2000; Sato et al., 1993; Shigemoto et al., 1992) and are found on peripheral primary afferent processes (Carlton and Hargett, 1995; Cincotta et al., 1989; Coggeshall and Carlton, 1998a; Kinkelin et al., 2000). It has also

been shown that glutamate receptors are predominantly expressed on small- and medium-sized neurons (Lee et al., 2001; Sato et al., 1993; Willcockson and Valtchanoff, 2008). Our data came in agreement with these previous works and further illustrated that DAPK1 and NMDA NR2B are co-localized in normal and injured small- to medium- sized neurons in the DRG.

Yan et al., 2013 have reported the up-regulation of NMDA NR2B 7 days after injury using a western blot, which came aligned with our gene expression analysis results. Surprisingly, our studies using immunohistochemistry staining have revealed that the proportion of NMDA NR2B labelled neurons decreased at 7 days and 14 days after sciatic nerve injury. However, this down regulation of NMDA NR2B in the neurons was accompanied with an up-regulation in the population of glial cells surrounding large-sized injured neurons ($> 30 \mu\text{m}$). This observation may provide an explanation of why we have found an increase in NMDA NR2B mRNA.

Although not much is known about the responses of satellite cell to nerve injury, there is evidence that these cells increase the expression of the glial marker GFAP in DRG (Woodham et al., 1989; Zhou et al., 1996; Hu et al., 2007). Our immunofluorescent double labelling staining revealed that GFAP and NMDA NR2B are co-localized in satellite cells of the large injured neurons in L4 and L5 DRG 14 days after injury, as they were also ATF3 positive.

It has been demonstrated that NR2B receptors are the main activators for most death signaling transduction pathways (Lai and Wang, 2010; Martin and Wang, 2010). Moreover, it has been reported that uncoupling of activated DAPK1 from the NMDA

receptor complex protects against brain damage in stroke without affecting the physiological actions of the NMDA receptors, as total blockade of NMDA receptor could be deleterious to animals and humans. Thus, targeting DAPK1-NMDA NR2B receptor interaction or inhibition of DAPK1 has been considered as a practical strategy for stroke therapy (Tu et al., 2010; Martin and Wang, 2010; Shu et al 2014). These findings are in line with our results, which demonstrate that DAPK1 might have a physical and functional association with NMDA NR2B in the PNS. We proposed that down-regulation of DAPK1 and NMDA NR2B following sciatic nerve injury might be the pathway that promotes neuronal survival and sheds light on the mechanisms of peripheral nerve regeneration.

4.5 DAPK1-NMDA NR2B and Pain

There is considerable evidence that glutamate receptors are major contributors to the development and maintenance of neuropathic pain (Carlton and Hargett, 1995;Coderre and Melzack, 1992; Dickenson and Sullivan, 1987; Keast and Stephensen, 2000; Woolf and Thompson, 1991). It is known that peripheral NMDA receptor activation contributes to nociception activation, mechanical sensitivity and heat sensitization after PNS injury (Du et al., 2003a). The NMDA NR2B receptor has been shown to play a crucial role in neuropathic pain (Boyce et al., 1999; Laurie et al., 1997; Nagy et al., 2004). Yet, we know little about how nerve injury alters responses to this neurotransmitter in primary sensory neurons.

One important component in the findings of this study was that both DAPK1 and NMDA NR2B were down-regulated in L4 and L5 DRG neurons 14 days after peripheral

nerve injury. This has been confirmed by NMDA NR2B and ATF3 double immunofluorescence staining, which showed that both DAPK1 and NMDA NR2B were negative in many positively labeled ATF3 injured neurons 14 days after nerve injury. It has been known that after this postoperative period of time following sciatic nerve injury the inflammatory response is turned off 2-3 weeks after injury and macrophages are rapidly eliminated (Mueller et al., 2003; Omura et al., 2005; Taskinen and Røyttä, 1997).

Another striking finding that DAPK1-expressing dorsal root ganglion cells co-stained for markers of nociceptive neurons, such as CGRP and IB4. Furthermore, our data showed complete co-localization of DAPK1 and NMDA NR2B in small-sized diameter neurons in the DRG which are responsible for pain transmission from the periphery to the spinal cord. Several studies documented that small diameter peptidergic neurons co-express NMDA receptors and knockdown of NMDA receptors in DRGs reduced pain behavior (Ma and Hargreaves, 2000; McRoberts et al., 2011). Most DRG nociceptive neurons are small diameter neurons (Basbaum et al., 2009).

Although, convincing evidence from several studies anticipated NR2B as the major subunit involved in mediating the development and maintenance of neuropathic pain (Abe et al., 2005; Geng et al., 2010; Iwata et al., 2007; Li et al., 2011), extensive controversies have been reported regarding NMDAR's roles in nociception in the peripheral and central terminals of primary afferent neurons. Indeed, whether NR2B has a pro-nociceptive or anti-nociceptive role remains an unsettled issue (Bardoni et al., 2004; Coggeshall and Carlton, 1998b; Du et al., 2003b; Liu et al., 1997).

Thus, the most parsimonious explanation for our finding was that DAPK1 activated NMDA NR2B in the early stage in this study of nerve injury, conversely, both become down-regulated at later stages after 7 and 14 days, which might indicate their participation in modulating nociception in the PNS.

4.6 The Relationship of the Expression of NMDA NR2B in Satellite Cells and Pain

As in our gene expression analysis, previous study (Yan et al., 2013) showed that NMDA NR2B is up-regulated in DRG and suggested its role in development of neuropathic pain. However, our results using an immunohistochemical method distinctively showed that NMDA NR2B is down-regulated in DRG neurons which might argue against its involvement in neuropathic pain. Interestingly, we found an up-regulation of NMDA NR2B in satellite cells 7-14 days following sciatic nerve injury suggesting the involvement of NMDA NR2B in neuropathic pain via these glial cells and not neurons.

A series of older and recent studies have emphasized the importance of glial cells in the maintenance of neuropathic pain (Hanani et al., 2002; Pannese et al., 2003; Xu et al., 2008; Xie et al., 2009). These glial cells, as well as neurons have been documented to contribute in neuropathic pain. Shinder and Devor (1994) observed that satellite cells formed several layers around neurons after axotomy of the sciatic nerve and having the appearance of “onion bulbs”. The satellite cell phenomena was seen only around large neurons (Liu et al., 2012).

GFAP was used based on its well-known role as satellite cells activation marker

(Dubový et al., 2010; Gunjigake et al., 2009; Ohara et al., 2008; Stephenson and Byers, 1995; Takeda et al., 2007; Vit et al., 2006; Xie et al., 2009; Xu et al., 2008; Zhou et al., 1996; Hu et al., 2007), and found that after spinal nerve ligation (SNL) satellite cell activated and wrapped all types of DRG neurons as early as 12 hours postoperatively, but the distribution of GFAP shifted from small and medium to be concentrated around large DRG neurons 7 days after injury (Liu et al., 2012).

This expression of GFAP in satellite cells of large DRG neurons has been also shown after chronic constriction (CCI) in previous studies or transection of the rat sciatic nerve (Zhou et al., 1996; Hu et al., 2007). Although, it is known that A β fibers which originate from large neurons in the DRG are involved in the transmission of innocuous sensory information, it has been reported that a significant number (about 20% in rats and >50% in rodents) of large fibres are also nociceptive (Djoughri and Lawson, 2004). This observation has been described in other studies which have shown that large A β -fiber DRG neurons contributed in mechanical allodynia in SNL rats (Song et al., 2003; Xie et al., 2005), although very little is known about the underlying molecular mechanism of how they initiate pain.

In support of this observation, Hanani et al. (2002) have examined satellite cells in mouse DRG 14 days after PNS injury, and they found a great increase in gap junction-mediated coupling between satellite cells enveloping different neurons reached 6-fold suggesting that new gap junctions formed between satellite cells and suggested possible interaction of satellite cells in neuropathic pain. Further research has documented the up-regulation of the gap junction protein, connexin 43 and a decrease in membrane resistance (Ohara et al., 2008; Pannese et al., 2003). Inhibition of such

phenomena from occurring by injection gap junction blockers led to significant reduction of pain behaviour and opened the door to studies on gap junction blockers that may have a potential in pain therapy (Huang et al., 2010; Warwick et al., 2014).

Our results showed that NMDA NR2B co-expressed with GFAP in activated satellite cells that surrounded large DRG neurons 7 and 14 days after injury. Taken together, we show here for the first time the up-regulation of NMDA NR2B in satellite cells 7 days and 14 days after PNS injury. This up-regulation may be the link between the mechanisms of NMDA NR2B and satellite cells in the development and maintenance of neuropathic pain.

CHAPTER 5: CONCLUSION

In this study, the DAPK1 expression pattern has been investigated for the first time in normal L4 and L5 DRG neurons and after sciatic nerve injury. This study showed that:

- DAPK1 is present in normal DRG neurons. The down-regulation of DAPK1 following sciatic nerve injury along with other vital pro-apoptotic players and NMDA NR2B down-regulations might promote neuronal survival and shed light on the mechanisms of peripheral nerve regeneration.
- Peripheral DAPK1-NMDA NR2B co-localization in nociceptive neurons might indicate DAPK1-NMDA NR2B participation in modulating nociception in the PNS. NMDA NR2B expression in the satellite cells might suggest that NMDA NR2B modulate neuropathic pain through satellite cell, and not through neurons, after 7 and 14 days of peripheral nerve injury.

In conclusion, the results of this study have shown that DAPK1 is present in the sensory neurons and it might be a master regulator of neuropathic pain as well as play a role in driving the intrinsic growth ability and recovery from injury. A deeper understanding of the complex regulation of peripheral DAPK1-NMDA NR2B as a regulator of both axonal regeneration and neuropathic pain might help in designing genetic and pharmacological strategies to promote faster neuronal repair and regeneration. The field of study would benefit immensely from investigation into the cellular, molecular, systemic, and behavioural levels underlying these processes. Understanding neuronal and glial mechanisms in neuropathic pain offers hope of

developing drugs targeting DAPK1-NMDA NR2B or NMDA NR2B satellite cell-related molecules.

5.1 DAPK1 in Nervous system

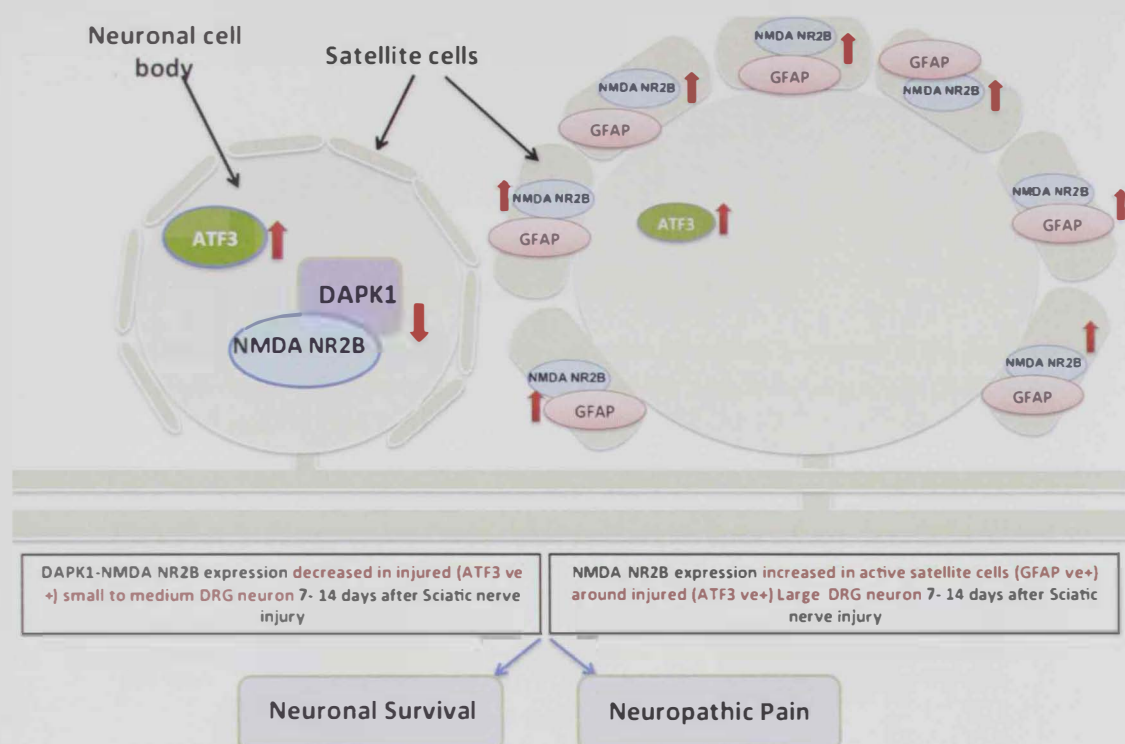


Figure 22: Diagrams illustrate DAPK1 and NMDA NR2B protein expression in DRG neurons following peripheral nerve injury

The down-regulation of DAPK1-NMDA NR2B in small to medium neurons may have a significant contribution in intrinsic regulation of PNS regeneration. The up-regulation of NMDA NR2B in activated satellite cells 7-14 days after PNS injury may indicate that NMDA NR2B modulate neuropathic pain through satellite cells in this time period.

DAPK1 in CNS

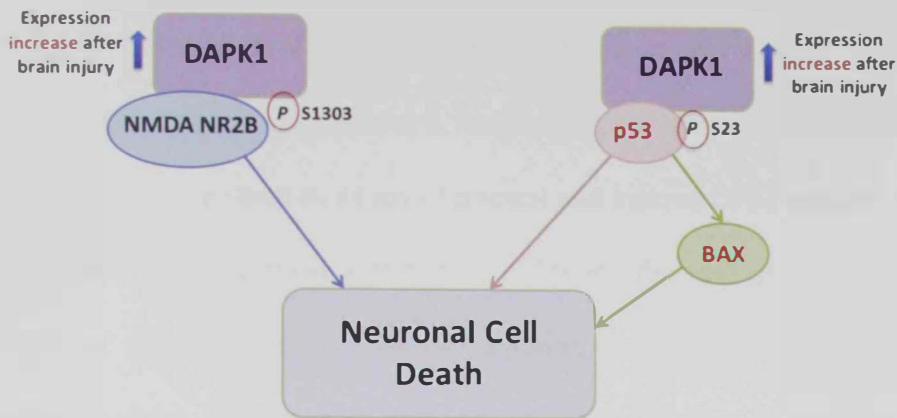


Figure 23: DAPK1 in CNS

DAPK1-NMDA NR2B and DAPK1- p53 physically and functionally interact in the brain and their expression level up-regulated in brain injury/diseases which leads to neuronal cell death (modified from Tu et al., 2010 and Pei et al., 2014).

DAPK1 Suggested Working Model in PNS

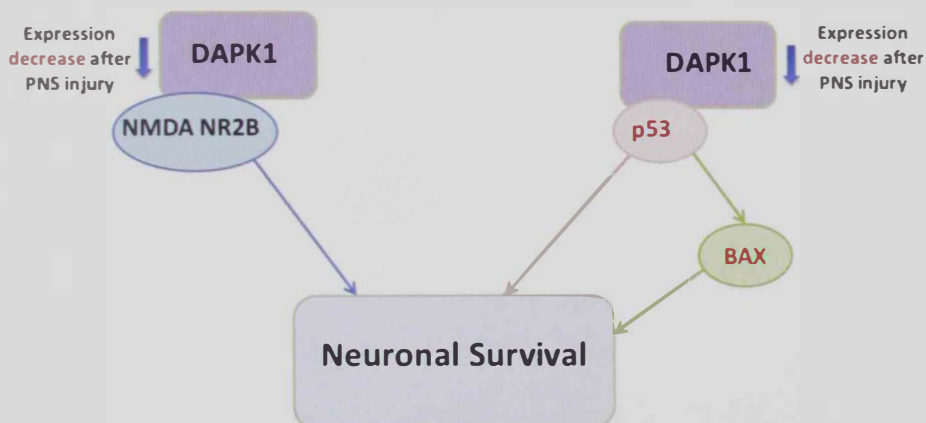


Figure 24: DAPK1 suggested working model in PNS in this study

Suggested working model of DAPK1 in the PNS. DAPK1- NMDA NR2B and DAPK1- p53 expressions are down-regulated in the DRG after PNS injury to rescue cells from death.

5.2 Future Direction

The establishment of the expression pattern of DAPK1 in normal DRG neurons and following PNS injury in this study, has opened doors for further research to explore DAPK1 signal transduction pathways, mechanism and its role in the PNS. Investigating DAPK1 expression pattern in axons of normal and injured DRG neurons and its possible role in growth cone function will further enlighten the role of DAPK1 in PNS axonal regrowth and regeneration. In addition, studying the effect of DAPK1 inhibition in the expression pattern of key pro- and anti-apoptotic molecules such as p53, Bax and NMDA NR2B will enhance our understanding of its molecular activities in PNS. Moreover, DAPK1 knockout mice will help us to settle its contribution in PNS nociception and regeneration. Indeed, this study put the first stone in shedding light to understand the possible role of DAPK1 in the peripheral nervous system.

BIBLIOGRAPHY

A. Journal Articles

- Abe, N., Borson, S.H., Gambello, M.J., Wang, F., and Cavalli, V. (2010). Mammalian target of rapamycin (mTOR) activation increases axonal growth capacity of injured peripheral nerves. *J. Biol. Chem.* 285, 28034–28043.
- Abe, T., Matsumura, S., Katano, T., Mabuchi, T., Takagi, K., Xu, L., ... & Ito, S. (2005). Fyn kinase-mediated phosphorylation of NMDA receptor NR2B subunit at Tyr1472 is essential for maintenance of neuropathic pain. *Eur. J. Neurosci.* 22, 1445–1454.
- Aley, K.O., and Levine, J.D. (2002). Different peripheral mechanisms mediate enhanced nociception in metabolic/toxic and traumatic painful peripheral neuropathies in the rat. *Neuroscience* 111, 389–397.
- Ali, D.W., and Salter, M.W. (2001). NMDA receptor regulation by Src kinase signalling in excitatory synaptic transmission and plasticity. *Curr. Opin. Neurobiol.* 11, 336–342.
- Alonso, M.E., Bello, M.J., Gonzalez-Gomez, P., Arjona, D., Lomas, J., de Campos, J.M., Isla, A., Sarasa, J.L., and Rey, J.A. (2003). Aberrant promoter methylation of multiple genes in oligodendrogliomas and ependymomas. *Cancer Genet. Cytogenet.* 144, 134–142.
- Ambron, R.T., and Walters, E.T. (1996). Priming events and retrograde injury signals. A new perspective on the cellular and molecular biology of nerve regeneration. *Mol. Neurobiol.* 13, 61–79.
- André, S., Boukhaddaoui, H., Campo, B., Al-Jumaily, M., Mayeux, V., Greuet, D., Valmier, J., and Scamps, F. (2003). Axotomy-induced expression of calcium-

activated chloride current in subpopulations of mouse dorsal root ganglion neurons. *J. Neurophysiol.* *90*, 3764–3773.

Anjum, R., Roux, P.P., Ballif, B.A., Gygi, S.P., and Blenis, J. (2005). The tumor suppressor DAP kinase is a target of RSK-mediated survival signaling. *Curr. Biol. CB* *15*, 1762–1767.

Annis, M.G., Soucie, E.L., Dlugosz, P.J., Cruz-Aguado, J.A., Penn, L.Z., Leber, B., and Andrews, D.W. (2005). Bax forms multispinning monomers that oligomerize to permeabilize membranes during apoptosis. *EMBO J.* *24*, 2096–2103.

Anson, L.C., Chen, P.E., Wyllie, D.J., Colquhoun, D., and Schoepfer, R. (1998). Identification of amino acid residues of the NR2A subunit that control glutamate potency in recombinant NR1/NR2A NMDA receptors. *J. Neurosci. Off. J. Soc. Neurosci.* *18*, 581–589.

Araki, T., Nagarajan, R., and Milbrandt, J. (2001). Identification of genes induced in peripheral nerve after injury. Expression profiling and novel gene discovery. *J. Biol. Chem.* *276*, 34131–34141.

Araki, T., Shinoda, S., Schindler, C.K., Quan-Lan, J., Meller, R., Taki, W., Simon, R.P., and Henshall, D.C. (2004). Expression, interaction, and proteolysis of death-associated protein kinase and p53 within vulnerable and resistant hippocampal subfields following seizures. *Hippocampus* *14*, 326–336.

Arkipova, S.S., Raginov, I.S., Mukhitov, A.R., and Chelyshev, Y.A. (2010). Satellite cells of sensory neurons after various types of sciatic nerve trauma in the rat. *Neurosci. Behav. Physiol.* *40*, 609–614.

Arvidsson, J., Ygge, J., and Grant, G. (1986). Cell loss in lumbar dorsal root ganglia and transganglionic degeneration after sciatic nerve resection in the rat. *Brain Res.* *373*, 15–21.

- Averill, S., Michael, G.J., Shortland, P.J., Leavesley, R.C., King, V.R., Bradbury, E.J., McMahon, S.B., and Priestley, J.V. (2004). NGF and GDNF ameliorate the increase in ATF3 expression which occurs in dorsal root ganglion cells in response to peripheral nerve injury. *Eur. J. Neurosci.* *19*, 1437–1445.
- Asano, T., Tanaka, K., Suemasu, S., Ishihara, T., Tahara, K., Suzuki, T., Suzuki, H., Fukudo, S., and Mizushima, T. (2012). Effects of β -(1,3-1,6)-D-glucan on irritable bowel syndrome-related colonic hypersensitivity. *Biochem. Biophys. Res. Commun.* *420*, 444–449.
- Assmus, H. (1978). [Somatosensory evoked cortical potentials (SSEP) in regenerating nerves following suture (author's transl)]. *EEG-EMG Z. Für Elektroenzephalographie Elektromyographie Verwandte Geb.* *9*, 167–171. [Abstract in English]
- Augustinack, J.C., Schneider, A., Mandelkow, E.-M., and Hyman, B.T. (2002). Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease. *Acta Neuropathol. (Berl.)* *103*, 26–35.
- Bahadori, M.H., Al-Tiraihi, T., and Valojerdi, M.R. (2001). Sciatic nerve transection in neonatal rats induces apoptotic neuronal death in L5 dorsal root ganglion. *J. Neurocytol.* *30*, 125–130.
- Bajbouj, K., Poehlmann, A., Kuester, D., Drewes, T., Haase, K., Hartig, R., Teller, A., Kliche, S., Walluscheck, D., Ivanovska, J., et al. (2009). Identification of phosphorylated p38 as a novel DAPK-interacting partner during TNF α -induced apoptosis in colorectal tumor cells. *Am. J. Pathol.* *175*, 557–570.
- Ballif, B.A., and Blenis, J. (2001). Molecular mechanisms mediating mammalian mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK cell survival signals. *Cell Growth Differ. Mol. Biol. J. Am. Assoc. Cancer Res.* *12*, 397–408.
- Banerjee, B., Medda, B.K., Zheng, Y., Miller, H., Miranda, A., Sengupta, J.N., and Shaker, R. (2009). Alterations in N-methyl-D-aspartate receptor subunits in

primary sensory neurons following acid-induced esophagitis in cats. *Am. J. Physiol. Gastrointest. Liver Physiol.* 296, G66–G77.

- Bardoni, R., Torsney, C., Tong, C.-K., Prandini, M., and MacDermott, A.B. (2004). Presynaptic NMDA receptors modulate glutamate release from primary sensory neurons in rat spinal cord dorsal horn. *J. Neurosci.* 24, 2774–2781.
- Barnard, E.A., and Henley, J.M. (1990). The non-NMDA receptors: types, protein structure and molecular biology. *Trends Pharmacol. Sci.* 11, 500–507.
- Barrette, B., Hébert, M.-A., Filali, M., Lafortune, K., Vallières, N., Gowing, G., Julien, J.-P., and Lacroix, S. (2008). Requirement of myeloid cells for axon regeneration. *J. Neurosci.* 28, 9363–9376.
- Basbaum, A.I., Bautista, D.M., Scherrer, G., and Julius, D. (2009). Cellular and molecular mechanisms of pain. *Cell* 139, 267–284.
- Bendszus, M., and Stoll, G. (2003). Caught in the act: in vivo mapping of macrophage infiltration in nerve injury by magnetic resonance imaging. *J. Neurosci. Off. J. Soc. Neurosci.* 23, 10892–10896.
- Bennett, G.J., and Xie, Y.K. (1988). A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* 33, 87–107.
- Berta, T., Poirot, O., Pertin, M., Ji, R.-R., Kellenberger, S., and Decosterd, I. (2008). Transcriptional and functional profiles of voltage-gated Na(+) channels in injured and non-injured DRG neurons in the SNI model of neuropathic pain. *Mol. Cell. Neurosci.* 37, 196–208.
- Bhave, G., and Gereau, R.W. (2004). Posttranslational mechanisms of peripheral sensitization. *J. Neurobiol.* 61, 88–106.
- Bialik, S., and Kimchi, A. (2004). DAP-kinase as a target for drug design in cancer and diseases associated with accelerated cell death. *Semin. Cancer Biol.* 14, 283–294.

- Bialik, S., and Kimchi, A. (2006). The death-associated protein kinases: structure, function, and beyond. *Annu. Rev. Biochem.* 75, 189–210.
- Bialik, S., and Kimchi, A. (2010). Lethal weapons: DAP-kinase, autophagy and cell death: DAP-kinase regulates autophagy. *Curr. Opin. Cell Biol.* 22, 199–205.
- Bialik, S., Bresnick, A.R., and Kimchi, A. (2004). DAP-kinase-mediated morphological changes are localization dependent and involve myosin-II phosphorylation. *Cell Death Differ.* 11, 631–644.
- Bleakman, D., Alt, A., and Nisenbaum, E.S. (2006). Glutamate receptors and pain. *Semin. Cell Dev. Biol.* 17, 592–604.
- Boivin, A., Pineau, I., Barrette, B., Filali, M., Vallières, N., Rivest, S., and Lacroix, S. (2007). Toll-like receptor signaling is critical for Wallerian degeneration and functional recovery after peripheral nerve injury. *J. Neurosci. Off. J. Soc. Neurosci.* 27, 12565–12576.
- Bomze, H.M., Bulsara, K.R., Iskandar, B.J., Caroni, P., and Skene, J.H. (2001). Spinal axon regeneration evoked by replacing two growth cone proteins in adult neurons. *Nat. Neurosci.* 4, 38–43.
- Bonilla, I.E., Tanabe, K., and Strittmatter, S.M. (2002). Small proline-rich repeat protein 1A is expressed by axotomized neurons and promotes axonal outgrowth. *J. Neurosci.* 22, 1303–1315.
- Bouvard, V., Zaitchouk, T., Vacher, M., Duthu, A., Canivet, M., Choisy-Rossi, C., Nieruchalski, M., and May, E. (2000). Tissue and cell-specific expression of the p53-target genes: bax, fas, mdm2 and waf1/p21, before and following ionising irradiation in mice. *Oncogene* 19, 649–660.
- Boyce, S., Wyatt, A., Webb, J.K., O'Donnell, R., Mason, G., Rigby, M., Sirinathsinghji, D., Hill, R.G., and Rupniak, N.M. (1999). Selective NMDA NR2B antagonists

induce antinociception without motor dysfunction: correlation with restricted localisation of NR2B subunit in dorsal horn. *Neuropharmacology* 38, 611–623.

Brattgård, S.O., and Thulin, C.A. (1965). Ultrastructural changes of feline ventral horn cells during the sequences of axon regeneration with relation to their physiological function. *Acta Anat. (Basel)* 62, 563–567.

Brodsky, M.H., Weinert, B.T., Tsang, G., Rong, Y.S., McGinnis, N.M., Golic, K.G., Rio, D.C., and Rubin, G.M. (2004). *Drosophila melanogaster* MNK/Chk2 and p53 regulate multiple DNA repair and apoptotic pathways following DNA damage. *Mol. Cell. Biol.* 24, 1219–1231.

Broude, E., McAtee, M., Kelley, M.S., and Bregman, B.S. (1997). c-Jun expression in adult rat dorsal root ganglion neurons: differential response after central or peripheral axotomy. *Exp. Neurol.* 148, 367–377.

Byers, M.R., and Costello, R.J. (1988). Cation-binding sites in trigeminal ganglia and maxillary nerve: unusual reactivity of perikarya, stem axons and satellite cells. *Brain Res.* 443, 125–136.

Campbell, J.N., and Meyer, R.A. (2006). Mechanisms of neuropathic pain. *Neuron* 52, 77–92.

Cao, Y., and Klionsky, D.J. (2007). Physiological functions of Atg6/Beclin 1: a unique autophagy-related protein. *Cell Res.* 17, 839–849.

Carey, D.J., and Bunge, R.P. (1981). Factors influencing the release of proteins by cultured Schwann cells. *J. Cell Biol.* 91, 666–672.

Carlsson, M., and Carlsson, A. (1990). Interactions between glutamatergic and monoaminergic systems within the basal ganglia--implications for schizophrenia and Parkinson's disease. *Trends Neurosci.* 13, 272–276.

- Carlton, S.M., Hargett, G.L., and Coggeshall, R.E. (1995). Localization and activation of glutamate receptors in unmyelinated axons of rat glabrous skin. *Neurosci. Lett.* 197, 25–28.
- Caroni, P. (1998). Neuro-regeneration: plasticity for repair and adaptation. *Essays Biochem.* 33, 53–64.
- Castroman, P.J., and Ness, T.J. (2002). Ketamine, an N-methyl-D-aspartate receptor antagonist, inhibits the spinal neuronal responses to distension of the rat urinary bladder. *Anesthesiology* 96, 1410–1419.
- Cavalli, V., Kujala, P., Klumperman, J., and Goldstein, L.S.B. (2005). Sunday Driver links axonal transport to damage signaling. *J. Cell Biol.* 168, 775–787.
- Al-Chaer, E.D., Kawasaki, M., and Pasricha, P.J. (2000). A new model of chronic visceral hypersensitivity in adult rats induced by colon irritation during postnatal development. *Gastroenterology* 119, 1276–1285.
- Chaigne-Delalande, B., Moreau, J.-F., and Legembre, P. (2008). Rewinding the DISC. *Arch. Immunol. Ther. Exp. (Warsz.)* 56, 9–14.
- Chan, E.Y. (2009). mTORC1 phosphorylates the ULK1-mAtg13-FIP200 autophagy regulatory complex. *Sci. Signal.* 2, pe51.
- Charton, J.P., Herkert, M., Becker, C.M., and Schröder, H. (1999). Cellular and subcellular localization of the 2B-subunit of the NMDA receptor in the adult rat telencephalon. *Brain Res.* 816, 609–617.
- Chen, C.-H., Wang, W.-J., Kuo, J.-C., Tsai, H.-C., Lin, J.-R., Chang, Z.-F., and Chen, R.-H. (2005). Bidirectional signals transduced by DAPK-ERK interaction promote the apoptotic effect of DAPK. *EMBO J.* 24, 294–304.

- Chen, H.-S., Qu, F., He, X., Kang, S.-M., Liao, D., and Lu, S.-J. (2010). Differential roles of peripheral metabotropic glutamate receptors in bee venom-induced nociception and inflammation in conscious rats. *J. Pain* *11*, 321–329.
- Chen, M., Lu, T.-J., Chen, X.-J., Zhou, Y., Chen, Q., Feng, X.-Y., Xu, L., Duan, W.-H., and Xiong, Z.-Q. (2008). Differential roles of NMDA receptor subtypes in ischemic neuronal cell death and ischemic tolerance. *Stroke J. Cereb. Circ.* *39*, 3042–3048.
- Chen, Y., Aulia, S., and Tang, B.L. (2006). Myelin-associated glycoprotein-mediated signaling in central nervous system pathophysiology. *Mol. Neurobiol.* *34*, 81–91.
- Cherkas, P.S., Huang, T.-Y., Pannicke, T., Tal, M., Reichenbach, A., and Hanani, M. (2004). The effects of axotomy on neurons and satellite glial cells in mouse trigeminal ganglion. *Pain* *110*, 290–298.
- Cheung, Y.-T., Zhang, N.Q., Hung, C.H.-L., Lai, C.S.-W., Yu, M.-S., So, K.-F., and Chang, R.C.-C. (2011). Temporal relationship of autophagy and apoptosis in neurons challenged by low molecular weight β -amyloid peptide. *J. Cell. Mol. Med.* *15*, 244–257.
- Chierzi, S., Ratto, G.M., Verma, P., and Fawcett, J.W. (2005). The ability of axons to regenerate their growth cones depends on axonal type and age, and is regulated by calcium, cAMP and ERK. *Eur. J. Neurosci.* *21*, 2051–2062.
- Chipuk, J.E., and Green, D.R. (2008). How do BCL-2 proteins induce mitochondrial outer membrane permeabilization? *Trends Cell Biol.* *18*, 157–164.
- Choi, D.W. (1988). Glutamate neurotoxicity and diseases of the nervous system. *Neuron* *1*, 623–634.
- Choi, D.W., and Rothman, S.M. (1990). The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. *Annu. Rev. Neurosci.* *13*, 171–182.

- Chong, M.S., Woolf, C.J., Haque, N.S., and Anderson, P.N. (1999). Axonal regeneration from injured dorsal roots into the spinal cord of adult rats. *J. Comp. Neurol.* *410*, 42–54.
- Cincotta, M., Beart, P.M., Summers, R.J., and Lodge, D. (1989). Bidirectional transport of NMDA receptor and ionophore in the vagus nerve. *Eur. J. Pharmacol.* *160*, 167–171.
- Coderre, T.J., and Melzack, R. (1992). The role of NMDA receptor-operated calcium channels in persistent nociception after formalin-induced tissue injury. *J. Neurosci.* *12*, 3671–3675.
- Coggeshall, R.E., and Carlton, S.M. (1998a). Ultrastructural analysis of NMDA, AMPA, and kainate receptors on unmyelinated and myelinated axons in the periphery. *J. Comp. Neurol.* *391*, 78–86.
- Coggeshall, R.E., and Carlton, S.M. (1998b). Ultrastructural analysis of NMDA, AMPA, and kainate receptors on unmyelinated and myelinated axons in the periphery. *J. Comp. Neurol.* *391*, 78–86.
- Cohen, O., and Kimchi, A. (2001). DAP-kinase: from functional gene cloning to establishment of its role in apoptosis and cancer. *Cell Death Differ.* *8*, 6–15.
- Cohen, O., Feinstein, E., and Kimchi, A. (1997). DAP-kinase is a Ca²⁺/calmodulin-dependent, cytoskeletal-associated protein kinase, with cell death-inducing functions that depend on its catalytic activity. *EMBO J.* *16*, 998–1008.
- Cohen, O., Inbal, B., Kissil, J.L., Raveh, T., Berissi, H., Spivak-Kroizaman, T., Feinstein, E., and Kimchi, A. (1999). DAP-kinase participates in TNF- α - and Fas-induced apoptosis and its function requires the death domain. *J. Cell Biol.* *146*, 141–148.
- Coleman, M. (2005). Axon degeneration mechanisms: commonality amid diversity. *Nat. Rev. Neurosci.* *6*, 889–898.

- Coleman, M.P., and Freeman, M.R. (2010). Wallerian degeneration, wld(s), and nmnat. *Annu. Rev. Neurosci.* *33*, 245–267.
- Coleman, M.P., and Perry, V.H. (2002). Axon pathology in neurological disease: a neglected therapeutic target. *Trends Neurosci.* *25*, 532–537.
- Collingridge, G.L., Peineau, S., Howland, J.G., and Wang, Y.T. (2010). Long-term depression in the CNS. *Nat. Rev. Neurosci.* *11*, 459–473.
- Costigan, M., Mannion, R.J., Kendall, G., Lewis, S.E., Campagna, J.A., Coggeshall, R.E., Meridith-Middleton, J., Tate, S., and Woolf, C.J. (1998). Heat shock protein 27: developmental regulation and expression after peripheral nerve injury. *J. Neurosci.* *18*, 5891–5900.
- Costigan, M., Scholz, J., and Woolf, C.J. (2009). Neuropathic pain: a maladaptive response of the nervous system to damage. *Annu. Rev. Neurosci.* *32*, 1–32.
- Dansranjavin, T., Möbius, C., Tannapfel, A., Bartels, M., Wittekind, C., Hauss, J., and Witzigmann, H. (2006). E-cadherin and DAP kinase in pancreatic adenocarcinoma and corresponding lymph node metastases. *Oncol. Rep.* *15*, 1125–1131.
- Davar, G., Hama, A., Deykin, A., Vos, B., and Maciewicz, R. (1991). MK-801 blocks the development of thermal hyperalgesia in a rat model of experimental painful neuropathy. *Brain Res.* *553*, 327–330.
- Davies, S.N., and Lodge, D. (1987). Evidence for involvement of N-methylaspartate receptors in “wind-up” of class 2 neurones in the dorsal horn of the rat. *Brain Res.* *424*, 402–406.
- Deckwerth, T.L., Elliott, J.L., Knudson, C.M., Johnson, E.M., Snider, W.D., and Korsmeyer, S.J. (1996). BAX is required for neuronal death after trophic factor deprivation and during development. *Neuron* *17*, 401–411.

- Decosterd, I., and Woolf, C.J. (2000). Spared nerve injury: an animal model of persistent peripheral neuropathic pain. *Pain* 87, 149–158.
- Deiss, L.P., Feinstein, E., Berissi, H., Cohen, O., and Kimchi, A. (1995). Identification of a novel serine/threonine kinase and a novel 15-kD protein as potential mediators of the gamma interferon-induced cell death. *Genes Dev.* 9, 15–30.
- DeRidder, M.N., Simon, M.J., Siman, R., Auberson, Y.P., Raghupathi, R., and Meaney, D.F. (2006). Traumatic mechanical injury to the hippocampus in vitro causes regional caspase-3 and calpain activation that is influenced by NMDA receptor subunit composition. *Neurobiol. Dis.* 22, 165–176.
- Devor, M., Wall, P.D., and McMahon, S.B. (1984). Dichotomizing somatic nerve fibers exist in rats but they are rare. *Neurosci. Lett.* 49, 187–192.
- De Vos, K.J., Grierson, A.J., Ackerley, S., and Miller, C.C.J. (2008). Role of axonal transport in neurodegenerative diseases. *Annu. Rev. Neurosci.* 31, 151–173.
- Dewson, G., and Kluck, R.M. (2009). Mechanisms by which Bak and Bax permeabilise mitochondria during apoptosis. *J. Cell Sci.* 122, 2801–2808.
- Dickenson, A.H., and Sullivan, A.F. (1987). Evidence for a role of the NMDA receptor in the frequency dependent potentiation of deep rat dorsal horn nociceptive neurones following C fibre stimulation. *Neuropharmacology* 26, 1235-1238.
- Djouhri, L., and Lawson, S.N. (2004). Abeta-fiber nociceptive primary afferent neurons: a review of incidence and properties in relation to other afferent A-fiber neurons in mammals. *Brain Res. Brain Res. Rev.* 46, 131–145.
- Dumoulin, F.L., Raivich, G., Streit, W.J., Kreutzberg, G.W. (1991). Differential regulation of calcitonin gene-related peptide (CGRP) in regenerating rat facial nucleus and dorsal root ganglion. *Eur. J. Neurosci.* 3, 338–342.

- Dougherty, P.M., Paleček, J., Palečková, V., Sorkin, L.S., and Willis, W.D. (1992). The role of NMDA and non-NMDA excitatory amino acid receptors in the excitation of primate spinothalamic tract neurons by mechanical, chemical, thermal, and electrical stimuli. *J. Neurosci.* *12*, 3025–3041.
- Du, J., Gray, N.A., Falke, C., Yuan, P., Szabo, S., and Manji, H.K. (2003a). Structurally dissimilar antimanic agents modulate synaptic plasticity by regulating AMPA glutamate receptor subunit GluR1 synaptic expression. *Ann. N. Y. Acad. Sci.* *1003*, 378–380.
- Du, J., Zhou, S., Coggeshall, R.E., and Carlton, S.M. (2003b). N-methyl-D-aspartate-induced excitation and sensitization of normal and inflamed nociceptors. *Neuroscience* *118*, 547–562.
- Du, J., Zhou, S., and Carlton, S.M. (2006). Kainate-induced excitation and sensitization of nociceptors in normal and inflamed rat glabrous skin. *Neuroscience* *137*, 999–1013.
- Dubner, R., and Ruda, M.A. (1992). Activity-dependent neuronal plasticity following tissue injury and inflammation. *Trends Neurosci.* *15*, 96–103.
- Dubový, P., Jancálek, R., Klusáková, I., Svízenská, I., and Pejchalová, K. (2006). Intra- and extraneuronal changes of immunofluorescence staining for TNF-alpha and TNFR1 in the dorsal root ganglia of rat peripheral neuropathic pain models. *Cell. Mol. Neurobiol.* *26*, 1205–1217.
- Dubový, P., Klusáková, I., Svízenská, I., and Brázda, V. (2010). Spatio-temporal changes of SDF1 and its CXCR4 receptor in the dorsal root ganglia following unilateral sciatic nerve injury as a model of neuropathic pain. *Histochem. Cell Biol.* *133*, 323–337.
- Duce, I.R., and Keen, P. (1977). An ultrastructural classification of the neuronal cell bodies of the rat dorsal root ganglion using zinc iodide-osmium impregnation. *Cell Tissue Res.* *185*, 263–277.

- Decosterd, I., and Woolf, C.J. (2000). Spared nerve injury: an animal model of persistent peripheral neuropathic pain. *Pain* 87, 149–158.
- DeiSs, L.P., Feinstein, E., Berissi, H., Cohen, O., and Kimchi, A. (1995). Identification of a novel serine/threonine kinase and a novel 15-kD protein as potential mediators of the gamma interferon-induced cell death. *Genes Dev.* 9, 15–30.
- DeRidder, M.N., Simon, M.J., Siman, R., Auberson, Y.P., Raghupathi, R., and Meaney, D.F. (2006). Traumatic mechanical injury to the hippocampus in vitro causes regional caspase-3 and calpain activation that is influenced by NMDA receptor subunit composition. *Neurobiol. Dis.* 22, 165–176.
- Devor, M., Wall, P.D., and McMahon, S.B. (1984). Dichotomizing somatic nerve fibers exist in rats but they are rare. *Neurosci. Lett.* 49, 187–192.
- De Vos, K.J., Grierson, A.J., Ackerley, S., and Miller, C.C.J. (2008). Role of axonal transport in neurodegenerative diseases. *Annu. Rev. Neurosci.* 31, 151–173.
- Dewson, G., and Kluck, R.M. (2009). Mechanisms by which Bak and Bax permeabilise mitochondria during apoptosis. *J. Cell Sci.* 122, 2801–2808.
- Dickenson, A.H., and Sullivan, A.F. (1987). Evidence for a role of the NMDA receptor in the frequency dependent potentiation of deep rat dorsal horn nociceptive neurones following C fibre stimulation. *Neuropharmacology* 26, 1235-1238.
- Djoughri, L., and Lawson, S.N. (2004). Abeta-fiber nociceptive primary afferent neurons: a review of incidence and properties in relation to other afferent A-fiber neurons in mammals. *Brain Res. Brain Res. Rev.* 46, 131–145.
- Dumoulin, F.L., Raivich, G., Streit, W.J., Kreutzberg, G.W. (1991). Differential regulation of calcitonin gene-related peptide (CGRP) in regenerating rat facial nucleus and dorsal root ganglion. *Eur. J. Neurosci.* 3, 338–342.

- Decosterd, I., and Woolf, C.J. (2000). Spared nerve injury: an animal model of persistent peripheral neuropathic pain. *Pain* 87, 149–158.
- Deiss, L.P., Feinstein, E., Berissi, H., Cohen, O., and Kimchi, A. (1995). Identification of a novel serine/threonine kinase and a novel 15-kD protein as potential mediators of the gamma interferon-induced cell death. *Genes Dev.* 9, 15–30.
- DeRidder, M.N., Simon, M.J., Siman, R., Auberson, Y.P., Raghupathi, R., and Meaney, D.F. (2006). Traumatic mechanical injury to the hippocampus in vitro causes regional caspase-3 and calpain activation that is influenced by NMDA receptor subunit composition. *Neurobiol. Dis.* 22, 165–176.
- Devor, M., Wall, P.D., and McMahon, S.B. (1984). Dichotomizing somatic nerve fibers exist in rats but they are rare. *Neurosci. Lett.* 49, 187–192.
- De Vos, K.J., Grierson, A.J., Ackerley, S., and Miller, C.C.J. (2008). Role of axonal transport in neurodegenerative diseases. *Annu. Rev. Neurosci.* 31, 151–173.
- Dewson, G., and Kluck, R.M. (2009). Mechanisms by which Bak and Bax permeabilise mitochondria during apoptosis. *J. Cell Sci.* 122, 2801–2808.
- Dickenson, A.H., and Sullivan, A.F. (1987). Evidence for a role of the NMDA receptor in the frequency dependent potentiation of deep rat dorsal horn nociceptive neurones following C fibre stimulation. *Neuropharmacology* 26, 1235–1238.
- Djouhri, L., and Lawson, S.N. (2004). Abeta-fiber nociceptive primary afferent neurons: a review of incidence and properties in relation to other afferent A-fiber neurons in mammals. *Brain Res. Brain Res. Rev.* 46, 131–145.
- Dumoulin, F.L., Raivich, G., Streit, W.J., Kreutzberg, G.W. (1991). Differential regulation of calcitonin gene-related peptide (CGRP) in regenerating rat facial nucleus and dorsal root ganglion. *Eur. J. Neurosci.* 3, 338–342.

- Dougherty, P.M., Palecek, J., Paleckova, V., Sorkin, L.S., and Willis, W.D. (1992). The role of NMDA and non-NMDA excitatory amino acid receptors in the excitation of primate spinothalamic tract neurons by mechanical, chemical, thermal, and electrical stimuli. *J. Neurosci.* *12*, 3025–3041.
- Du, J., Gray, N.A., Falke, C., Yuan, P., Szabo, S., and Manji, H.K. (2003a). Structurally dissimilar antimanic agents modulate synaptic plasticity by regulating AMPA glutamate receptor subunit GluR1 synaptic expression. *Ann. N. Y. Acad. Sci.* *1003*, 378–380.
- Du, J., Zhou, S., Coggeshall, R.E., and Carlton, S.M. (2003b). N-methyl-D-aspartate-induced excitation and sensitization of normal and inflamed nociceptors. *Neuroscience* *118*, 547–562.
- Du, J., Zhou, S., and Carlton, S.M. (2006). Kainate-induced excitation and sensitization of nociceptors in normal and inflamed rat glabrous skin. *Neuroscience* *137*, 999–1013.
- Dubner, R., and Ruda, M.A. (1992). Activity-dependent neuronal plasticity following tissue injury and inflammation. *Trends Neurosci.* *15*, 96–103.
- Dubový, P., Jancálek, R., Klusáková, I., Svízenská, I., and Pejchalová, K. (2006). Intra- and extraneuronal changes of immunofluorescence staining for TNF-alpha and TNFR1 in the dorsal root ganglia of rat peripheral neuropathic pain models. *Cell. Mol. Neurobiol.* *26*, 1205–1217.
- Dubový, P., Klusáková, I., Svízenská, I., and Brázda, V. (2010). Spatio-temporal changes of SDF1 and its CXCR4 receptor in the dorsal root ganglia following unilateral sciatic nerve injury as a model of neuropathic pain. *Histochem. Cell Biol.* *133*, 323–337.
- Duce, I.R., and Keen, P. (1977). An ultrastructural classification of the neuronal cell bodies of the rat dorsal root ganglion using zinc iodide-osmium impregnation. *Cell Tissue Res.* *185*, 263–277.

- Ducker, T.B., Kempe, L.G., and Hayes, G.J. (1969). The metabolic background for peripheral nerve surgery. *J. Neurosurg.* 30, 270–280.
- Eisenberg-Lerner, A., and Kimchi, A. (2007). DAP kinase regulates JNK signaling by binding and activating protein kinase D under oxidative stress. *Cell Death Differ.* 14, 1908–1915.
- Engidawork, E., Gulesserian, T., Seidl, R., Cairns, N., and Lubec, G. (2001). Expression of apoptosis related proteins in brains of patients with Alzheimer's disease. *Neurosci. Lett.* 303, 79–82.
- Fang, X., McMullan, S., Lawson, S.N., and Djouhri, L. (2005). Electrophysiological differences between nociceptive and non-nociceptive dorsal root ganglion neurones in the rat in vivo. *J. Physiol.* 565, 927–943.
- Feinstein, E., Kimchi, A., Wallach, D., Boldin, M., and Varfolomeev, E. (1995). The death domain: a module shared by proteins with diverse cellular functions. *Trends Biochem. Sci.* 20, 342–344.
- Fields, R.D., and Ellisman, M.H. (1986a). Axons regenerated through silicone tube splices. I. Conduction properties. *Exp. Neurol.* 92, 48–60.
- Fields, R.D., and Ellisman, M.H. (1986b). Axons regenerated through silicone tube splices. II. Functional morphology. *Exp. Neurol.* 92, 61–74.
- Filbin, M.T. (2003). Myelin-associated inhibitors of axonal regeneration in the adult mammalian CNS. *Nat. Rev. Neurosci.* 4, 703–713.
- Finch, P.M., Knudsen, L., and Drummond, P.D. (2009). Reduction of allodynia in patients with complex regional pain syndrome: A double-blind placebo-controlled trial of topical ketamine. *Pain* 146, 18–25.
- Finn, J.T., Weil, M., Archer, F., Siman, R., Srinivasan, A., and Raff, M.C. (2000). Evidence that Wallerian degeneration and localized axon degeneration induced

by local neurotrophin deprivation do not involve caspases. *J. Neurosci. Off. J. Soc. Neurosci.* *20*, 1333–1341.

Fitzgerald, P.J. (2012). The NMDA receptor may participate in widespread suppression of circuit level neural activity, in addition to a similarly prominent role in circuit level activation. *Behav. Brain Res.* *230*, 291–298.

Fitzgerald, M.J., Martin, F., and Paletta, F.X. (1967). Innervation of skin grafts. *Surg. Gynecol. Obstet.* *124*, 808–812.

Forrest, D., Yuzaki, M., Soares, H.D., Ng, L., Luk, D.C., Sheng, M., Stewart, C.L., Morgan, J.I., Connor, J.A., and Curran, T. (1994). Targeted disruption of NMDA receptor 1 gene abolishes NMDA response and results in neonatal death. *Neuron* *13*, 325–338.

Frostick, S.P., Yin, Q., and Kemp, G.J. (1998). Schwann cells, neurotrophic factors, and peripheral nerve regeneration. *Microsurgery* *18*, 397–405.

Fu, S.Y., and Gordon, T. (1997). The cellular and molecular basis of peripheral nerve regeneration. *Mol. Neurobiol.* *14*, 67–116.

Fujita, Y., Taniguchi, J., Uchikawa, M., Endo, M., Hata, K., Kubo, T., Mueller, B.K., and Yamashita, T. (2008). Neogenin regulates neuronal survival through DAP kinase. *Cell Death Differ.* *15*, 1593–1608.

Fujita, Y., and Yamashita, T. (2014). Role of DAPK in neuronal cell death. *Apoptosis.* *19*:339-345.

Funakoshi, H., Frisé, J., Barbany, G., Timmusk, T., Zachrisson, O., Verge, V.M., and Persson, H. (1993). Differential expression of mRNAs for neurotrophins and their receptors after axotomy of the sciatic nerve. *J. Cell Biol.* *123*, 455–465.

Gandesiri, M., Chakilam, S., Ivanovska, J., Benderska, N., Ocker, M., Di Fazio, P., Feoktistova, M., Gali-Muhtasib, H., Rave-Fränk, M., Prante, O., et al. (2012).

- DAPK plays an important role in panobinostat-induced autophagy and commits cells to apoptosis under autophagy deficient conditions. *Apoptosis* 17, 1300-1315.
- Gaudreau, G.-A., and Plourde, V. (2004). Involvement of N-methyl-d-aspartate (NMDA) receptors in a rat model of visceral hypersensitivity. *Behav. Brain Res.* 150, 185–189.
- Gaunitz, C., Schüttler, A., Gillen, C., and Allgaier, C. (2002). Formalin-induced changes of NMDA receptor subunit expression in the spinal cord of the rat. *Amino Acids* 23, 177–182.
- Gavathiotis, E., Suzuki, M., Davis, M.L., Pitter, K., Bird, G.H., Katz, S.G., Tu, H.-C., Kim, H., Cheng, E.H.-Y., Tjandra, N., et al. (2008). BAX activation is initiated at a novel interaction site. *Nature* 455, 1076–1081.
- Geng, S.-J., Liao, F.-F., Dang, W.-H., Ding, X., Liu, X.-D., Cai, J., Han, J.-S., Wan, Y., and Xing, G.-G. (2010). Contribution of the spinal cord BDNF to the development of neuropathic pain by activation of the NR2B-containing NMDA receptors in rats with spinal nerve ligation. *Exp. Neurol.* 222, 256–266.
- George, R., and Griffin, J.W. (1994). Delayed macrophage responses and myelin clearance during Wallerian degeneration in the central nervous system: the dorsal radiculotomy model. *Exp. Neurol.* 129, 225–236.
- Glass, J.D., Culver, D.G., Levey, A.I., and Nash, N.R. (2002). Very early activation of m-calpain in peripheral nerve during Wallerian degeneration. *J. Neurol. Sci.* 196, 9-20.
- Gold, B.G., Storm-Dickerson, T., and Austin, D.R. (1993). Regulation of the transcription factor c-JUN by nerve growth factor in adult sensory neurons. *Neurosci. Lett.* 154, 129–133.

- Goff, J.R., Burkey, A.R., Goff, D.J., Jasmin, L. (1998). Reorganization of the spinal dorsal horn in models of chronic pain: correlation with behaviour. *Neuroscience* 82, 559-574.
- Goldberg, J.L., Klassen, M.P., Hua, Y., and Barres, B.A. (2002). Amacrine-signaled loss of intrinsic axon growth ability by retinal ganglion cells. *Science* 296, 1860–1864.
- Gonda, X. (2012). Basic pharmacology of NMDA receptors. *Curr. Pharm. Des.* 18, 1558-1567.
- Gonzalez-Gomez, P., Bello, M.J., Lomas, J., Arjona, D., Alonso, M.E., Amiñoso, C., Lopez-Marin, I., Anselmo, N.P., Sarasa, J.L., Gutierrez, M., et al. (2003a). Aberrant methylation of multiple genes in neuroblastic tumours: Relationship with MYCN amplification and allelic status at 1p. *Eur. J. Cancer Oxf. Engl.* 1990 39, 1478–1485.
- Gonzalez-Gomez, P., Bello, M.J., Arjona, D., Alonso, M.E., Lomas, J., De Campos, J.M., Kusak, M.E., Gutierrez, M., Sarasa, J.L., and Rey, J.A. (2003b). Aberrant CpG island methylation in neurofibromas and neurofibrosarcomas. *Oncol. Rep.* 10, 1519–1523.
- Goping, I.S., Gross, A., Lavoie, J.N., Nguyen, M., Jemmerson, R., Roth, K., Korsmeyer, S.J., and Shore, G.C. (1998). Regulated targeting of BAX to mitochondria. *J. Cell Biol.* 143, 207–215.
- Grafstein, B. (1975). The nerve cell body response to axotomy. *Exp. Neurol.* 48, 32–51.
- Griffiths, G.J., Dubrez, L., Morgan, C.P., Jones, N.A., Whitehouse, J., Corfe, B.M., Dive, C., and Hickman, J.A. (1999). Cell damage-induced conformational changes of the pro-apoptotic protein Bak in vivo precede the onset of apoptosis. *J. Cell Biol.* 144, 903–914.

- Gross, A., Jockel, J., Wei, M.C., and Korsmeyer, S.J. (1998). Enforced dimerization of BAX results in its translocation, mitochondrial dysfunction and apoptosis. *EMBO J.* 17, 3878–3885.
- Groves, M.J., Christopherson, T., Giometto, B., and Scaravilli, F. (1997). Axotomy-induced apoptosis in adult rat primary sensory neurons. *J. Neurocytol.* 26, 615–624.
- Gunjigake, K.K., Goto, T., Nakao, K., Kobayashi, S., and Yamaguchi, K. (2009). Activation of satellite glial cells in rat trigeminal ganglion after upper molar extraction. *Acta Histochem. Cytochem.* 42, 143–149.
- Haley, J.E., Sullivan, A.F., and Dickenson, A.H. (1990). Evidence for spinal N-methyl-D-aspartate receptor involvement in prolonged chemical nociception in the rat. *Brain Res.* 518, 218–226.
- Hall, S. (2001). Nerve repair: a neurobiologist's view. *J. Hand Surg. Edinb. Scotl.* 26, 129–136.
- Hall, S. (2005). The response to injury in the peripheral nervous system. *J. Bone Joint Surg. Br.* 87, 1309–1319.
- Halpain, S., and Dehmelt, L. (2006). The MAPI family of microtubule-associated proteins. *Genome Biol.* 7, 224.
- Hammarberg, H., Piehl, F., Cullheim, S., Fjell, J., Hökfelt, T., and Fried, K. (1996). GDNF mRNA in Schwann cells and DRG satellite cells after chronic sciatic nerve injury. *Neuroreport* 7, 857–860.
- Hammarlund, M., Nix, P., Hauth, L., Jorgensen, E.M., and Bastiani, M. (2009). Axon regeneration requires a conserved MAP kinase pathway. *Science* 323, 802–806.
- Hanani, M. (2005). Satellite glial cells in sensory ganglia: from form to function. *Brain Res. Brain Res. Rev.* 48, 457–476.

- Hanani, M., Huang, T.Y., Cherkas, P.S., Ledda, M., and Pannese, E. (2002). Glial cell plasticity in sensory ganglia induced by nerve damage. *Neuroscience* 114, 279–283.
- Hanz, S., Perlson, E., Willis, D., Zheng, J.-Q., Massarwa, R., Huerta, J.J., Koltzenburg, M., Kohler, M., van-Minnen, J., Twiss, J.L., et al. (2003). Axoplasmic importins enable retrograde injury signaling in lesioned nerve. *Neuron* 40, 1095–1104.
- Harrison, B., Kraus, M., Burch, L., Stevens, C., Craig, A., Gordon-Weeks, P., and Hupp, T.R. (2008). DAPK-1 binding to a linear peptide motif in MAP1B stimulates autophagy and membrane blebbing. *J. Biol. Chem.* 283, 9999–10014.
- Hawthorne, A.L., and Popovich, P.G. (2011). Emerging concepts in myeloid cell biology after spinal cord injury. *Neurother. J. Am. Soc. Exp. Neurother.* 8, 252–261.
- Henshall, D.C., Araki, T., Schindler, C.K., Shinoda, S., Lan, J.-Q., and Simon, R.P. (2003). Expression of death-associated protein kinase and recruitment to the tumor necrosis factor signaling pathway following brief seizures. *J. Neurochem.* 86, 1260–1270.
- Henshall, D.C., Schindler, C.K., So, N.K., Lan, J.-Q., Meller, R., and Simon, R.P. (2004). Death-associated protein kinase expression in human temporal lobe epilepsy. *Ann. Neurol.* 55, 485–494.
- Herdegen, T., Kummer, W., Fiallos, C.E., Leah, J., and Bravo, R. (1991). Expression of c-JUN, JUN B and JUN D proteins in rat nervous system following transection of vagus nerve and cervical sympathetic trunk. *Neuroscience* 45, 413–422.
- Hetman, M., Kanning, K., Cavanaugh, J.E., and Xia, Z. (1999). Neuroprotection by brain-derived neurotrophic factor is mediated by extracellular signal-regulated kinase and phosphatidylinositol 3-kinase. *J. Biol. Chem.* 274, 22569–22580.

- Hirai, H., Kirsch, J., Laube, B., Betz, H., and Kuhse, J. (1996). The glycine binding site of the N-methyl-D-aspartate receptor subunit NR1: identification of novel determinants of co-agonist potentiation in the extracellular M3-M4 loop region. *Proc. Natl. Acad. Sci. U. S. A.* *93*, 6031–6036.
- Hoeflich, A., Bünger, L., Nedbal, S., Renne, U., Elmlinger, M.W., Blum, W.F., Bruley, C., Kolb, H.J., and Wolf, E. (2004). Growth selection in mice reveals conserved and redundant expression patterns of the insulin-like growth factor system. *Gen. Comp. Endocrinol.* *136*, 248–259.
- Hoffman, P. N. (1989). Expression of GAP-43, a rapidly transported growth-associated protein, and class II beta tubulin, a slowly transported cytoskeletal protein, are coordinated in regenerating neurons. *J Neurosci.* *9*, 893-897.
- Hoökfelt, T., Wiesenfeld-Hallin, Z., Villar, M., Melander, T. (1987). Increase of galanin-like immunoreactivity in rat dorsal root ganglion cells after peripheral axotomy. *Neurosci. Lett.* *83*, 217–220.
- Hökfelt T, Zhang X, Wiesenfeld-Hallin Z. (1994). Messenger plasticity in primary sensory neurons following axotomy and its functional implications. *Trends Neurosci.* *17*, 22–30.
- Hu, J., Van den Steen, P.E., Sang, Q.-X.A., and Opdenakker, G. (2007). Matrix metalloproteinase inhibitors as therapy for inflammatory and vascular diseases. *Nat. Rev. Drug Discov.* *6*, 480–498.
- Hu, J., Wang, Z., Guo, Y.-Y., Zhang, X.-N., Xu, Z.-H., Liu, S.-B., Guo, H.-J., Yang, Q., Zhang, F.-X., Sun, X.-L., et al. (2009). A role of periaqueductal grey NR2B-containing NMDA receptor in mediating persistent inflammatory pain. *Mol. Pain* *5*, 71.
- Huang, T.-Y., Belzer, V., and Hanani, M. (2010). Gap junctions in dorsal root ganglia: possible contribution to visceral pain. *Eur. J. Pain Lond. Engl.* *14*, 49.e1–e11.

- Ide, C. (1996). Peripheral nerve regeneration. *Neurosci. Res.* 25, 101–121.
- Ikonomidou, C., Bosch, F., Miksa, M., Bittigau, P., Vöckler, J., Dikranian, K., Tenkova, T.I., Stefovská, V., Turski, L., and Olney, J.W. (1999). Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. *Science* 283, 70–74.
- Inaishi, Y., Kashihara, Y., Sakaguchi, M., Nawa, H., and Kuno, M. (1992). Cooperative regulation of calcitonin gene-related peptide levels in rat sensory neurons via their central and peripheral processes. *J. Neurosci.* 12, 518–524.
- Inbal, B., Cohen, O., Polak-Charcon, S., Kopolovic, J., Vadai, E., Eisenbach, L., and Kimchi, A. (1997). DAP kinase links the control of apoptosis to metastasis. *Nature* 390, 180–184.
- Inbal, B., Shani, G., Cohen, O., Kissil, J.L., and Kimchi, A. (2000). Death-associated protein kinase-related protein 1, a novel serine/threonine kinase involved in apoptosis. *Mol. Cell. Biol.* 20, 1044–1054.
- Inbal, B., Bialik, S., Sabanay, I., Shani, G., and Kimchi, A. (2002). DAP kinase and DRP-1 mediate membrane blebbing and the formation of autophagic vesicles during programmed cell death. *J. Cell Biol.* 157, 455–468.
- Ishii, D.N., Glazner, G.W., and Pu, S.F. (1994). Role of insulin-like growth factors in peripheral nerve regeneration. *Pharmacol. Ther.* 62, 125–144.
- Iwata, H., Takasusuki, T., Yamaguchi, S., and Hori, Y. (2007). NMDA receptor 2B subunit-mediated synaptic transmission in the superficial dorsal horn of peripheral nerve-injured neuropathic mice. *Brain Res.* 1135, 92–101.
- Jacob, J.M., and McQuarrie, I.G. (1993). Acceleration of axonal outgrowth in rat sciatic nerve at one week after axotomy. *J. Neurobiol.* 24, 356–367.

- Jang, C.-W., Chen, C.-H., Chen, C.-C., Chen, J., Su, Y.-H., and Chen, R.-H. (2002). TGF-beta induces apoptosis through Smad-mediated expression of DAP-kinase. *Nat. Cell Biol.* 4, 51–58.
- JaSmin, L., Vit, J.-P., Bhargava, A., and Ohara, P.T. (2010). Can satellite glial cells be therapeutic targets for pain control? *Neuron Glia Biol.* 6, 63–71.
- Jenkins, R., and Hunt, S.P. (1991). Long-term increase in the levels of c-jun mRNA and jun protein-like immunoreactivity in motor and sensory neurons following axon damage. *Neurosci. Lett.* 129, 107–110.
- Jenkins, J.R., Rudge, K., and Currie, G.A. (1984). Cellular immortalization by a cDNA clone encoding the transformation-associated phosphoprotein p53. *Nature* 312, 651–654.
- Ji, R.-R., Kohno, T., Moore, K.A., and Woolf, C.J. (2003a). Central sensitization and LTP: do pain and memory share similar mechanisms? *Trends Neurosci.* 26, 696–705.
- Jin, Y., Blue, E.K., Dixon, S., Hou, L., Wysolmerski, R.B., and Gallagher, P.J. (2001). Identification of a new form of death-associated protein kinase that promotes cell survival. *J. Biol. Chem.* 276, 39667–39678.
- Jin, Y., Blue, E.K., Dixon, S., Shao, Z., and Gallagher, P.J. (2002). A death-associated protein kinase (DAPK)-interacting protein, DIP-1, is an E3 ubiquitin ligase that promotes tumor necrosis factor-induced apoptosis and regulates the cellular levels of DAPK. *J. Biol. Chem.* 277, 46980–46986.
- Jivan, S., Novikova, L.N., Wiberg, M., and Novikov, L.N. (2006). The effects of delayed nerve repair on neuronal survival and axonal regeneration after seventh cervical spinal nerve axotomy in adult rats. *Exp. Brain Res.* 170, 245–254.
- Ju G, Hokfelt T, Brodin E, et al. (1987a). Primary sensory neurons of the rat showing calcitonin gene-related peptide immunoreactivity and their relation to substance

- P-, somatostatin-, galanin-, vasoactive intestinal polypeptide-, and cholecystokinin-immunoreactive ganglion cells, *Cell and Tissue Research* 247:417–431.
- Ju G, Melander T, Ceccatelli S, et al. (1987b). Immunohistochemical evidence for a spinothalamic pathway co-containing cholecystokinin- and galanin-like immunoreactivities in the rat, *Neuroscience* 20:439–456.
- Kai-Kai, M.A. (1989). Cytochemistry of the trigeminal and dorsal root ganglia and spinal cord of the rat. *Comp. Biochem. Physiol. A* 93, 183–193.
- Kalina, M., and Bubis, J.J. (1968). Histochemical studies on the distribution of acid phosphatases in neurones of sensory ganglia; light and electron microscopy. *Histochem. Histochem. Histochem.* 14, 103–112.
- Kalina, M., and Wolman, M. (1970). Correlative histochemical and morphological study on the maturation of sensory ganglion cells in the rat. *Histochem. Histochem. Histochem.* 22, 100–108.
- Kang, B.N., Ahmad, A.S., Saleem, S., Patterson, R.L., Hester, L., Doré, S., and Snyder, S.H. (2010). Death-associated protein kinase-mediated cell death modulated by interaction with DANGER. *J. Neurosci.* 30, 93–98.
- Kashiba, H., Senba, E., Ueda, Y., and Tohyama, M. (1992). Co-localized but target-unrelated expression of vasoactive intestinal polypeptide and galanin in rat dorsal root ganglion neurons after peripheral nerve crush injury. *Brain Res.* 582, 47–57.
- Kawai, T., Nomura, F., Hoshino, K., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., and Akira, S. (1999). Death-associated protein kinase 2 is a new calcium/calmodulin-dependent protein kinase that signals apoptosis through its catalytic activity. *Oncogene* 18, 3471–3480.

- Keast, J.R., and Stephensen, T.M. (2000). Glutamate and aspartate immunoreactivity in dorsal root ganglion cells supplying visceral and somatic targets and evidence for peripheral axonal transport. *J. Comp. Neurol.* 424, 577–587.
- Kerezoudi, E., King, R.H., Muddle, J.R., O'Neill, J.A., and Thomas, P.K. (1995). Influence of age on the late retrograde effects of sciatic nerve section in the rat. *J. Anat.* 187 (Pt 1), 27–35.
- Kew, J.N., Koester, A., Moreau, J.L., Jenck, F., Ouagazzal, A.M., Mutel, V., Richards, J.G., Trube, G., Fischer, G., Montkowski, A., et al. (2000). Functional consequences of reduction in NMDA receptor glycine affinity in mice carrying targeted point mutations in the glycine binding site. *J. Neurosci.* 20, 4037–4049.
- Kim, B.M., you, M-H., Chen, C.H., Lee, S., Hong, Y., Hong, Y., Kimchi, A., Zhou, XZ., and Lee, T.H. (2014). Death-associated protein kinase 1 has a critical role in aberrant tau protein regulation and function. *Cell Death Dis.* 5, 216.
- Kim, S.H., and Chung, J.M. (1992). An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. *Pain* 50, 355–363.
- Kim, D.H., Nelson, H.H., Wiencke, J.K., Christiani, D.C., Wain, J.C., Mark, E.J., and Kelsey, K.T. (2001). Promoter methylation of DAP-kinase: association with advanced stage in non-small cell lung cancer. *Oncogene* 20, 1765–1770.
- Kinkelin, I., Bröcker, E.B., Koltzenburg, M., and Carlton, S.M. (2000). Localization of ionotropic glutamate receptors in peripheral axons of human skin. *Neurosci. Lett.* 283, 149–152.
- Kinugasa, T., Ozaki, S., Hamanaka, S., and Kudo, N. (2002). The effects of sciatic nerve axotomy on spinal motoneurons in neonatal Bax-deficient mice. *NeuroSci. Res.* 44, 439–446.

- Kiryu, S., Morita, N., Ohno, K., Maeno, H., and Kiyama, H. (1995). Regulation of mRNA expression involved in Ras and PKA signal pathways during rat hypoglossal nerve regeneration. *Brain Res. Mol. Brain Res.* 29, 147–156.
- Kishino, M., Yukawa, K., Hoshino, K., Kimura, A., Shirasawa, N., Otani, H., Tanaka, T., Owada-Makabe, K., Tsubota, Y., Maeda, M., et al. (2004). Deletion of the kinase domain in death-associated protein kinase attenuates tubular cell apoptosis in renal ischemia-reperfusion injury. *J. Am. Soc. Nephrol. JASN* 15, 1826-1834.
- Kline, D.G., Hayes, G.J., and Morse, A.S. (1964a). A comparative study of response of species to peripheral-nerve injury. II: Crush and severance with primary suture. *J. Neurosurg.* 21, 980–988.
- Kline, D.G., Hayes, G.J., and Morse, A.S. (1964b). A comparative study of response of species to peripheral-nerve injury. I: SEVERANCE. *J. Neurosurg.* 21, 968–979.
- Koeppen, A.H. (2004). Wallerian degeneration: history and clinical significance. *J. Neurol. Sci.* 220, 115–117.
- Kögel, D., Plöttner, O., Landsberg, G., Christian, S., and Scheidtmann, K.H. (1998). Cloning and characterization of Dlk, a novel serine/threonine kinase that is tightly associated with chromatin and phosphorylates core histones. *Oncogene* 17, 2645-2654.
- Kovalsky, Y., Amir, R., and Devor, M. (2009). Simulation in sensory neurons reveals a key role for delayed Na⁺ current in subthreshold oscillations and ectopic discharge: implications for neuropathic pain. *J. Neurophysiol.* 102, 1430–1442.
- Kress, M., May, E., Cassingena, R., and May, P. (1979). Simian virus 40-transformed cells express new species of proteins precipitable by anti-simian virus 40 tumor serum. *J. Virol.* 31, 472–483.

- Kuo, J.-C., Wang, W.-J., Yao, C.-C., Wu, P.-R., and Chen, R.-H. (2006). The tumor suppressor DAPK inhibits cell motility by blocking the integrin-mediated polarity pathway. *J. Cell Biol.* 172, 619–631.
- Lai, T.W., and Wang, Y.T. (2010). Fashioning drugs for stroke. *Nat. Med.* 16, 1376–1378.
- Lane, D.P., and Crawford, L.V. (1979). T antigen is bound to a host protein in SV40-transformed cells. *Nature* 278, 261–263.
- Lankford, K.L., Waxman, S.G., and Kocsis, J.D. (1998). Mechanisms of enhancement of neurite regeneration in vitro following a conditioning sciatic nerve lesion. *J. Comp. Neurol.* 391, 11–29.
- Laurie, D.J., Bartke, I., Schoepfer, R., Naujoks, K., and Seeburg, P.H. (1997). Regional, developmental and interspecies expression of the four NMDAR2 subunits, examined using monoclonal antibodies. *Brain Res. Mol. Brain Res.* 51, 23–32.
- Laube, B., Hirai, H., Sturgess, M., Betz, H., and Kuhse, J. (1997). Molecular determinants of agonist discrimination by NMDA receptor subunits: analysis of the glutamate-binding site on the NR2B subunit. *Neuron* 18, 493–503.
- Lawson, S.N. (1979). The postnatal development of large light and small dark neurons in mouse dorsal root ganglia: a statistical analysis of cell numbers and size. *J. Neurocytol.* 8, 275–294.
- Lawson, S.N., Caddy, K.W., and Biscoe, T.J. (1974). Development of rat dorsal root ganglion neurones. Studies of cell birthdays and changes in mean cell diameter. *Cell Tissue Res.* 153, 399–413.
- Leber, B., Lin, J., and Andrews, D.W. (2010). Still embedded together binding to membranes regulates Bcl-2 protein interactions. *Oncogene* 29, 5221–5230.

- LeBlanc, A.C., and Poduslo, J.F. (1990). Axonal modulation of myelin gene expression in the peripheral nerve. *J. Neurosci. Res.* 26, 317–326.
- Lee, C.J., Kong, H., Manzini, M.C., Albuquerque, C., Chao, M.V., and MacDermott, A.B. (2001). Kainate receptors expressed by a subpopulation of developing nociceptors rapidly switch from high to low Ca²⁺ permeability. *J. Neurosci.* 21, 4572–4581.
- Lee, J.H., Rho, S.B., and Chun, T. (2005). Programmed cell death 6 (PDCD6) protein interacts with death-associated protein kinase 1 (DAPk1): additive effect on apoptosis via caspase-3 dependent pathway. *Biotechnol. Lett.* 27, 1011–1015.
- Lee, J.M., Zipfel, G.J., and Choi, D.W. (1999). The changing landscape of ischaemic brain injury mechanisms. *Nature* 399, A7–A14.
- Lee, S.E., Shen, H., Tagliabatella, G., Chung, J.M., and Chung, K. (1998). Expression of nerve growth factor in the dorsal root ganglion after peripheral nerve injury. *Brain Res.* 796, 99–106.
- Li, S., Cao, J., Yang, X., Suo, Z.-W., Shi, L., Liu, Y.-N., Yang, H.-B., and Hu, X.-D. (2011a). NR2B phosphorylation at tyrosine 1472 in spinal dorsal horn contributed to N-methyl-D-aspartate-induced pain hypersensitivity in mice. *J. Neurosci. Res.* 89, 1869–1876.
- Li, X.-Y., Ko, H.-G., Chen, T., Descalzi, G., Koga, K., Wang, H., Kim, S.S., Shang, Y., Kwak, C., Park, S.-W., et al. (2010). Alleviating neuropathic pain hypersensitivity by inhibiting PKMzeta in the anterior cingulate cortex. *Science* 330, 1400–1404.
- Li, X.-Y., Ko, H.-G., Chen, T., Collingridge, G.L., Kaang, B.-K., and Zhuo, M. (2011b). Erasing injury-related cortical synaptic potentiation as a new treatment for chronic pain. *J. Mol. Med. Berl. Ger.* 89, 847–855.

- Li, Y., Grupe, A., Rowland, C., Nowotny, P., Kauwe, J.S.K., Smemo, S., Hinrichs, A., Tacey, K., Toombs, T.A., Kwok, S., et al. (2006). DAPK1 variants are associated with Alzheimer's disease and allele-specific expression. *Hum. Mol. Genet.* *15*, 2560–2568.
- Libby, R.T., Li, Y., Savinova, O.V., Barter, J., Smith, R.S., Nickells, R.W., and John, S.W.M. (2005). Susceptibility to neurodegeneration in a glaucoma is modified by Bax gene dosage. *PLoS Genet.* *1*, 17–26.
- Lieberman, A.R. (1971). The axon reaction: a review of the principal features of perikaryal responses to axon injury. *Int. Rev. Neurobiol.* *14*, 49–124.
- Lin, Y., Hupp, T.R., and Stevens, C. (2010). Death-associated protein kinase (DAPK) and signal transduction: additional roles beyond cell death. *FEBS J.* *277*, 48–57.
- Lindholm, D., Heumann, R., Meyer, M., and Thoenen, H. (1987). Interleukin-1 regulates synthesis of nerve growth factor in non-neuronal cells of rat sciatic nerve. *Nature* *330*, 658–659.
- Lindwall, C., and Kanje, M. (2005). The role of p-c-Jun in survival and outgrowth of developing sensory neurons. *Neuroreport* *16*, 1655–1659.
- Linzer, D.I., and Levine, A.J. (1979). Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. *Cell* *17*, 43–52.
- Liot, G., Gabriel, C., Cacquevel, M., Ali, C., MacKenzie, E.T., Buisson, A., and Vivien, D. (2004). Neurotrophin-3-induced PI-3 kinase/Akt signaling rescues cortical neurons from apoptosis. *Exp. Neurol.* *187*, 38–46.
- Lipton, S.A., and Rosenberg, P.A. (1994). Excitatory amino acids as a final common pathway for neurologic disorders. *N. Engl. J. Med.* *330*, 613–622.

- Liss, A.G., af Ekenstam, F.W., and Wiberg, M. (1994). Liss, A. G., af Ekenstam, F. W., & Wiberg, M. (1994). Cell loss in sensory ganglia after peripheral nerve injury: An anatomical tracer study using lectin-coupled horseradish peroxidase in cats. *Scand. J. Plast. Reconstr. Surg. Hand Surg.* 28, 177–188.
- Liss, A.G., af Ekenstam, F.W., and Wiberg, M. (1996). Loss of neurons in the dorsal root ganglia after transection of a peripheral sensory nerve. An anatomical study in monkeys. *Scand. J. Plast. Reconstr. Surg. Hand Surg.* 30, 1–6.
- Liu, G., Parant, J.M., Lang, G., Chau, P., Chavez-Reyes, A., El-Naggar, A.K., Multani, A., Chang, S., and Lozano, G. (2004). Chromosome stability, in the absence of apoptosis, is critical for suppression of tumorigenesis in Trp53 mutant mice. *Nat. Genet.* 36, 63–68.
- Liu, F.-Y., Sun, Y.-N., Wang, F.-T., Li, Q., Su, L., Zhao, Z.-F., Meng, X.-L., Zhao, H., Wu, X., Sun, Q., et al. (2012). Activation of satellite glial cells in lumbar dorsal root ganglia contributes to neuropathic pain after spinal nerve ligation. *Brain Res.* 1427, 65–77.
- Liu, H., Mantyh, P.W., and Basbaum, A.I. (1997). NMDA-receptor regulation of substance P release from primary afferent nociceptors. *Nature* 386, 721–724.
- Liu, S., Zhang, N., Guo, Y., Zhao, R., Shi, T., Feng, S., Wang, S., Yang, Q., Li, X., Wu, Y., et al. (2012). G-protein-coupled receptor 30 mediates rapid neuroprotective effects of estrogen via depression of NR2B-containing NMDA receptors. *J. Neurosci.* 32, 4887–4900.
- Liu, Y., Wong, T.P., Aarts, M., Rooyackers, A., Liu, L., Lai, T.W., Wu, D.C., Lu, J., Tymianski, M., Craig, A.M., et al. (2007). NMDA receptor subunits have differential roles in mediating excitotoxic neuronal death both in vitro and in vivo. *J. Neurosci.* 27, 2846–2857.
- Lohrum, M.A., and Vousden, K.H. (1999). Regulation and activation of p53 and its family members. *Cell Death Differ.* 6, 1162–1168.

- Longstreth, G.F. (2005). Definition and classification of irritable bowel syndrome: current consensus and controversies. *Gastroenterol. Clin. North Am.* *34*, 173–187.
- Lu, X., and Richardson, P.M. (1991). Inflammation near the nerve cell body enhances axonal regeneration. *J. Neurosci.* *11*, 972–978.
- Lundborg, G. (2000). A 25-year perspective of peripheral nerve surgery: evolving neuroscientific concepts and clinical significance. *J. Hand Surg.* *25*, 391–414.
- Lundborg, G., and Rosén, B. (2007). Hand function after nerve repair. *Acta Physiol.* *189*, 207–217.
- Luo, J., Wang, Y., Yasuda, R.P., Dunah, A.W., and Wolfe, B.B. (1997). The majority of N-methyl-D-aspartate receptor complexes in adult rat cerebral cortex contain at least three different subunits (NR1/NR2A/NR2B). *Mol. Pharmacol.* *51*, 79–86.
- Ma, Q.P., and Hargreaves, R.J. (2000). Localization of N-methyl-D-aspartate NR2B subunits on primary sensory neurons that give rise to small-caliber sciatic nerve fibers in rats. *Neuroscience* *101*, 699–707.
- Ma, J., Novikov, L.N., Kellerth, J.-O., and Wiberg, M. (2003). Early nerve repair after injury to the postganglionic plexus: an experimental study of sensory and motor neuronal survival in adult rats. *Scand. J. Plast. Reconstr. Surg. Hand Surg. Nord. Plast. Foren. Nord. Klubb Handkirurgi* *37*, 1–9.
- Maher, M.P., Wu, N.-T., Guo, H.-Q., Dubin, A.E., Chaplan, S.R., and Wickenden, A.D. (2009). HCN channels as targets for drug discovery. *Comb. Chem. High Throughput Screen.* *12*, 64–72.
- Makwana, M., and Raivich, G. (2005). Molecular mechanisms in successful peripheral regeneration. *FEBS J.* *272*, 2628–2638.

- Malenka, R.C., and Nicoll, R.A. (1999). Long-term potentiation--a decade of progress? *Science* 285, 1870–1874.
- Mao, J., Price, D.D., Mayer, D.J., Lu, J., and Hayes, R.L. (1992). Intrathecal MK-801 and local nerve anesthesia synergistically reduce nociceptive behaviors in rats with experimental peripheral mononeuropathy. *Brain Res.* 576, 254–262.
- Mao, J., Mayer, D.J., Hayes, R.L., and Price, D.D. (1993). Spatial patterns of increased spinal cord membrane-bound protein kinase C and their relation to increases in 14C-2-deoxyglucose metabolic activity in rats with painful peripheral mononeuropathy. *J. Neurophysiol.* 70, 470–481.
- Markus, A., Patel, T.D., and Snider, W.D. (2002). Neurotrophic factors and axonal growth. *Curr. Opin. Neurobiol.* 12, 523–531.
- Martin, H.G.S., and Wang, Y.T. (2010). Blocking the deadly effects of the NMDA receptor in stroke. *Cell* 140, 174–176.
- Martinet, W., Schrijvers, D.M., De Meyer, G.R.Y., Thielemans, J., Knaapen, M.W.M., Herman, A.G., and Kockx, M.M. (2002). Gene expression profiling of apoptosis-related genes in human atherosclerosis: upregulation of death-associated protein kinase. *Arterioscler. Thromb. Vasc. Biol.* 22, 2023–2029.
- Martoriati, A., Doumont, G., Alcalay, M., Bellefroid, E., Pelicci, P.G., and Marine, J.-C. (2005). *dapk1*, encoding an activator of a p19ARF-p53-mediated apoptotic checkpoint, is a transcription target of p53. *Oncogene* 24, 1461–1466.
- Marvizón, J.C.G., McRoberts, J.A., Ennes, H.S., Song, B., Wang, X., Jinton, L., Corneliussen, B., and Mayer, E.A. (2002). Two N-methyl-D-aspartate receptors in rat dorsal root ganglia with different subunit composition and localization. *J. Comp. Neurol.* 446, 325–341.

- Mason MR, Lieberman AR, Grenningloh G, Anderson PN. (2002). Transcriptional upregulation of SCG10 and CAP-23 is correlated with regeneration of the axons of peripheral and central neurons in vivo. *Mol Cell Neurosci* 20(4):595-615.
- Mathur, P., Graybeal, C., Feyder, M., Davis, M.I., and Holmes, A. (2009). Fear memory impairing effects of systemic treatment with the NMDA NR2B subunit antagonist, Ro 25-6981, in mice: attenuation with ageing. *Pharmacol. Biochem. Behav.* 91, 453–460.
- Matsuoka, I., Meyer, M., and Thoenen, H. (1991). Cell-type-specific regulation of nerve growth factor (NGF) synthesis in non-neuronal cells: comparison of Schwann cells with other cell types. *J. Neurosci. Off. J. Soc. Neurosci.* 11, 3165–3177.
- May, P., and May, E. (1999). Twenty years of p53 research: structural and functional aspects of the p53 protein. *Oncogene* 18, 7621–7636.
- Mayer, M.L., and Miller, R.J. (1990). Excitatory amino acid receptors, second messengers and regulation of intracellular Ca²⁺ in mammalian neurons. *Trends Pharmacol. Sci.* 11, 254–260.
- McGee, A.W., and Strittmatter, S.M. (2003). The Nogo-66 receptor: focusing myelin inhibition of axon regeneration. *Trends Neurosci.* 26, 193–198.
- McGregor, G.P., Gibson, S.J., Sabate, I.M., Blank, M.A., Christofides, N.D., Wall, P.D., Polak, J.M., and Bloom, S.R. (1984). Effect of peripheral nerve section and nerve crush on spinal cord neuropeptides in the rat; increased VIP and PHI in the dorsal horn. *Neuroscience* 13, 207–216.
- McKay Hart, A., Brannstrom, T., Wiberg, M., and Terenghi, G. (2002). Primary sensory neurons and satellite cells after peripheral axotomy in the adult rat: timecourse of cell death and elimination. *Exp. Brain Res.* 142, 308–318.
- McRoberts, J.A., Ennes, H.S., Marvizón, J.C.G., Fanselow, M.S., Mayer, E.A., and Vissel, B. (2011). Selective knockdown of NMDA receptors in primary afferent

neurons decreases pain during phase 2 of the formalin test. *Neuroscience* 172, 474–482.

- Méchaly, I., Bourane, S., Piquemal, D., Al-Jumaily, M., Ventéo, S., Puech, S., Scamps, F., Valmier, J., and Carroll, P. (2006). Gene profiling during development and after a peripheral nerve traumatism reveals genes specifically induced by injury in dorsal root ganglia. *Mol. Cell. Neurosci.* 32, 217–229.
- Meguro, H., Mori, H., Araki, K., Kushiya, E., Kutsuwada, T., Yamazaki, M., Kumanishi, T., Arakawa, M., Sakimura, K., and Mishina, M. (1992). Functional characterization of a heteromeric NMDA receptor channel expressed from cloned cDNAs. *Nature* 357, 70–74.
- Mendrysa, S.M., Ghassemifar, S., and Malek, R. (2011). p53 in the CNS: Perspectives on Development, Stem Cells, and Cancer. *Genes Cancer* 2, 431–442.
- Michie, A.M., McCaig, A.M., Nakagawa, R., and Vukovic, M. (2010). Death-associated protein kinase (DAPK) and signal transduction: regulation in cancer. *FEBS J.* 277, 74–80.
- Millan, M.J. (1999). The induction of pain: an integrative review. *Prog. Neurobiol.* 57, 1–164.
- Mirsky, R., and Jessen, K.R. (1999). The neurobiology of Schwann cells. *Brain Pathol.* 9, 293–311.
- Mitoma, J., Ito, M., Furuya, S., and Hirabayashi, Y. (1998). Bipotential roles of ceramide in the growth of hippocampal neurons: promotion of cell survival and dendritic outgrowth in dose- and developmental stage-dependent manners. *J. Neurosci. Res.* 51, 712–722.
- Miwa, H., Fukaya, M., Watabe, A.M., Watanabe, M., and Manabe, T. (2008). Functional contributions of synaptically localized NR2B subunits of the NMDA

receptor to synaptic transmission and long-term potentiation in the adult mouse CNS. *J. Physiol.* 586, 2539–2550.

- Miyagi, M., Ohtori, S., Ishikawa, T., Aoki, Y., Ozawa, T., Doya, H., Saito, T., Moriya, H., and Takahashi, K. (2006). Up-regulation of TNF α in DRG satellite cells following lumbar facet joint injury in rats. *Eur. Spine J. Off. Publ. Eur. Spine Soc. Eur. Spinal Deform.* 15, 953–958.
- Moalem, G., and Tracey, D.J. (2006). Immune and inflammatory mechanisms in neuropathic pain. *Brain Res. Rev.* 51, 240–264.
- Molliver, D.C., Wright, D.E., Leitner, M.L., Parsadanian, A.S., Doster, K., Wen, D., Yan, Q., and Snider, W.D. (1997). IB4-binding DRG neurons switch from NGF to GDNF dependence in early postnatal life. *Neuron* 19, 849–861.
- Monyer, H., Burnashev, N., Laurie, D.J., Sakmann, B., and Seeburg, P.H. (1994). Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron* 12, 529–540.
- Mori, H., and Mishina, M. (1995). Structure and function of the NMDA receptor channel. *Neuropharmacology* 34, 1219–1237.
- Mosahebi, A., Woodward, B., Wiberg, M., Martin, R., and Terenghi, G. (2001). Retroviral labeling of Schwann cells: in vitro characterization and in vivo transplantation to improve peripheral nerve regeneration. *Glia* 34, 8–17.
- Morris, E.J., Keramaris, E., Rideout, H.J., Slack, R.S., Dyson, N.J., Stefanis, L., and Park, D.S. (2001). Cyclin-dependent kinases and P53 pathways are activated independently and mediate Bax activation in neurons after DNA damage. *J. Neurosci.* 21, 5017–5026.
- Mosavi, L.K., Cammett, T.J., Desrosiers, D.C., and Peng, Z.-Y. (2004). The ankyrin repeat as molecular architecture for protein recognition. *Protein* 13, 1435–1448.

- Mueller, M., Leonhard, C., Wacker, K., Ringelstein, E.B., Okabe, M., Hickey, W.F., and Kiefer, R. (2003). Macrophage response to peripheral nerve injury: the quantitative contribution of resident and hematogenous macrophages. *Lab. Investig. J. Tech. Methods Pathol.* 83, 175–185.
- Müller, M., Wilder, S., Bannasch, D., Israeli, D., Lehlbach, K., Li-Weber, M., Friedman, S.L., Galle, P.R., Stremmel, W., Oren, M., et al. (1998). p53 activates the CD95 (APO-1/Fas) gene in response to DNA damage by anticancer drugs. *J. Exp. Med.* 188, 2033–2045.
- Mueller, M., Leonhard, C., Wacker, K., Ringelstein, E.B., Okabe, M., Hickey, W.F., and Kiefer, R. (2003). Macrophage response to peripheral nerve injury: the quantitative contribution of resident and hematogenous macrophages. *Lab. Investig. J. Tech. Methods Pathol.* 83, 175–185.
- Nagy, G.G., Watanabe, M., Fukaya, M., and Todd, A.J. (2004). Synaptic distribution of the NR1, NR2A and NR2B subunits of the N-methyl-d-aspartate receptor in the rat lumbar spinal cord revealed with an antigen-unmasking technique. *Eur. J. Neurosci.* 20, 3301–3312.
- Naik, A.K., Pathirathna, S., and Jevtovic-Todorovic, V. (2008). GABAA receptor modulation in dorsal root ganglia in vivo affects chronic pain after nerve injury. *Neuroscience* 154, 1539–1553.
- Nakagomi, S., Suzuki, Y., Namikawa, K., Kiryu-Seo, S., and Kiyama, H. (2003). Expression of the activating transcription factor 3 prevents c-Jun N-terminal kinase-induced neuronal death by promoting heat shock protein 27 expression and Akt activation. *J. Neurosci.* 23, 5187–5196.
- Namikawa, K., Honma, M., Abe, K., Takeda, M., Mansur, K., Obata, T., Miwa, A., Okado, H., and Kiyama, H. (2000). Akt/protein kinase B prevents injury-induced motoneuron death and accelerates axonal regeneration. *J. Neurosci.* 20, 2875–2886.

- Nave, K.-A., and Trapp, B.D. (2008). Axon-glia signaling and the glial support of axon function. *Annu. Rev. Neurosci.* 31, 535–561.
- Neumann, S., and Woolf, C.J. (1999). Regeneration of dorsal column fibers into and beyond the lesion site following adult spinal cord injury. *Neuron* 23, 83–91.
- Nikolaidis, N., Chalkia, D., Watkins, D.N., Barrow, R.K., Snyder, S.H., van Rossum, D.B., and Patterson, R.L. (2007). Ancient origin of the new developmental superfamily DANGER. *PLoS One* 2, e204.
- Noguchi, K., Senba, E., Morita, Y., Sato, M., Tohyama, M. (1990). Alpha-CGRP and beta-CGRP mRNAs are differentially regulated in the rat spinal cord and dorsal root ganglion. *Brain Res. Mol. Brain Res.* 7, 299–304.
- Novikoff, P.M., Novikoff, A.B., Quintana, N., and Hauw, J.J. (1971). Golgi apparatus, GERL, and lysosomes of neurons in rat dorsal root ganglia, studied by thick section and thin section cytochemistry. *J. Cell Biol.* 50, 859–886.
- O'Brien, J.J., and Nathanson, N.M. (2007). Retrograde activation of STAT3 by leukemia inhibitory factor in sympathetic neurons. *J. Neurochem.* 103, 288–302.
- Ohara, P.T., Vit, J.-P., Bhargava, A., and Jasmin, L. (2008). Evidence for a role of connexin 43 in trigeminal pain using RNA interference in vivo. *J. Neurophysiol.* 100, 3064–3073.
- Ohba, N., Maeda, M., Nakagomi, S., Muraoka, M., and Kiyama, H. (2003). Biphasic expression of activating transcription factor-3 in neurons after cerebral infarction. *Brain Res. Mol. Brain Res.* 115, 147–156.
- Oliveira, A.L., Risling, M., Deckner, M., Lindholm, T., Langone, F., and Cullheim, S. (1997). Neonatal sciatic nerve transection induces TUNEL labeling of neurons in the rat spinal cord and DRG. *Neuroreport* 8, 2837–2840.

- Omura, T., Omura, K., Sano, M., Sawada, T., Hasegawa, T., and Nagano, A. (2005). Spatiotemporal quantification of recruit and resident macrophages after crush nerve injury utilizing immunohistochemistry. *Brain Res.* 1057, 29–36.
- Orgel, M., Aguayo, A., and Williams, H.B. (1972). Sensory nerve regeneration: an experimental study of skin grafts in the rabbit. *J. Anat.* 111, 121–135.
- Ossipov, M.H., Zhang, E.-T., Carvajal, C., Gardell, L., Quirion, R., Dumont, Y., Lai, J., and Porreca, F. (2002). Selective mediation of nerve injury-induced tactile hypersensitivity by neuropeptide Y. *J. Neurosci. Off. J. Soc. Neurosci.* 22, 9858–9867.
- Otoshi, K., Kikuchi, S., Konno, S., and Sekiguchi, M. (2010). The reactions of glial cells and endoneurial macrophages in the dorsal root ganglion and their contribution to pain-related behavior after application of nucleus pulposus onto the nerve root in rats. *Spine* 35, 264–271.
- Pagadala, P., Park, C.-K., Bang, S., Xu, Z.-Z., Xie, R.-G., Liu, T., Han, B.-X., Tracey, W.D., Wang, F., and Ji, R.-R. (2013). Loss of NR1 subunit of NMDARs in primary sensory neurons leads to hyperexcitability and pain hypersensitivity: involvement of Ca(2+)-activated small conductance potassium channels. *J. Neurosci.* 33, 13425–13430.
- Pannese, E. (1974). The histogenesis of the spinal ganglia. *Adv. Anat. Embryol. Cell Biol.* 47, 7–97.
- Pannese, E. (1978). The response of the satellite and other non-neuronal cells to the degeneration of neuroblasts in chick embryo spinal ganglia. *Cell Tissue Res.* 190, 1–14.
- Pannese, E. (1981). The satellite cells of the sensory ganglia. *Adv. Anat. Embryol. Cell Biol.* 65, 1–111.

- Pannese, E., Ledda, M., Cherkas, P.S., Huang, T.Y., and Hanani, M. (2003). Satellite cell reactions to axon injury of sensory ganglion neurons: increase in number of gap junctions and formation of bridges connecting previously separate perineuronal sheaths. *Anat. Embryol. (Berl.)* 206, 337–347.
- Paoletti, P., and Neyton, J. (2007). NMDA receptor subunits: function and pharmacology. *Curr. Opin. Pharmacol.* 7, 39–47.
- Park, K.K., Liu, K., Hu, Y., Smith, P.D., Wang, C., Cai, B., Xu, B., Connolly, L., Kramvis, I., Sahin, M., et al. (2008). Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway. *Science* 322, 963–966.
- Pearce, J.M. (2000). Wallerian degeneration. *J. Neurol. Neurosurg. Psychiatry* 69, 791.
- Pearson, A.G., Gray, C.W., Pearson, J.F., Greenwood, J.M., During, M.J., and Dragunow, M. (2003). ATF3 enhances c-Jun-mediated neurite sprouting. *Brain Res. Mol. Brain Res.* 120, 38–45.
- Pedersen, L.M., and Gjerstad, J. (2008). Spinal cord long-term potentiation is attenuated by the NMDA-2B receptor antagonist Ro 25-6981. *Acta Physiol.* 192, 421–427.
- Pei, L., Shang, Y., Jin, H., Wang, S., Wei, N., Yan, H., ... & Lu, Y. (2014). DAPK1-p53 Interaction Converges Necrotic and Apoptotic Pathways of Ischemic Neuronal Death. *The Journal of Neuroscience*, 34(19), 6546-6556.
- Peles, S., Miranda, A., Shaker, R., and Sengupta, J.N. (2004). Acute nociceptive somatic stimulus sensitizes neurones in the spinal cord to colonic distension in the rat. *J. Physiol.* 560, 291–302.
- Pelled, D., Raveh, T., Riebeling, C., Fridkin, M., Berissi, H., Futerman, A.H., and Kimchi, A. (2002). Death-associated protein (DAP) kinase plays a central role in ceramide-induced apoptosis in cultured hippocampal neurons. *J. Biol. Chem.* 277, 1957–1961.

- Perlson, E., Hanz, S., Ben-Yaakov, K., Segal-Ruder, Y., Seger, R., and Fainzilber, M. (2005). Vimentin-dependent spatial translocation of an activated MAP kinase in injured nerve. *Neuron* 45, 715–726.
- Perrin, F.E., Lacroix, S., Avilés-Trigueros, M., and David, S. (2005). Involvement of monocyte chemoattractant protein-1, macrophage inflammatory protein-1alpha and interleukin-1beta in Wallerian degeneration. *Brain J. Neurol.* 128, 854–866.
- Perry, V.H., Brown, M.C., and Gordon, S. (1987). The macrophage response to central and peripheral nerve injury. A possible role for macrophages in regeneration. *J. Exp. Med.* 165, 1218–1223.
- Le Pichon, C.E., Valley, M.T., Polymenidou, M., Chesler, A.T., Sagdullaev, B.T., Aguzzi, A., and Firestein, S. (2009). Olfactory behavior and physiology are disrupted in prion protein knockout mice. *Nat. Neurosci.* 12, 60–69.
- Price, J. (1985). An immunohistochemical and quantitative examination of dorsal root ganglion neuronal subpopulations. *J. Neurosci.* 5, 2051–2059.
- Proud, C.G. (2007). Signalling to translation: how signal transduction pathways control the protein synthetic machinery. *Biochem. J.* 403, 217–234.
- Raivich, G., Bohatschek, M., Da Costa, C., Iwata, O., Galiano, M., Hristova, M., Nateri, A.S., Makwana, M., Riera-Sans, L., Wolfer, D.P., et al. (2004). The AP-1 transcription factor c-Jun is required for efficient axonal regeneration. *Neuron* 43, 57–67.
- Raivich, G., and Makwana, M. (2007). The making of successful axonal regeneration: genes, molecules and signal transduction pathways. *Brain Res. Rev.* 53, 287–311.
- Raivich, G., Gehrmann, J., and Kreutzberg, G.W. (1991). Increase of macrophage colony-stimulating factor and granulocyte-macrophage colony-stimulating factor receptors in the regenerating rat facial nucleus. *J. Neurosci. Res.* 30, 682–686.

- Rambourg, A., Clermont, Y., and Beaudet, A. (1983). Ultrastructural features of six types of neurons in rat dorsal root ganglia. *J. Neurocytol.* *12*, 47–66.
- Raveh, T., Droguett, G., Horwitz, M.S., DePinho, R.A., and Kimchi, A. (2001). DAP kinase activates a p19ARF/p53-mediated apoptotic checkpoint to suppress oncogenic transformation. *Nat. Cell Biol.* *3*, 1–7.
- Ren, K., and Dubner, R. (1993). NMDA receptor antagonists attenuate mechanical hyperalgesia in rats with unilateral inflammation of the hindpaw. *Neurosci. Lett.* *163*, 22–26.
- Ren, K., Hylden, J.L., Williams, G.M., Ruda, M.A., and Dubner, R. (1992). The effects of a non-competitive NMDA receptor antagonist, MK-801, on behavioral hyperalgesia and dorsal horn neuronal activity in rats with unilateral inflammation. *Pain* *50*, 331–344.
- Renganathan, M., Cummins, T.R., and Waxman, S.G. (2001). Contribution of Na(v)1.8 sodium channels to action potential electrogenesis in DRG neurons. *J. Neurophysiol.* *86*, 629–640.
- Renno, W.M. (1998). Prolonged noxious stimulation increases periaqueductal gray NMDA mRNA expression: a hybridization study using two different rat models for nociception. *Neurobiol. Bp. Hung.* *6*, 333–357.
- Rich, K.M., Disch, S.P., and Eichler, M.E. (1989). The influence of regeneration and nerve growth factor on the neuronal cell body reaction to injury. *J. Neurocytol.* *18*, 569–576.
- Richardson, P.M., and Ebendal, T. (1982). Nerve growth activities in rat peripheral nerve. *Brain Res.* *246*, 57–64.
- Robinson, L.R. (2000). Traumatic injury to peripheral nerves. *Muscle Nerve* *23*, 863–873.

- Rossi, F., Gianola, S., and Corvetto, L. (2007). Regulation of intrinsic neuronal properties for axon growth and regeneration. *Prog. Neurobiol.* *81*, 1–28.
- Van Rossum, D.B., Patterson, R.L., Cheung, K.-H., Barrow, R.K., Syrovatkina, V., Gessell, G.S., Burkholder, S.G., Watkins, D.N., Foskett, J.K., and Snyder, S.H. (2006). DANGER, a novel regulatory protein of inositol 1,4,5-trisphosphate-receptor activity. *J. Biol. Chem.* *281*, 37111–37116.
- Rothman, S.M., and Olney, J.W. (1986). Glutamate and the pathophysiology of hypoxic-ischemic brain damage. *Ann. Neurol.* *19*, 105–111.
- Rothman, S.M., and Olney, J.W. (1995). Excitotoxicity and the NMDA receptor--still lethal after eight years. *Trends Neurosci.* *18*, 57–58.
- Rotshenker, S. (2011). Wallerian degeneration: the innate-immune response to traumatic nerve injury. *J. Neuroinflammation* *8*, 109.
- Rotter, V., Witte, O.N., Coffman, R., and Baltimore, D. (1980). Abelson murine leukemia virus-induced tumors elicit antibodies against a host cell protein, P50. *J. Virol.* *36*, 547–555.
- Ryan, T.J., Emes, R.D., Grant, S.G., and Komiyama, N.H. (2008). Evolution of NMDA receptor cytoplasmic interaction domains: implications for organisation of synaptic signalling complexes. *BMC Neurosci.* *9*, 6.
- Saadé, N.E., and Jabbur, S.J. (2008). Nociceptive behavior in animal models for peripheral neuropathy: spinal and supraspinal mechanisms. *Prog. Neurobiol.* *86*, 22–47.
- Sakagami, H., and Kondo, H. (1997). Molecular cloning and developmental expression of a rat homologue of death-associated protein kinase in the nervous system. *Brain Res. Mol. Brain Res.* *52*, 249–256.

- Sanchez-Cespedes, M., Esteller, M., Wu, L., Nawroz-Danish, H., Yoo, G.H., Koch, W.M., Jen, J., Herman, J.G., and Sidransky, D. (2000). Gene promoter hypermethylation in tumors and serum of head and neck cancer patients. *Cancer Res.* *60*, 892–895.
- Sanders, V.M., and Jones, K.J. (2006). Role of immunity in recovery from a peripheral nerve injury. *J. Neuroimmune Pharmacol. Off. J. Soc. Neuroimmune Pharmacol.* *1*, 11–19.
- Sandkühler, J. (2009). Models and mechanisms of hyperalgesia and allodynia. *Physiol. Rev.* *89*, 707–758.
- Saraste, M., Sibbald, P.R., and Wittinghofer, A. (1990). The P-loop--a common motif in ATP- and GTP-binding proteins. *Trends Biochem. Sci.* *15*, 430–434.
- Sato, K., Kiyama, H., Park, H.T., and Tohyama, M. (1993a). AMPA, KA and NMDA receptors are expressed in the rat DRG neurones. *Neuroreport* *4*, 1263–1265.
- Sato, K., Kiyama, H., Park, H.T., and Tohyama, M. (1993b). AMPA, KA and NMDA receptors are expressed in the rat DRG neurones. *Neuroreport* *4*, 1263–1265.
- Sawynok, J., and Reid, A. (2002). Modulation of formalin-induced behaviors and edema by local and systemic administration of dextromethorphan, memantine and ketamine. *Eur. J. Pharmacol.* *450*, 153–162.
- Schmalbruch, H. (1987). Loss of sensory neurons after sciatic nerve section in the rat. *Anat. Rec.* *219*, 323–329.
- Schmitt, C.A., Fridman, J.S., Yang, M., Baranov, E., Hoffman, R.M., and Lowe, S.W. (2002). Dissecting p53 tumor suppressor functions in vivo. *Cancer Cell* *1*, 289–298.
- Schoepp, D.D., and Conn, P.J. (1993). Metabotropic glutamate receptors in brain function and pathology. *Trends Pharmacol. Sci.* *14*, 13–20.

- Schori, H., Yoles, E., Wheeler, L.A., Raveh, T., Kimchi, A., and Schwartz, M. (2002). Immune-related mechanisms participating in resistance and susceptibility to glutamate toxicity. *Eur. J. Neurosci.* *16*, 557–564.
- Schumacher, A.M., Velentza, A.V., Watterson, D.M., and Wainwright, M.S. (2002). DAPK catalytic activity in the hippocampus increases during the recovery phase in an animal model of brain hypoxic-ischemic injury. *Biochim. Biophys. Acta* *1600*, 128–137.
- Schumacher, B., Hofmann, K., Boulton, S., and Gartner, A. (2001). The *C. elegans* homolog of the p53 tumor suppressor is required for DNA damage-induced apoptosis. *Curr. Biol. CB* *11*, 1722–1727.
- Schwab, M.E. (2004). Nogo and axon regeneration. *Curr. Opin. Neurobiol.* *14*, 118–124.
- Schwab, M.E., and Bartholdi, D. (1996). Degeneration and regeneration of axons in the lesioned spinal cord. *Physiol. Rev.* *76*, 319–370.
- Schwarcz, R., Whetsell, W.O., and Mangano, R.M. (1983). Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain. *Science* *219*, 316–318.
- Seddon, H.J. (1950). Peripheral nerve injuries in Great Britain during World War II; a review. *Arch. Neurol. Psychiatry* *63*, 171–173.
- Seddon, H.J. (1954). Methods of investigating nerve injuries. *Spec. Rep. Ser. Med. Res. Counc. G. B.* *282*, 1–15.
- Seddon, H.J., Medawar, P.B., and Smith, H. (1943). Rate of regeneration of peripheral nerves in man. *J. Physiol.* *102*, 191–215.
- Seiffers, R., Mills, C.D., and Woolf, C.J. (2007). ATF3 increases the intrinsic growth state of DRG neurons to enhance peripheral nerve regeneration. *J. Neurosci.* *27*, 7911–7920.

- Seltzer, Z., Dubner, R., and Shir, Y. (1990). A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. *Pain* 43, 205–218.
- Shadiack, A.M., Sun, Y., and Zigmond, R.E. (2001). Nerve growth factor antiserum induces axotomy-like changes in neuropeptide expression in intact sympathetic and sensory neurons. *J. Neurosci.* 21, 363–371.
- Shamash, S., Reichert, F., and Rotshenker, S. (2002). The cytokine network of Wallerian degeneration: tumor necrosis factor- α , interleukin-1 α , and interleukin-1 β . *J. Neurosci.* 22, 3052–3060.
- Shamloo, M., Soriano, L., Wieloch, T., Nikolich, K., Urfer, R., and Oksenberg, D. (2005). Death-associated protein kinase is activated by dephosphorylation in response to cerebral ischemia. *J. Biol. Chem.* 280, 42290–42299.
- Shehab, S.A., and Atkinson, M.E. (1986). Vasoactive intestinal polypeptide (VIP) increases in the spinal cord after peripheral axotomy of the sciatic nerve originate from primary afferent neurons. *Brain Res.* 372, 37–44.
- Shehab, S. a. S., Spike, R.C., and Todd, A.J. (2003). Evidence against cholera toxin B subunit as a reliable tracer for sprouting of primary afferents following peripheral nerve injury. *Brain Res.* 964, 218–227.
- Shehab, S.A.S., Spike, R.C., and Todd, A.J. (2004). Do central terminals of intact myelinated primary afferents sprout into the superficial dorsal horn of rat spinal cord after injury to a neighboring peripheral nerve? *J. Comp. Neurol.* 474, 427–437.
- Shehab, S.A.S., Al-Marashda, K., Al-Zahmi, A., Abdul-Kareem, A., and Al-Sultan, M.A.H. (2008). Unmyelinated primary afferents from adjacent spinal nerves intermingle in the spinal dorsal horn: a possible mechanism contributing to neuropathic pain. *Brain Res.* 1208, 111–119.

- Shehab, S.A.S., (2011). Fifth lumbar spinal nerve injury causes neurochemical changes in corresponding as well as adjacent spinal segments. A possible mechanism underlying neuropathic pain. In: 8th IBRO World Congress of Neuroscience, Florence, Italy.
- Sheikh, M.S., Burns, T.F., Huang, Y., Wu, G.S., Amundson, S., Brooks, K.S., Fornace, A.J., and el-Deiry, W.S. (1998). p53-dependent and -independent regulation of the death receptor KILLER/DR5 gene expression in response to genotoxic stress and tumor necrosis factor alpha. *Cancer Res.* 58, 1593–1598.
- Shigemoto, R., Ohishi, H., Nakanishi, S., and Mizuno, N. (1992). Expression of the mRNA for the rat NMDA receptor (NMDAR1) in the sensory and autonomic ganglion neurons. *Neurosci. Lett.* 144, 229–232.
- Shinder, V., and Devor, M. (1994). Structural basis of neuron-to-neuron cross-excitation in dorsal root ganglia. *J. Neurocytol.* 23, 515–531.
- Shinder, V., Govrin-Lippmann, R., Cohen, S., Belenky, M., Ilin, P., Fried, K., Wilkinson, H.A., and Devor, M. (1999). Structural basis of sympathetic-sensory coupling in rat and human dorsal root ganglia following peripheral nerve injury. *J. Neurocytol.* 28, 743–761.
- Shohat, G., Spivak-Kroizman, T., Cohen, O., Bialik, S., Shani, G., Berrisi, H., Eisenstein, M., and Kimchi, A. (2001). The pro-apoptotic function of death-associated protein kinase is controlled by a unique inhibitory autophosphorylation-based mechanism. *J. Biol. Chem.* 276, 47460–47467.
- Shu, S., Pei, L., and Lu, Y. (2014). Promising targets of cell death signaling of NR2B receptor subunit in stroke pathogenesis. *Regenerative Medicine Research*, 2:8.

- Simon, R.P., Swan, J.H., Griffiths, T., and Meldrum, B.S. (1984). Blockade of N-methyl-D-aspartate receptors may protect against ischemic damage in the brain. *Science* 226, 850–852.
- Skene, J.H. (1984). Growth-associated proteins and the curious dichotomies of nerve regeneration. *Cell* 37, 697–700.
- Skene, J.H., and Shooter, E.M. (1983). Denervated sheath cells secrete a new protein after nerve injury. *Proc. Natl. Acad. Sci. U. S. A.* 80, 4169–4173.
- Skene, J.H., and Willard, M. (1981). Axonally transported proteins associated with axon growth in rabbit central and peripheral nervous systems. *J. Cell Biol.* 89, 96–103.
- Sloviter, R.S. (1991). Permanently altered hippocampal structure, excitability, and inhibition after experimental status epilepticus in the rat: the “dormant basket cell” hypothesis and its possible relevance to temporal lobe epilepsy. *Hippocampus* 1, 41–66.
- Smith, D.S., and Skene, J.H. (1997). A transcription-dependent switch controls competence of adult neurons for distinct modes of axon growth. *J. Neurosci.* 17, 646–658.
- Snider, W.D., Zhou, F.-Q., Zhong, J., and Markus, A. (2002). Signaling the pathway to regeneration. *Neuron* 35, 13–16.
- Soler, R.M., Dolcet, X., Encinas, M., Egea, J., Bayascas, J.R., and Comella, J.X. (1999). Receptors of the glial cell line-derived neurotrophic factor family of neurotrophic factors signal cell survival through the phosphatidylinositol 3-kinase pathway in spinal cord motoneurons. *J. Neurosci.* 19, 9160–9169.
- Sommer, B., and Seeburg, P.H. (1992). Glutamate receptor channels: novel properties and new clones. *Trends Pharmacol. Sci.* 13, 291–296.

- Son, Y.J., and Thompson, W.J. (1995). Schwann cell processes guide regeneration of peripheral axons. *Neuron* *14*, 125–132.
- Song, X.-J., Zhang, J.-M., Hu, S.-J., and LaMotte, R.H. (2003). Somata of nerve-injured sensory neurons exhibit enhanced responses to inflammatory mediators. *Pain* *104*, 701–709.
- Spater, H.W., Novikoff, A.B., Spater, S.H., and Quintana, N. (1978). Pyridoxal phosphatase: cytochemical localization in GERL and other organelles of rat neurons. *J. Histochem. Cytochem.* *26*, 809–821.
- Stephenson, J.L., and Byers, M.R. (1995). GFAP immunoreactivity in trigeminal ganglion satellite cells after tooth injury in rats. *Exp. Neurol.* *131*, 11–22.
- Steckley, D., Karajgikar, M., Dale, L.B., Fuerth, B., Swan, P., Drummond-Main, C., Poulter, M.O., Ferguson, S.S.G., Strasser, A., and Cregan, S.P. (2007). Puma is a dominant regulator of oxidative stress induced Bax activation and neuronal apoptosis. *J. Neurosci.* *27*, 12989–12999.
- Stucky, C.L., and Lewin, G.R. (1999). Isolectin B(4)-positive and -negative nociceptors are functionally distinct. *J. Neurosci.* *19*, 6497–6505.
- Sun, F., and He, Z. (2010). Neuronal intrinsic barriers for axon regeneration in the adult CNS. *Curr. Opin. Neurobiol.* *20*, 510–518.
- Sung, Y.J., Chiu, D.T.W., and Ambron, R.T. (2006). Activation and retrograde transport of protein kinase G in rat nociceptive neurons after nerve injury and inflammation. *Neuroscience* *141*, 697–709.
- Swaim, S.F. (1972). Peripheral nerve surgery in the dog. *J. Am. Vet. Med. Assoc.* *161*, 905–911.

- Symonds, H., Krall, L., Remington, L., Saenz-Robles, M., Lowe, S., Jacks, T., and Van Dyke, T. (1994). p53-dependent apoptosis suppresses tumor growth and progression in vivo. *Cell* 78, 703–711.
- Takeda, M., Tanimoto, T., Kadoi, J., Nasu, M., Takahashi, M., Kitagawa, J., and Matsumoto, S. (2007). Enhanced excitability of nociceptive trigeminal ganglion neurons by satellite glial cytokine following peripheral inflammation. *Pain* 129, 155–166.
- Tal, M., and Bennett, G.J. (1993). Dextrorphan relieves neuropathic heat-evoked hyperalgesia in the rat. *Neurosci. Lett.* 151, 107–110.
- Tanaka, K., Zhang, Q.L., and Webster, H.D. (1992). Myelinated fiber regeneration after sciatic nerve crush: morphometric observations in young adult and aging mice and the effects of macrophage suppression and conditioning lesions. *Exp. Neurol.* 118, 53–61.
- Tandrup, T. (1993a). A method for unbiased and efficient estimation of number and mean volume of specified neuron subtypes in rat dorsal root ganglion. *J. Comp. Neurol.* 329, 269–276.
- Tandrup, T. (1993b). A method for unbiased and efficient estimation of number and mean volume of specified neuron subtypes in rat dorsal root ganglion. *J. Comp. Neurol.* 329, 269–276.
- Tang, Y.P., Shimizu, E., Dube, G.R., Rampon, C., Kerchner, G.A., Zhuo, M., Liu, G., and Tsien, J.Z. (1999). Genetic enhancement of learning and memory in mice. *Nature* 401, 63–69.
- Taniuchi, M., Clark, H.B., and Johnson, E.M. (1986). Induction of nerve growth factor receptor in Schwann cells after axotomy. *Proc. Natl. Acad. Sci. U. S. A.* 83, 4094–4098.

- Taniguchi, K., Shinjo, K., Mizutani, M., Shimada, K., Ishikawa, T., Menniti, F.S., and Nagahisa, A. (1997). Antinociceptive activity of CP-101,606, an NMDA receptor NR2B subunit antagonist. *Br. J. Pharmacol.* *122*, 809–812.
- Taskinen, H.S., and Røyttä, M. (1997). The dynamics of macrophage recruitment after nerve transection. *Acta Neuropathol. (Berl.)* *93*, 252–259.
- Terasaki, Y., Sasaki, T., Yagita, Y., Okazaki, S., Sugiyama, Y., Oyama, N., Omura-Matsuoka, E., Sakoda, S., and Kitagawa, K. (2010). Activation of NR2A receptors induces ischemic tolerance through CREB signaling. *J. Cereb. Blood Flow Metab. Off. J. Int. Soc. Cereb. Blood Flow Metab.* *30*, 1441–1449.
- Terenghi, G. (1999). Peripheral nerve regeneration and neurotrophic factors. *J. Anat.* *194 (Pt 1)*, 1–14.
- Tereshko, V., Teplova, M., Brunzelle, J., Watterson, D.M., and Egli, M. (2001). Crystal structures of the catalytic domain of human protein kinase associated with apoptosis and tumor suppression. *Nat. Struct. Biol.* *8*, 899–907.
- Tian, J.-H., Das, S., and Sheng, Z.-H. (2003a). Ca²⁺-dependent phosphorylation of syntaxin-1A by the death-associated protein (DAP) kinase regulates its interaction with Munc18. *J. Biol. Chem.* *278*, 26265–26274.
- Tian, J.-H., Das, S., and Sheng, Z.-H. (2003b). Ca²⁺-dependent phosphorylation of syntaxin-1A by the death-associated protein (DAP) kinase regulates its interaction with Munc18. *J. Biol. Chem.* *278*, 26265–26274.
- Toews, A.D., Barrett, C., and Morell, P. (1998). Monocyte chemoattractant protein 1 is responsible for macrophage recruitment following injury to sciatic nerve. *J. Neurosci. Res.* *53*, 260–267.
- Tong, A., Lynn, G., Ngo, V., Wong, D., Moseley, S.L., Ewbank, J.J., Goncharov, A., Wu, Y.-C., Pujol, N., and Chisholm, A.D. (2009). Negative regulation of

- Caenorhabditis elegans epidermal damage responses by death-associated protein kinase. *Proc. Natl. Acad. Sci. U. S. A.* *106*, 1457–1461.
- Tovar, K.R., and Westbrook, G.L. (1999). The incorporation of NMDA receptors with a distinct subunit composition at nascent hippocampal synapses in vitro. *J. Neurosci.* *19*, 4180–4188.
- Traynelis, S.F., Wollmuth, L.P., McBain, C.J., Menniti, F.S., Vance, K.M., Ogden, K.K., Hansen, K.B., Yuan, H., Myers, S.J., and Dingledine, R. (2010). Glutamate-receptor ion channels: structure, regulation, and function. *Pharmacol. Rev.* *62*, 405–496.
- Tsujino, H., Kondo, E., Fukuoka, T., Dai, Y., Tokunaga, A., Miki, K., Yonenobu, K., Ochi, T., and Noguchi, K. (2000). Activating transcription factor 3 (ATF3) inductions by axotomy in sensory and motoneurons: A novel neuronal marker of nerve injury. *Mol. Cell. Neurosci.* *15*, 170–182.
- Tu, W., Xu, X., Peng, L., Zhong, X., Zhang, W., Soundarapandian, M.M., Balel, C., Wang, M., Jia, N., Zhang, W., et al. (2010). DAPK1 interaction with NMDA receptor NR2B subunits mediates brain damage in stroke. *Cell* *140*, 222–234.
- Turski, L., Bressler, K., Rettig, K.J., Löschmann, P.A., and Wachtel, H. (1991). Protection of substantia nigra from MPP⁺ neurotoxicity by N-methyl-D-aspartate antagonists. *Nature* *349*, 414–418.
- Ulas, J., Brunner, L.C., Geddes, J.W., Choe, W., and Cotman, C.W. (1992). N-methyl-D-aspartate receptor complex in the hippocampus of elderly, normal individuals and those with Alzheimer's disease. *Neuroscience* *49*, 45–61.
- Ulfenius, C., Linderöth, B., Meyerson, B.A., and Wallin, J. (2006). Spinal NMDA receptor phosphorylation correlates with the presence of neuropathic signs following peripheral nerve injury in the rat. *Neurosci. Lett.* *399*, 85–90.

- Urch, C.E., Rahman, W., and Dickenson, A.H. (2001). Electrophysiological studies on the role of the NMDA receptor in nociception in the developing rat spinal cord. *Brain Res. Dev. Brain Res.* 126, 81–89.
- Vargas, M.E., and Barres, B.A. (2007). Why is Wallerian degeneration in the CNS so slow? *Annu. Rev. Neurosci.* 30, 153–179.
- Varon, S., Manthorpe, M., and Williams, L.R. (1983). Neuronotrophic and neurite-promoting factors and their clinical potentials. *Dev. Neurosci.* 6, 73–100.
- Velentza, A.V., Schumacher, A.M., Weiss, C., Egli, M., and Watterson, D.M. (2001). A protein kinase associated with apoptosis and tumor suppression: structure, activity, and discovery of peptide substrates. *J. Biol. Chem.* 276, 38956–38965.
- Verdú, E., Ceballos, D., Vilches, J.J., and Navarro, X. (2000). Influence of aging on peripheral nerve function and regeneration. *J. Peripher. Nerv. Syst. JPNS* 5, 191–208.
- Villar, M.J., Cortés, R., Theodorsson, E., Wiesenfeld-Hallin, Z., Schalling, M., Fahrenkrug, J., Emson, P.C., and Hökfelt, T. (1989). Neuropeptide expression in rat dorsal root ganglion cells and spinal cord after peripheral nerve injury with special reference to galanin. *Neuroscience* 33, 587–604.
- Villar, M.J., Wiesenfeld-Hallin, Z., Xu, X.J., Theodorsson, E., Emson, P.C., and Hökfelt, T. (1991). Further studies on galanin-, substance P-, and CGRP-like immunoreactivities in primary sensory neurons and spinal cord: effects of dorsal rhizotomies and sciatic nerve lesions. *Exp. Neurol.* 112, 29–39.
- Vit, J.-P., Jasmin, L., Bhargava, A., and Ohara, P.T. (2006). Satellite glial cells in the trigeminal ganglion as a determinant of orofacial neuropathic pain. *Neuron Glia Biol.* 2, 247–257.
- Vit, J.-P., Ohara, P.T., Sundberg, C., Rubi, B., Maechler, P., Liu, C., Puntel, M., Lowenstein, P., Castro, M., and Jasmin, L. (2009). Adenovector GAD65 gene

- delivery into the rat trigeminal ganglion produces orofacial analgesia. *Mol. Pain* 5, 42.
- Vogelstein, B., Lane, D., and Levine, A.J. (2000). Surfing the p53 network. *Nature* 408, 307–310.
- Vousden, K.H., and Lu, X. (2002). Live or let die: the cell's response to p53. *Nat. Rev. Cancer* 2, 594–604.
- Wafford, K.A., Kathoria, M., Bain, C.J., Marshall, G., Le Bourdellès, B., Kemp, J.A., and Whiting, P.J. (1995). Identification of amino acids in the N-methyl-D-aspartate receptor NR1 subunit that contribute to the glycine binding site. *Mol. Pharmacol.* 47, 374–380.
- Wakisaka, S., Kajander, K.C., and Bennett, G.J. (1991). Increased neuropeptide Y (NPY)-like immunoreactivity in rat sensory neurons following peripheral axotomy. *Neurosci. Lett.* 124, 200–203.
- Walensky, L.D., and Gavathiotis, E. (2011). BAX unleashed: the biochemical transformation of an inactive cytosolic monomer into a toxic mitochondrial pore. *Trends Biochem. Sci.* 36, 642–652.
- Waller AV. (1850). Experiments on the section of the glossopharyngeal and hypoglossal nerves of the frog, and observations of the alterations produced thereby in the structure of their primitive fibres. *Philosophical Trans. Royal Soc. London-Biol. Sci.* 140, 423-429.
- Wang, W.-J., Kuo, J.-C., Ku, W., Lee, Y.-R., Lin, F.-C., Chang, Y.-L., Lin, Y.-M., Chen, C.-H., Huang, Y.-P., Chiang, M.-J., et al. (2007). The tumor suppressor DAPK is reciprocally regulated by tyrosine kinase Src and phosphatase LAR. *Mol. Cell* 27, 701–716.
- Waris, T. (1978). Reinnervation of free skin autografts in the rat. *Scand. J. Plast. Reconstr. Surg.* 12, 85–93.

- Warwick, R.A., and Hanani, M. (2013). The contribution of satellite glial cells to chemotherapy-induced neuropathic pain. *Eur. J. Pain Lond. Engl.* *17*, 571–580.
- Warwick, R.A., Ledgerwood, C.J., Brenner, T., and Hanani, M. (2014). Satellite glial cells in dorsal root ganglia are activated in experimental autoimmune encephalomyelitis. *Neurosci. Lett.* *569*, 59–62.
- Watanabe, M., Inoue, Y., Sakimura, K., and Mishina, M. (1993). Distinct spatio-temporal distributions of the NMDA receptor channel subunit mRNAs in the brain. *Ann. N. Y. Acad. Sci.* *707*, 463–466.
- White, D.M., Walker, S., Brenneman, D.E., and Gozes, I. (2000). CREB contributes to the increased neurite outgrowth of sensory neurons induced by vasoactive intestinal polypeptide and activity-dependent neurotrophic factor. *Brain Res.* *868*, 31–38.
- White, F.A., Keller-Peck, C.R., Knudson, C.M., Korsmeyer, S.J., and Snider, W.D. (1998). Widespread elimination of naturally occurring neuronal death in Bax-deficient mice. *J. Neurosci.* *18*, 1428–1439.
- Wickenden, A.D., Maher, M.P., and Chaplan, S.R. (2009). HCN pacemaker channels and pain: a drug discovery perspective. *Curr. Pharm. Des.* *15*, 2149–2168.
- Willcockson, H., and Valtschanoff, J. (2008). AMPA and NMDA glutamate receptors are found in both peptidergic and non-peptidergic primary afferent neurons in the rat. *Cell Tissue Res.* *334*, 17–23.
- Willert, R.P., Woolf, C.J., Hobson, A.R., Delaney, C., Thompson, D.G., and Aziz, Q. (2004). The development and maintenance of human visceral pain hypersensitivity is dependent on the N-methyl-D-aspartate receptor. *Gastroenterology* *126*, 683–692.
- Willis, S.N., and Adams, J.M. (2005). Life in the balance: how BH3-only proteins induce apoptosis. *Curr. Opin. Cell Biol.* *17*, 617–625.

- Wilson, J.A., Garry, E.M., Anderson, H.A., Rosie, R., Colvin, L.A., Mitchell, R., and Fleetwood-Walker, S.M. (2005). NMDA receptor antagonist treatment at the time of nerve injury prevents injury-induced changes in spinal NR1 and NR2B subunit expression and increases the sensitivity of residual pain behaviours to subsequently administered NMDA receptor antagonists. *Pain* 117, 421–432.
- Wolter, K.G., Hsu, Y.T., Smith, C.L., Nechushtan, A., Xi, X.G., and Youle, R.J. (1997). Movement of Bax from the cytosol to mitochondria during apoptosis. *J. Cell Biol.* 139, 1281–1292.
- Woodham, P., Anderson, P.N., Nadim, W., and Turmaine, M. (1989a). Satellite cells surrounding axotomised rat dorsal root ganglion cells increase expression of a GFAP-like protein. *Neurosci. Lett.* 98, 8–12.
- Woolf, C.J., and Salter, M.W. (2000). Neuronal plasticity: increasing the gain in pain. *Science* 288, 1765–1769.
- Woolf, C.J., and Thompson, S.W. (1991). The induction and maintenance of central sensitization is dependent on N-methyl-D-aspartic acid receptor activation; implications for the treatment of post-injury pain hypersensitivity states. *Pain* 44, 293–299.
- Wu, L.-J., and Zhuo, M. (2009). Targeting the NMDA receptor subunit NR2B for the treatment of neuropathic pain. *Neurother. J. Am. Soc. Exp. Neurother.* 6, 693–702.
- Wu, P.-R., Tsai, P.-I., Chen, G.-C., Chou, H.-J., Huang, Y.-P., Chen, Y.-H., Lin, M.-Y., Kimchi, A., Chien, C.-T., and Chen, R.-H. (2011). DAPK activates MARK1/2 to regulate microtubule assembly, neuronal differentiation, and tau toxicity. *Cell Death Differ.* 18, 1507–1520.
- Xiao, H.-S., Huang, Q.-H., Zhang, F.-X., Bao, L., Lu, Y.-J., Guo, C., Yang, L., Huang, W.-J., Fu, G., Xu, S.-H., et al. (2002). Identification of gene expression profile of

dorsal root ganglion in the rat peripheral axotomy model of neuropathic pain. *Proc. Natl. Acad. Sci. U. S. A.* 99, 8360–8365.

- Xie, W., Strong, J.A., Meij, J.T.A., Zhang, J.-M., and Yu, L. (2005). Neuropathic pain: early spontaneous afferent activity is the trigger. *Pain* 116, 243–256.
- Xie, F., and Zheng, B. (2008). White matter inhibitors in CNS axon regeneration failure. *Exp. Neurol.* 209, 302–312.
- Xie, W., Strong, J.A., and Zhang, J.-M. (2009a). Early blockade of injured primary sensory afferents reduces glial cell activation in two rat neuropathic pain models. *Neuroscience* 160, 847–857.
- Xu, M., Aita, M., and Chavkin, C. (2008). Partial infraorbital nerve ligation as a model of trigeminal nerve injury in the mouse: behavioral, neural, and glial reactions. *J. Pain* 9, 1036–1048.
- Yamamoto, M., Takahashi, H., Nakamura, T., Hioki, T., Nagayama, S., Ooashi, N., Sun, X., Ishii, T., Kudo, Y., Nakajima-Iijima, S., et al. (1999). Developmental changes in distribution of death-associated protein kinase mRNAs. *J. Neurosci. Res.* 58, 674–683.
- Yamamoto, M., Hioki, T., Ishii, T., Nakajima-Iijima, S., and Uchino, S. (2002). DAP kinase activity is critical for C(2)-ceramide-induced apoptosis in PC12 cells. *Eur. J. Biochem. FEBS* 269, 139–147.
- Yan, X., Jiang, E., Gao, M., and Weng, H.-R. (2013). Endogenous activation of presynaptic NMDA receptors enhances glutamate release from the primary afferents in the spinal dorsal horn in a rat model of neuropathic pain. *J. Physiol.* 591, 2001–2019.
- Ygge, J. (1989). Neuronal loss in lumbar dorsal root ganglia after proximal compared to distal sciatic nerve resection: a quantitative study in the rat. *Brain Res.* 478, 193–195.

- Yoshimura, Y., Ohmura, T., and Komatsu, Y. (2003). Two forms of synaptic plasticity with distinct dependence on age, experience, and NMDA receptor subtype in rat visual cortex. *J. Neurosci.* *23*, 6557–6566.
- Youle, R.J., and Strasser, A. (2008). The BCL-2 protein family: opposing activities that mediate cell death. *Nat. Rev. Mol. Cell Biol.* *9*, 47–59.
- Yu, J., and Zhang, L. (2005). The transcriptional targets of p53 in apoptosis control. *Biochem. Biophys. Res. Commun.* *331*, 851–858.
- Yukawa, K., Hoshino, K., Kishino, M., Mune, M., Shirasawa, N., Kimura, A., Tsubota, Y., Owada-Makabe, K., Tanaka, T., Ichinose, M., et al. (2004). Deletion of the kinase domain in death-associated protein kinase attenuates renal tubular cell apoptosis in chronic obstructive uropathy. *Int. J. Mol. Med.* *13*, 515–520.
- Yukawa, K., Kishino, M., Hoshino, K., Shirasawa, N., Kimura, A., Tsubota, Y., Owada-Makabe, K., Bai, T., Tanaka, T., Ueyama, T., et al. (2005). The kinase domain of death-associated protein kinase is inhibitory for tubulointerstitial fibrosis in chronic obstructive nephropathy. *Int. J. Mol. Med.* *15*, 73–78.
- Yukawa, K., Tanaka, T., Bai, T., Li, L., Tsubota, Y., Owada-Makabe, K., Maeda, M., Hoshino, K., Akira, S., and Iso, H. (2006). Deletion of the kinase domain from death-associated protein kinase enhances spatial memory in mice. *Int. J. Mol. Med.* *17*, 869–873.
- Zachary, R.B., and Holmes, W. (1946). Primary suture of nerves. *Surg. Gynecol. Obstet.* *82*, 632–651.
- Zalckvar, E., Berissi, H., Mizrachy, L., Idelchuk, Y., Koren, I., Eisenstein, M., Sabanay, H., Pinkas-Kramarski, R., and Kimchi, A. (2009). DAP-kinase-mediated phosphorylation on the BH3 domain of beclin 1 promotes dissociation of beclin 1 from Bcl-XL and induction of autophagy. *EMBO Rep.* *10*, 285–292.

- Zhang, X., Aman, K., Hoffelt, T. (1995b). Secretory pathways of neuropeptides in rat lumbar dorsal root ganglion neurons and effects of peripheral axotomy. *J. Comp. Neurol.* 352, 481–500.
- Zhao, S., and Fernald, R.D. (2005). Comprehensive algorithm for quantitative real-time polymerase chain reaction. *J. Comput. Biol. J. Comput. Mol. Cell Biol.* 12, 1047–1064.
- Zhou, M., and Baudry, M. (2006). Developmental changes in NMDA neurotoxicity reflect developmental changes in subunit composition of NMDA receptors. *J. Neurosci.* 26, 2956–2963.
- Zhou, S., Bonasera, L., and Carlton, S.M. (1996). Peripheral administration of NMDA, AMPA or KA results in pain behaviors in rats. *Neuroreport* 7, 895–900.
- Zhou, X.F., Deng, Y.S., Chie, E., Xue, Q., Zhong, J.H., McLachlan, E.M., Rush, R.A., and Xian, C.J. (1999). Satellite-cell-derived nerve growth factor and neurotrophin-3 are involved in noradrenergic sprouting in the dorsal root ganglia following peripheral nerve injury in the rat. *Eur. J. Neurosci.* 11, 1711–1722.
- Zhuo, M. (2007). A synaptic model for pain: long-term potentiation in the anterior cingulate cortex. *Mol. Cells* 23, 259–271.
- Zuo, Y., Perkins, N.M., Tracey, D.J., and Gezy, C.L. (2003). Inflammation and hyperalgesia induced by nerve injury in the rat: a key role of mast cells. *Pain* 105, 467–479.

B. Book Chapters:

- Birch, R. Nerve repair. Green, Hotchkiss Pederson Wolfe. (2005). *Green's Operative Hand Surgery* 5th Ed.. Elsevier, Churchill Livingstone.
- Bunge, R. P., & Bunge, M. B. (1981). Cues and constraints in Schwann cell development. In W. M. Cowan (Ed.), *Studies in Developmental Neurobiology* (pp. 322-353). New York; Oxford University Press.
- Cajal, S. R. (1928). *Degeneration and Regeneration of the Nervous System*, Vols. 1 & 2. London, Oxford University Press.
- Devor, M., (2006). Response of nerves to injury in relation to neuropathic pain. In: McMahon, S.B., Koltzenburg, M. (Eds.), *Wall Melzack's Textbook of Pain*. Elsevier Inc., London.
- Diamond J. and Jackson P.C. (1980). Regeneration and collateral sprouting of peripheral nerves. In: Jewett D.L. and Relton MC Carroll J. Jr (Eds). *Nerve Repair and Regeneration, its Clinical and Experimental Basis*. Mosby Company. St. Louis.
- Kreutzberg GW (1995) Reaction of the neuronal cell body to axonal damage. In: Waxman SG KJSP (Ed). *The Axon: Structure, Function and Pathophysiology*. Oxford University Press, New York,
- Lawson SN. 1992. *Morphological and Biochemical Cell Types of Sensory Neurons: Sensory Neurons, Diversity, Development and Plasticity*. New York: Oxford University Press.
- Peacock EE, VanWinkle W. (1972). *Wound Repair*. Philadelphia, WB Saunders.
- Richardson EP, De Girolami U. (1995). Pathology of the peripheral nerve. In: *General Reactions of Peripheral Nerve to Disease*. Philadelphia: Saunders, (p.8-21).
- Sunderland S. (1978). *Nerves and Nerve Injuries*. 2nd Ed. Baltimore: Williams & Wilkins.

Swaim S.F. (1978). Peripheral nerve surgery. In B.F. Hoerlein: *Canine Neurology*. Philadelphia, WB Saunders.

Zochodne D. (2008). *Neurobiology of Peripheral Nerve Regeneration*. Cambridge: Cambridge University Press.

- Swaim S.F. (1978). Peripheral nerve surgery. In B.F. Hoerlein: *Canine Neurology*. Philadelphia, WB Saunders.
- Zochodne D. (2008). *Neurobiology of Peripheral Nerve Regeneration*. Cambridge: Cambridge University Pres.

PUBLICATIONS

- Alsaadi, M., Shehab, S., and Al Hammadi, R. (2014). The Expression Pattern of Death Associated Protein Kinase 1 (DAPK1) In Dorsal Root Ganglion Neurons Following Peripheral Nerve Injury. Society for Neuroscience (SFN) Conference 2014 (*Abstract #2652*).
- Alsaadi, M., Bajbouj, K., and Al Hammadi, R. (2013). DAPK Signal Transduction Pathways and Its Role In The Nervous System. *European Journal of Biological and Life Sciences*, 10:2.
- Alsaadi, M., Al Hammadi, R., Shehab, S. (2014). The Expression Pattern of Death Associated Protein Kinase 1 (DAPK1) in Normal Dorsal Root Ganglion Neurons and Following Peripheral Nerve Injury. *Brain Research* [Under Review]
- Alsaadi, M., Anwer, M., Al Hammadi, R., Shehab, S. (2015). Characterization of neurons expressing Death Associated Protein Kinase 1 (DAPK1) in normal L4 and L5 Dorsal Root Ganglion [Forthcoming].