

# ASPECTS OF RETINAL AND OPTIC NERVE PATHOLOGY AFTER EXCITOTOXIC RETINAL INJURY

*DR. SARABJIT KAUR SAGGU*

Discipline of Ophthalmology and Visual Sciences  
The University of Adelaide, South Australia

&

Division of Neuropathology  
Institute of Medical and Veterinary Science  
Adelaide, South Australia

A thesis submitted to the University of Adelaide in fulfilment of the requirements  
for the degree of Doctor of Philosophy

**JUNE 2011**

*This thesis is dedicated to those  
who fight blindness with courage and dignity  
& my husband, Hiren, whose love I cherish.*

# TABLE OF CONTENTS

<b>ABSTRACT .....</b>	<b>VI</b>
<b>DECLARATION .....</b>	<b>VIII</b>
<b>PUBLICATIONS/ PRESENTATIONS RELATED TO THESIS.....</b>	<b>IX</b>
<b>ACKNOWLEDGEMENT .....</b>	<b>XI</b>
<b>ACRONYMS.....</b>	<b>XIII</b>
<b>LIST OF FIGURES.....</b>	<b>XVII</b>
<b>LIST OF TABLES .....</b>	<b>XIX</b>
<b>CHAPTER 1: INTRODUCTION.....</b>	<b>1</b>
<b>CHAPTER 2: BACKGROUND RATIONALE .....</b>	<b>5</b>
<b>2.1 DEFINITION OF TOPIC .....</b>	<b>5</b>
<b>2.2 RELEVANT ANATOMY .....</b>	<b>6</b>
2.2.1 EYEBALL AND RETINA.....	6
2.2.1.1 Microscopic structure of the vertebrate retina .....	7
2.2.2 OPTIC NERVE.....	8
2.2.3 CHARACTERISTIC FEATURES OF THE ALBINO RAT VISUAL SYSTEM .....	10
<b>2.3 MORPHOLOGY OF THE NEURON.....</b>	<b>11</b>
2.3.1 THE NEURONAL CELL BODY.....	12
2.3.1.1 Nucleus.....	12
2.3.1.2 Perikaryon .....	12
2.3.2 DENDRITES .....	13
2.3.3 THE AXON.....	13
2.3.3.1 The Axon Hillock and the Initial Segment.....	14
2.3.3.2 The Axon Proper.....	14
2.3.4 THE MYELIN .....	15
2.3.5 AXO-GLIAL RELATIONSHIPS AND DOMAINS OF A MYELINATED AXON.....	18
<b>2.4 GLIAL CELLS IN THE CNS.....</b>	<b>20</b>
2.4.1 OLIGODENDROCYTES .....	21
2.4.2 ASTROCYTES .....	22
2.4.3 MICROGLIA .....	23
<b>2.5 AXONAL TRANSPORT.....</b>	<b>24</b>
2.5.1 FAST AXONAL TRANSPORT .....	25
2.5.1.1 Mechanism for anterograde transport.....	26
2.5.1.2 Mechanism for retrograde transport.....	27
2.5.2 SLOW AXONAL TRANSPORT.....	28
2.5.2.1 Mechanism for slow transport.....	29
<b>2.6 EXCITOTOXICITY .....</b>	<b>33</b>
2.6.1 NMDA-RECEPTOR SYSTEM .....	35
2.6.2 MECHANISM OF EXCITOTOXICITY .....	36
2.6.2.1 NMDA receptor activation.....	36

2.6.2.2 Ca <sup>2+</sup> overload: cytoplasmic events.....	37
2.6.2.3 Mitochondrial cascade .....	37
2.6.2.4 Mitochondrial-nuclear interplay .....	38
<b>2.7 CELL DEATH.....</b>	<b>40</b>
2.7.1 NECROSIS.....	40
2.7.2 APOPTOSIS .....	41
2.7.3 AUTOPHAGY .....	41
<b>2.8 NERVE DEGENERATION .....</b>	<b>42</b>
2.8.1 WALLERIAN DEGENERATION .....	43
2.8.2 DYING-BACK DEGENERATION .....	46
2.8.3 SEGMENTAL DEMYELINATION .....	49
<b>2.9 OPTIC ATROPHY/ NEUROPATHY.....</b>	<b>49</b>
2.9.1 INHERITED OPTIC NEUROPATHY .....	50
2.9.2 ACQUIRED OPTIC NEUROPATHY .....	50
<b>2.10 EXPERIMENTAL METHODS OF RETINAL INJURY .....</b>	<b>53</b>
<b>2.11 JUSTIFICATION FOR THE USE OF NMDA MODEL.....</b>	<b>54</b>
<b>CHAPTER 3: AIM &amp; OBJECTIVES .....</b>	<b>56</b>
<b>CHAPTER 4: MATERIALS &amp; METHODS.....</b>	<b>58</b>
<b>4.1 INTRODUCTION .....</b>	<b>58</b>
<b>4.2 MATERIALS .....</b>	<b>60</b>
4.2.1 EXPERIMENTAL ANIMALS .....	60
4.2.2 GENERAL REAGENTS & EQUIPMENTS .....	60
<b>4.3 METHODS .....</b>	<b>64</b>
4.3.1 EXPERIMENTAL PLAN .....	64
4.3.2 MAKING NMDA SOLUTION FROM POWDER .....	64
4.3.3 INTRAVITREAL INJECTION TECHNIQUE .....	65
4.3.4 PERFUSION FIXATIVE PREPARATION .....	66
4.3.4.1 Paraformaldehyde fixative (4%) .....	67
4.3.4.2 Glutaraldehyde fixative (2.5%).....	68
4.3.5 ANIMAL PERFUSION.....	69
4.3.6 TISSUE DISSECTION .....	70
4.3.7 PARAFFIN PROCESSING AND SECTIONING .....	71
4.3.7.1 Cassette Preparation .....	71
4.3.7.2 Processing and embedding .....	72
4.3.7.3 Sectioning.....	72
4.3.8 HAEMATOXYLIN & EOSIN (H & E) STAINING .....	73
4.3.9 TAAB-RESIN PROCESSING AND SECTIONING .....	74
4.3.9.1 Processing and embedding .....	74
4.3.9.2 TAAB EPON Resin mixture preparation .....	76
4.3.9.3 Semi-thin sections and Toluidine blue staining.....	77
4.3.9.4 Grid preparation and staining .....	78
4.3.10 TUNEL LABELING .....	79
4.3.11 IMMUNOHISTOCHEMICAL STAINING .....	80
<b>4.4 DATA ANALYSIS .....</b>	<b>82</b>
4.4.1 HISTOLOGICAL ANALYSIS OF RAT RETINA.....	82
4.4.2 MORPHOLOGICAL ASSESSMENT OF OPTIC NERVE PATHWAY .....	83
4.4.3 QUANTITATIVE ANALYSIS OF OPTIC NERVE DAMAGE .....	83
4.4.4 STATISTICAL ANALYSIS .....	84
4.4.5 ULTRASTRUCTURAL STUDY OF RGCs AND OPTIC NERVE .....	85
4.4.6 ANALYSIS OF TUNEL STAINING .....	86

4.4.7 ANALYSIS OF IMMUNOLOGICAL MARKERS .....	87
<b>CHAPTER 5: PILOT STUDY .....</b>	<b>88</b>
<b>CHAPTER 6: THE SPATIOTEMPORAL PATTERN OF LIGHT MICROSCOPIC AND MORPHOMETRIC CHANGES .....</b>	<b>92</b>
<b>6.1 INTRODUCTION .....</b>	<b>92</b>
<b>6.2 RESULTS.....</b>	<b>92</b>
6.2.1 HISTOLOGICAL ANALYSIS OF RETINAL DAMAGE .....	92
6.2.2 MORPHOLOGICAL EXAMINATION OF THE OPTIC NERVE AND TRACT .....	98
6.2.3 QUANTITATIVE ANALYSIS OF OPTIC NERVE DAMAGE .....	105
6.2.4 CORRELATION OF RETINAL AND OPTIC NERVE DAMAGE .....	107
<b>6.3 DISCUSSION.....</b>	<b>107</b>
<b>CHAPTER 7: TUNEL STAINING OF THE RAT RETINA.....</b>	<b>115</b>
<b>7.1 INTRODUCTION .....</b>	<b>115</b>
<b>7.2 RESULTS.....</b>	<b>116</b>
<b>7.3 DISCUSSION.....</b>	<b>117</b>
<b>CHAPTER 8: ULTRASTRUCTURE OF RGCs AND OPTIC NERVE FIBRES AFTER INTRAVITREAL INJECTION OF NMDA.....</b>	<b>119</b>
<b>8.1 INTRODUCTION .....</b>	<b>119</b>
<b>8.2 RESULTS.....</b>	<b>120</b>
8.2.1 ULTRASTRUCTURAL CHANGES IN RGCs .....	121
8.2.2 ULTRASTRUCTURAL CHANGES IN OPTIC NERVE AXONS .....	129
<b>8.3 DISCUSSION.....</b>	<b>139</b>
<b>CHAPTER 9: EFFECTS OF EXCITOTOXIC PERIKARYAL INJURY ON THE AXON TRANSPORT SYSTEM.....</b>	<b>148</b>
<b>9.1 INTRODUCTION .....</b>	<b>148</b>
<b>9.2 RESULTS.....</b>	<b>150</b>
9.2.1 NF-L IMMUNOSTAINING IN THE RETINA .....	150
9.2.2 NF-L IMMUNOSTAINING IN THE OPTIC NERVE.....	154
9.2.3 B-APP IMMUNOSTAINING IN THE RETINA .....	159
9.2.4 B-APP IMMUNOSTAINING IN THE OPTIC NERVE.....	161
<b>9.3 DISCUSSION.....</b>	<b>164</b>
<b>CHAPTER 10: IMMUNOHISTOCHEMICAL CHANGES IN MICROGLIAL AND ASTROCYTIC CELL MARKERS.....</b>	<b>171</b>
<b>10.1 INTRODUCTION .....</b>	<b>171</b>
<b>10.2 RESULTS.....</b>	<b>174</b>
10.2.1 ED-1 IMMUNOSTAINING IN THE RETINA .....	174
10.2.2 ED-1 IMMUNOSTAINING IN THE OPTIC NERVE .....	176
10.2.3 GFAP IMMUNOSTAINING IN THE RETINA .....	178
10.2.4 GFAP IMMUNOSTAINING IN THE OPTIC NERVE .....	180

<b>10.3 DISCUSSION .....</b>	<b>184</b>
<b>CHAPTER 11: CONCLUSION AND FUTURE DIRECTIONS.....</b>	<b>189</b>
<b>CHAPTER 12 APPENDICES.....</b>	<b>193</b>
<b>12.1 APPENDIX 1: ETHICS APPROVAL .....</b>	<b>193</b>
<b>12.2 APPENDIX 2: PUBLICATION 1 .....</b>	<b>196</b>
<b>12.3 APPENDIX 3: PUBLICATION 2 .....</b>	<b>203</b>
<b>CHAPTER 13: BIBLIOGRAPHY.....</b>	<b>217</b>

## ABSTRACT

A large body of evidence supports the notion that excitotoxicity plays a major role in the pathogenesis of a number of neurological diseases, including central nervous system (CNS) ischaemia, Alzheimer's disease, motor neurone disease, and glaucoma. In the global population 60 years of age and over, these diseases are among the leading causes of mortality and morbidity. Although the site of excitotoxic injury is principally at the level of the cell body (perikaryal), understanding the secondary effects on the neuronal axon is important because axonopathy is a documented early feature of these common neurological conditions; hence, an understanding of the pattern and mechanisms of secondary axonal degeneration after excitotoxic perikaryal injury could provide novel detection and treatment strategies in the early phase of neurological disease. The retina and optic nerve, as approachable regions of the CNS, provide a unique anatomical substrate to investigate axonal degeneration after perikaryal excitotoxic injury.

Spatiotemporal changes in the retina and optic nerve were studied after injection of 20nM of N-methyl-D-Aspartate (NMDA) in the left eye of the rat with the saline-injected right eye serving as the control. Temporal changes in the morphology of retina and optic nerve were studied by light and electron microscopy. Progressive retinal damage beginning at 72 hrs, seen as thinning of the inner retina and cell loss in the ganglion cell layer, showed strong correlation ( $R= 0.949$ ) with degenerative changes in the optic nerve; the distal optic nerve segment displayed significantly more axon loss, axon swellings and myelin damage than the proximal segment ( $p<0.05$ ), suggestive of a 'dying-back type degeneration'. Beginning at 24 hrs, electron microscopy demonstrated various features of necrosis in retinal ganglion cells (RGCs): mitochondrial and endoplasmic reticulum swelling, disintegration of polyribosomes, rupture of membranous organelle and formation of myelin bodies. Ultrastructural damage in the optic nerve, which began at 72 hrs, mimicked the changes of Wallerian degeneration, where early nodal-paranodal disturbances were followed by the appearance of three major morphological variants: watery

degeneration, dark degeneration, and demyelination. Features suggestive of RGC regeneration in the form of dendritic sprouting after acute excitotoxic injury were also demonstrated at day 7.

Immunohistochemistry revealed glial cell responses and changes to the axon transport system. Excitotoxic injury resulted in progressive activation of macroglia (Müller cells and astrocytes) and microglial cells in the retina and optic nerve as demonstrated by increased glial-fibrillary-acidic protein (GFAP) and ED-1 immunolabelling as early as 72 hrs. Interaxonal glial cells in the optic nerve also showed increased  $\beta$ -amyloid precursor protein ( $\beta$ -APP) beginning at 72 hrs. Impairment of slow axonal transport at 72 hrs resulted in decrease anterograde transport of neurofilament-light (NF-L) to the axon terminal and hence their accumulation in proximal neuron (seen as NF-L rich spheroids).

This fundamental research revealed a pathological picture of Wallerian-like degeneration after perikaryal excitotoxic injury in the CNS. This novel finding is consistent with recent evidence of a labile axonal "survival" factor, nicotinamide mononucleotide adenylyltransferase 2, (Nmnat2) produced by the neuronal cell body. Further study is required to test the hypothesis that a lack of Nmnat2 is the mechanism by which axons degenerate after excitotoxic perikaryal injury.



## DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Dr. Sarabjit Kaur Saggu and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holder of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

**SARABJIT KAUR SAGGU**

## PUBLICATIONS/ PRESENTATIONS RELATED TO THESIS

### *Publication*

**Sarabjit K Saggu**, Hiren P Chotaliya, Peter C Blumbergs, Robert J Casson. Wallerian-like axonal degeneration in the optic nerve after excitotoxic retinal insult: an ultrastructural study. BMC Neuroscience 2010, 11:97. Copyright © 2010 Saggu et al; licensee BioMed Central Ltd.

**Saggu SK**, Chotaliya HP, Cai Z, Blumbergs P, Casson RJ. The spatiotemporal pattern of somal and axonal pathology after perikaryal excitotoxic injury to retinal ganglion cells: A histological and morphometric study. Experimental Neurology, 211 (2008), 52-58. Copyright © 2007 Elsevier Inc.

**Saggu SK**, Chotaliya HP, Manavis J, Blumbergs P, Casson RJ. The spatial and temporal pattern of optic nerve pathology after excitotoxic retinal injury. (abstract) Clinical and experimental ophthalmology, 34 (Suppl 1) 2006. Copyright © Blackwell Publishing Asia

### *Presentations*

**Saggu SK**, Chotaliya HP, Ghabriel MN. Will prevention of retinal excitotoxicity modulate optic nerve degeneration? An essay presented for Sir Grafton Elliot Smith Award, 2005 at the Annual Australian Neuroscience Society meeting: 31st January- 3rd February, 2006: Sydney, Australia.

**Saggu SK**, Chotaliya HP, Blumbergs P, Manavis J, Casson RJ. Optic nerve pathology after excitotoxic retinal injury. Presented at Australia and New Zealand Glaucoma Investigator Group meeting; 21-22<sup>nd</sup> July, 2006; Sydney, Australia.

**Saggu SK**, Chotaliya HP, Cai Z, Blumbergs P, Casson RJ. The spatial and temporal pattern of optic nerve degeneration after excitotoxic retinal injury. Presented at the International

Australasian Winter Conference on Brain Research; 26-30<sup>th</sup> August, 2006; Queentown, New Zealand.

**Saggu SK**, Chotaliya HP, Blumbergs P, Manavis J, Casson RJ. The spatial pattern of retinal ganglion cell axonal degeneration after intraocular excitotoxic injury. Presented at 38<sup>th</sup> Annual Scientific Congress of the Royal Australian and New Zealand College of Ophthalmologists; 4-8<sup>th</sup> November, 2006; Sydney, Australia.

**Saggu SK**, Chotaliya HP, Cai Z, Blumbergs P, Casson RJ. Autophagic and apoptotic perikaryal death: Role in “dying-back” axonal degeneration. Presented at the Australian Scientific Medical Research SA Scientific meeting; June 2007; Adelaide, Australia.

Casson RJ, **Saggu SK**, Childlow G, Wood J, Cai Z, Blumbergs P. Dying back-like axonopathy and early neurofilament degeneration in the optic nerve after perikaryal excitotoxic injury to retinal ganglion cells. Presented at the ARVO 2008 annual meeting; April-May 2008; Fort Lauderdale, Florida, USA.

## ACKNOWLEDGEMENT

Firstly, I would like to thank the University of Adelaide for providing me the opportunity to undertake this study and financially assisting me by awarding the invaluable International Postgraduate Research Scholarship, without which, the undertaking of this research would not have been possible for me.

Thanks to my Integrated Bridging Program lecturer, Christina Eira, for teaching the importance of language in research. Thanks to Dr. Mounir Ghabriel for stimulating my interest in laboratory-based Ophthalmology research and for invaluable advice during the initial phase of my research career. Thanks to Mr. Chris Leigh for teaching resin processing, ultratome sectioning and for his trustful friendship. Thanks to the Adelaide Microscopy staff for providing educational sessions and technical assistance in using electron microscope. Thanks to Gail Hermanis and Nadia Gagliardi for showing the perfect way of paraffin and cryosectioning as well as for explaining me the importance of Occupational Health and Safety measures.

Thanks to the staff of Neuropathology division, Institute of Medical and Veterinary Science for providing me an enjoyable and healthy working environment. Thanks to the laboratory manager, Jim Manavis, for letting me use equipments and reagents from neuropathology laboratory, for getting antibodies and stains in time, for teaching me immunohistochemistry techniques, for his cheerful nature and helping attitude. Thanks to the senior researcher, Dr. Zhao Cai, for showing the technique of animal perfusion and for his invaluable expert technical assistance.

I am greatly honoured and grateful to my co-supervisor, Professor Peter Blumbergs, who always guided me with his extraordinary scientific knowledge in the field of neuropathology. Thanks to him for his wisdom, stimulating and interesting discussions and encouragement.

Many thanks to my supervisor, Dr. Robert Casson, for so generously accepting me as a PhD student, for making research so interesting, for showing the technique of intravitreal injection, for providing independence while guiding me in a proper direction at each step, for his ever-encouraging words and for his friendly attitude. The work in this thesis could not have been performed without his guidance and dedication.

A special mention must also be made to my friends, Alan and Barbara Raine. A big thank for the kind words, constant support and encouragement during ups and downs in the study.

Finally, thanks to my husband, Dr. Hiren Chotaliya, for encouraging me to pursue my research interests, for providing scientific advice and for being a constant source of inspiration throughout this endeavour. The completion of this thesis is surely a reward of his hard work and support.

## ACRONYMS

AD	Alzheimer's Disease
ADP	Adenosine Di-Phosphate
AIF	Apoptosis Inducing Factor
ALS	Amyotrophic Lateral Sclerosis
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATP	Adenosine Tri-Phosphate
ATPase	Adenosine Tri-Phosphatase
AUD	Australian Dollars
B-APP	Beta-Amyloid Precursor Protein
BBB	Blood Brain Barrier
BL	Basal Lamina
CNS	Central Nervous System
CysLT1	Cysteinyl Leukotriene receptor 1
DAB	DiaminoBenzidine
DDSA	DoDecyl Succinic Anhydride
DMP	DiMethylaminomethyl Phenol
DNA	Deoxyribo Nucleic Acid
EDTA	Ethylene DiamineTetracetic Acid
EGF	Epidermal Growth Factor
EGFR	Epidermal Growth Factor Receptor
ER	Endoplasmic Reticulum
GA	Golgi apparatus
GCL	Ganglion Cell Layer
GFAP	Glial Fibrillary Acidic Protein
GTP	Guanosine Tri-Phosphate

H <sub>2</sub> O	Di-hydrogen oxide (Water)
H&E	Haematoxylin & Eosin
HIER	Heat Induced epitope Retrieval
HIV	Human Immunodeficiency Virus
HRP	HorseRadish Peroxidase
HSP	Hereditary Spastic Paraplegia
INL	Inner Nuclear Layer
IOP	Intra-Ocular Pressure
IPL	Inner Plexiform Layer
IRT	Inner Retinal Thickness
JNK	Jun N-terminal Kinase
LHON	Leber's Hereditary Optic Neuropathy
LIF	Leukaemia Inhibitory Factor
LM	Light Microscopy
LSAB	Labelled Streptavidin-Biotin
MAP	Microtubule-associated Proteins
MAPK	Mitogen Activated Protein Kinase
MCP-1	Monocyte Chemoattractant Protein-1
MHC-II	Major Histocompatibility Complex-II
MNA	Methyl Nadic Anhydride
MND	Motor Neuron Disease
mRNA	messenger Ribo Nucleic Acid
MTL	Myelin Terminal Loop
MTP	Mitochondrial Transition Pore
Na <sup>+</sup> -K <sup>+</sup> ATPase	Sodium-Potassium-Adenosine Tri-Phosphatase
NF-H	Neurofilament-heavy

NF-L	Neurofilament- light
NF-M	Neurofilament-medium
NGF	Nerve Growth Factor
NHS	Normal Horse Serum
NMDA	N-Methyl-D-Aspartate
NO	Nitric Oxide
NOS	Nitrous Oxide Synthase
NR1	NMDA receptor subunit 1
NR2	NMDA receptor subunit 2
NR3	NMDA receptor subunit 3
NT-3	Neurotrophin-3
OFL	Optic Fibre Layer
ONL	Outer Nuclear Layer
OPL	Outer Plexiform Layer
PARP-1	Poly (ADP-Ribose) Polymerase-1
PBS	Phosphate Buffer Saline
PGE2	Prostaglandin E2
PNS	Peripheral Nervous System
RGC	Retinal Ganglion Cell
rER	Rough Endoplasmic reticulum
ROS	Reactive Oxygen Species
SCa	Slow Component a
SCb	Slow Component b
Sec	Second
SPC	Streptavidin Peroxidase Conjugate
STAT	Signal Transducers and Activators of Transcription



TdT	Terminal deoxynucleotidyl Transferase
TEM	Transmission Electron Microscopy
TLR	Toll-like Receptor
TNF- $\alpha$	Tumour Necrosis Factor- $\alpha$
TUNEL	TdT-mediated dUTP-biotin Nick End Labeling
UTP	Uridine TriPhosphate
WD	Wallerian Degeneration

# LIST OF FIGURES

FIGURE 1. SCHEMATIC DIAGRAM OF THE EXCITOTOXIC INJURY MODEL. INTRAVITREAL INJECTION OF NMDA CAUSES PERIKARYAL RGC INJURY WITHOUT PRIMARY INJURY TO THE AXON.....	3
FIGURE 2. SAGITTAL SECTION OF THE EYEBALL SHOWING THE MAIN STRUCTURES IN THE ANTERIOR AND POSTERIOR SEGMENTS. ....	6
FIGURE 3. HISTOLOGY OF THE NORMAL RETINA.....	7
FIGURE 4. SCHEMATIC REPRESENTATION OF THE VISUAL SYSTEM.....	8
FIGURE 5. HISTOLOGICAL APPEARANCE OF TRANSVERSE SECTION OF OPTIC NERVE.....	9
FIGURE 6. SCHEMATIC DIAGRAM OF A TYPICAL MYELINATED NEURON REPRESENTING RGC SYSTEM. ....	11
FIGURE 7. SCHEMATIC DIAGRAM SHOWING THE PROCESS OF MYELINATION IN THE CNS. ....	17
FIGURE 8. LONGITUDINAL DOMAINS OF MYELINATED AXONS. ....	19
FIGURE 9. TYPES OF ASTROCYTES IN THE CENTRAL NERVOUS SYSTEM.....	23
FIGURE 10. MOTOR PROTEINS INVOLVED IN FAST AXONAL TRANSPORT.....	26
FIGURE 11. STEPS IN THE ASSEMBLY OF NF-L AND BASIC STRUCTURE OF NF-L MONOMER.....	30
FIGURE 12. STRUCTURE AND SYNTHESIS OF MICROTUBULES. ....	32
FIGURE 13. NMDA RECEPTOR.....	35
FIGURE 14. MECHANISM OF EXCITOTOXICITY.....	39
FIGURE 15. AXONAL SELF-DESTRUCTION AND NEURODEGENERATION. ....	46
FIGURE 16. CHEMICAL STRUCTURE OF N-METHYL-D-ASPARTATE.....	65
FIGURE 17. SYSTEMATIC SAMPLING METHOD FOR OBTAINING REPRESENTATIVE SAMPLE PHOTOGRAPHS FROM TRANSVERSE SECTION OF THE OPTIC NERVE. ....	84
FIGURE 18. PROGRESSIVE CHANGES IN THE RETINA AFTER NMDA INJECTION IN RAT.....	93
FIGURE 19. QUANTIFICATION OF RGCs IMMEDIATELY AFTER SALINE AND NMDA INJECTION.....	95
FIGURE 20. QUANTIFICATION OF IRT AFTER SALINE AND NMDA INJECTION. ....	97
FIGURE 21. TRANSVERSE SECTIONS OF THE NORMAL RAT OPTIC NERVE.....	99
FIGURE 22. TRANSVERSE SECTION OF THE NORMAL RAT OPTIC TRACT. ....	100
FIGURE 23. TRANSVERSE SECTIONS OF RAT OPTIC NERVE 24HRS AFTER NMDA INJECTION.....	100
FIGURE 24. TRANSVERSE SECTIONS OF RAT OPTIC TRACT 24HRS AFTER NMDA INJECTION.....	101
FIGURE 25. TRANSVERSE SECTIONS OF THE OPTIC NERVES 72HRS AFTER NMDA INJECTION.....	102
FIGURE 26. TRANSVERSE SECTIONS OF THE RAT OPTIC TRACT AFTER 72 HRS OF NMDA INJECTION. ....	103
FIGURE 27. TRANSVERSE SECTIONS OF OPTIC NERVE 7DAYS AFTER NMDA INJECTION.....	104
FIGURE 28. TRANSVERSE SECTIONS OF THE RAT OPTIC TRACT AFTER 7DAYS OF NMDA INJECTION. ....	104
FIGURE 29. CORRELATION BETWEEN THE RETINAL DAMAGE AND NUMBER OF AXONS IN THE OPTIC NERVE OF EXPERIMENTAL ANIMALS. ....	107
FIGURE 30. TUNEL STAINING OF THE SALINE-INJECTED RETINA.....	116
FIGURE 31. LIGHT MICROSCOPIC APPEARANCE OF RESIN-EMBEDDED SEMI-THIN SECTION OF SALINE CONTROL NORMAL INNER RETINA.....	122
FIGURE 32. ULTRASTRUCTURAL APPEARANCES OF NORMAL RGCs FROM SALINE INJECTED EYE. ....	123
FIGURE 33. ULTRASTRUCTURAL APPEARANCE OF RGCs 24HRS AFTER NMDA INJECTION).....	124
FIGURE 34. ULTRASTRUCTURE APPEARANCE OF RGCs 72HRS AFTER NMDA INJECTION.....	126
FIGURE 35. NMDA-INDUCED ULTRASTRUCTURAL CHANGES IN RGCs AT 7 DAYS.....	128

FIGURE 36. TEM OF THE PARALLEL RUNNING LONGITUDINAL SECTIONS OF THE INTRAORBITAL AXONS.....	129
FIGURE 37. TEM OF THE RETRO-ORBITAL DISTAL SEGMENT OF RAT OPTIC NERVE OF THE SALINE-INJECTED CONTROL ANIMAL IMMEDIATELY AFTER THE INJECTION.....	131
FIGURE 38. ULTRASTRUCTURAL APPEARANCES OF AXONAL SWELLINGS IN THE TRANSVERSE SECTIONS OF DISTAL SEGMENT OF RAT OPTIC NERVE AFTER 72HRS OF NMDA INJECTION.....	133
FIGURE 39. ULTRASTRUCTURAL APPEARANCE OF HYPERDENSE AXONS IN DISTAL OPTIC NERVES IN THE TRANSVERSE SECTIONS OF OPTIC NERVES SEEN 72HRS AFTER NMDA INJECTION .....	134
FIGURE 40. ULTRASTRUCTURE APPEARANCES OF THE LONGITUDINAL SECTIONS OF THE RAT OPTIC NERVE AFTER 72HRS OF NMDA INJECTION .....	136
FIGURE 41. TEM OF DISTAL SEGMENT OF RAT OPTIC NERVE AFTER 7 DAYS OF NMDA INJECTION.....	138
FIGURE 42. NF-L IMMUNOSTAINING IN THE RETINA AFTER INTRAVITREAL SALINE INJECTION IN RAT.....	151
FIGURE 43. PROGRESSIVE CHANGES IN NF-L IMMUNOSTAINING IN THE RETINA AFTER NMDA INJECTION IN RAT.....	153
FIGURE 44. NF-L IMMUNOREACTIVITY IN THE LONGITUDINAL AND TRANSVERSE SECTIONS OF THE SALINE INJECTED OPTIC NERVES AT VARIOUS TIME POINTS.....	155
FIGURE 45. NF-L IMMUNOREACTIVITY IN THE TRANSVERSE SECTIONS OF THE OPTIC NERVE OF THE NMDA INJECTED RAT. .	157
FIGURE 46. NF-L IMMUNOREACTIVITY IN THE LONGITUDINAL SECTIONS OF THE NMDA-INJECTED OPTIC NERVES. ....	158
FIGURE 47. B-APP IMMUNOREACTIVITY IN SALINE-INJECTED RAT RETINAS. ....	159
FIGURE 48. B-APP IMMUNOREACTIVITY IN NMDA-INJECTED RAT RETINAS.....	160
FIGURE 49. B-APP IMMUNOREACTIVITY IN THE LONGITUDINAL SECTIONS OF THE DISTAL SEGMENTS OF THE SALINE-INJECTED RATS. ....	161
FIGURE 50. B-APP IMMUNOREACTIVITY IN THE LONGITUDINAL SECTIONS OF NMDA INJECTED PROXIMAL AND DISTAL OPTIC NERVES .....	163
FIGURE 51. B-APP IMMUNOREACTIVITY IN THE LONGITUDINAL SECTIONS OF NMDA INJECTED DISTAL OPTIC NERVES AT DAY 7. .....	164
FIGURE 52. ED-1 IMMUNOREACTIVITY OF SALINE-INJECTED RETINAS OF RAT. ....	174
FIGURE 53. ED-1 IMMUNOREACTIVITY IN THE RETINAS OF NMDA-INJECTED RATS. ....	175
FIGURE 54. ED-1 IMMUNOREACTIVITY IN THE LONGITUDINAL SECTIONS OF SALINE-INJECTED OPTIC NERVES. ....	176
FIGURE 55. ED-1 IMMUNOREACTIVITY IN THE LONGITUDINAL SECTIONS OF NMDA-INJECTED OPTIC NERVES. ....	177
FIGURE 56. GFAP IMMUNOREACTIVITY IN THE SALINE-INJECTED RAT RETINAS. ....	178
FIGURE 57. GFAP IMMUNOREACTIVITY IN NMDA INJECTED RAT RETINAS.....	180
FIGURE 58. GFAP IMMUNOREACTIVITY IN THE TRANSVERSE SECTIONS OF SALINE-INJECTED RAT OPTIC NERVE. ....	181
FIGURE 59. GFAP IMMUNOREACTIVITY IN THE TRANSVERSE SECTIONS OF NMDA-INJECTED RAT OPTIC NERVE.....	183

## LIST OF TABLES

TABLE 1. MORPHOLOGICAL DIFFERENCES BETWEEN APOPTOTIC AND NECROTIC CELL DEATH .....	42
TABLE 2. CLASSIFICATION OF DYING-BACK NEUROPATHIES BASED ON THEIR MECHANISMS.....	48
TABLE 3. CHEMICAL/ REAGENTS AND THEIR SOURCES.....	60
TABLE 4. SOURCES OF SCIENTIFIC INSTRUMENTS.....	62
TABLE 5. EXPERIMENTAL PLAN OF THE RESEARCH SHOWING EXPERIMENTAL GROUPS AND NUMBER OF RATS USED IN EACH GROUP.....	64
TABLE 6. TABLE SHOWING DIFFERENT PHOSPHATE BUFFER SALTS, THEIR MOLECULAR WEIGHTS, AND THE AMOUNT USED TO PREPARE DIFFERENT VOLUMES OF 0.2M PHOSPHATE BUFFER STOCK SOLUTION. ....	68
TABLE 7. TISSUE SPECIMENS OBTAINED FROM INDIVIDUAL ANIMAL. ....	70
TABLE 8. AMOUNT OF INGREDIENTS NEEDED TO PREPARE TAAB EPON RESIN. ....	77
TABLE 9. DETAILS OF PRIMARY ANTIBODIES, ANTIGEN RETRIEVAL SOLUTIONS AND SECONDARY ANTIBODIES USED IN THE IMMUNOHISTOCHEMICAL STAINING. ....	82
TABLE 10. PROGRESSIVE CHANGES IN CELL COUNT IN THE GCL. EACH VALUE REPRESENTS MEAN $\pm$ S.E. ....	94
TABLE 11. PROGRESSIVE CHANGES IN INNER RETINAL THICKNESS. EACH VALUE REPRESENTS MEAN $\pm$ S.E. ....	96
TABLE 12. SHOWS PROGRESSIVE CHANGES IN PROXIMAL AND DISTAL OPTIC NERVE SEGMENTS IN CONTROL AND EXPERIMENTAL EYES. EACH VALUE REPRESENTS MEAN $\pm$ S.E.....	106