

SPECIFICITY OF THE REGENERATING AXONS FOLLOWING SPINAL CORD LESIONS

THESIS

Submitted for the partial fulfillment of

The M.D. Degree (Anatomy)

BY

GEORGE FAYEK BARSOUM HANNA

M. B. B. Ch., M. Sc.

Under Supervision of

Prof. Dr. NABIL NAWAR YOUSEF

Prof. of Anatomy
Faculty of Medicine
Ain Shams University

Prof. Dr. SANSAR SHARMA

Prof. of Anatomy
and Cell Biology
New York Medical College

Prof. Dr. YEHIA YOUSEF AHMED

Prof. of Anatomy
Faculty of Medicine
Ain Shams University

Prof. Dr. NAWAL FOUAD

Prof. of Anatomy
Faculty of Medicine
Ain Shams University

Prof. Dr. ABD-EL-AZIM MOUSTAFA

Assistant Prof. of Anatomy
Faculty of Medicine
Ain Shams University

**FACULTY OF MEDICINE
AIN SHAMS UNIVERSITY
1998**



ACKNOWLEDGEMENT

I would like to express my sincere thanks and profound gratitude to **Prof. Dr. SANSAR SHARMA**, *Professor of Anatomy and Cell Biology, New York Medical College*, who suggested the subject of the research, provided the necessary equipment for its fulfillment, and for his continuous guidance and criticism.

It is my great honor to express my deepest gratitude and grateful thanks to **Prof. Dr. NABIL NAWAR**, *Professor of Anatomy, Faculty of Medicine, Ain Shams University*, to whom I shall be always indebted for his guidance, support, and understanding. His kindness, patient supervision, and beneficial criticism can never be rewarded.

My deepest appreciation is also expressed to **Prof. Dr. YEHIA YOUSEF**, *Professor of Anatomy, Faculty of Medicine, Ain Shams University*, his supervision, sincere help and support.

I am indebted to **Prof. Dr. NAWAL FOUAD**, *Professor of Anatomy, Faculty of Medicine, Ain Shams University*, for her great help, assistance, and continuous enthusiastic encouragement throughout this work.

Also, I would like to express my thanks to late **Prof. Dr. ABD-EL-AZIM MOUSTAFA**, *Assistant Professor of Anatomy, Faculty of Medicine, Ain Shams University*, for his great help. To his memory, I pay my sincere respects.

CONTENTS

I. INTRODUCTION AND AIM OF WORK	i
II. REVIEW OF LITERATURE:	
1. Central Nervous System Regeneration	1
2. Descending Spinal Pathways In Various Animals:	12
A. In fishes	13
B. In amphibians	19
C. In reptiles	20
D. In mammals	20
3. Ascending Spinal Pathways In Various Animals:	21
A. In fishes	22
B. In amphibians	26
C. In reptiles	27
4. Spinocerebellar Projections In Various Animals:	28
A. In mammals	29
B. In reptiles	29
C. In amphibians	30
D. In fishes	32
III. MATERIAL AND METHODS:	
* Animals Used	38

* Acclimatization	38
* Surgery of Spinal Cord Transection:	38
1- Anaesthesia and preoperative preparation	
2- Exposure of spinal cord	
3- Transection of spinal cord	
4- Wound closure	
5- Postoperative care and observation	
* Labeling of Ascending and Descending Spinal Projections.	40
1- Labeling of normal spinal projections:	40
Ascending spinal projections	
Descending spinal projections	
2- Labeling of regenerated spinal projections:	41
Ascending spinal projections	
Descending spinal projections	
3- Control animals	42
* Biotinylated Dextran Amines Immunohistochemistry	42
* Calculation of The Area Covered By A Terminal Arbor	43

IV- RESULTS

* General Morphology of The Goldfish Brain	45
* Anatomy of The Cerebellum	45
* Normal Spinocerebellar Projection Pattern	46
* Regenerated Spinocerebellar Projection Pattern	48

* Terminal Arbors of Spinocerebellar Fibers	50
* Spino-toral Projection	51
*Reciprocal Projection Between The Spinal Cord And The Diencephalon:	52
<i>A. Hypothalamus</i>	52
<i>B. Thalamus</i>	53
*Spinotelencephalic Projection	55
 V. DISCUSSION:	
*Use of Biotinylated Dextran Amines As A Tracer	132
*Normal Spinocerebellar Projection	132
*Suggestion of Nucleus Lateralis Valvulae As A Deep Cerebellar Nucleus	136
*Terminal Arbors of Normal And Regenerated Spinocerebellar Fibers	138
*Other Ascending Spinal Pathways	139
*Spino- toral Projection	140
*Reciprocal Projection Between The Spinal Cord And The Diencephalon:	141
<i>A. Hypothalamus</i>	141
<i>B. Thalamus</i>	143
*Spinotelencephalic Projection	145

*Regenerated Descending And Ascending Spinal Projections After Spinal Cord Transection	146
VI. SUMMARY	149
VII. REFERENCES	152
ARABIC SUMMARY	

INTRODUCTION AND AIM OF WORK

INTRODUCTION AND AIM OF WORK

Transection of the spinal cord in man results in paralysis, loss of sensation below the lesion, and unfortunately, there is little regeneration or recovery of normal function (Windle, 1956; Clemente, 1964; Pettegrew and Windle, 1976; Puchala and Windle, 1977; Kiernan, 1979; Freed et al., 1985; Collins and West, 1989). In contrast to the limited regenerative capabilities of the mammalian spinal cord, in goldfish, regeneration of the transected spinal cord and recovery of function are well-documented. Out of 17 brain centers found to project to the goldfish spinal cord (Prasada Rao et al., 1987, 1993), only 11 participated in the process of regeneration of the descending spinal projections (Sharma et al., 1993).

Reviewing the literature, it was found that the sources of normal descending projections to the spinal cord were investigated in the goldfish (Sloan and Demski, 1985; Prasada Rao et al., 1987, 1993); adult zebrafish (Becker et al., 1997); Himé salmon (Oka et al., 1986a); European eel (Bosch and Roberts, 1994); lungfish (Ronan and Northcutt, 1985); electric fish *Eigenmammia* (Behrend and Donicht, 1990); agnathans (Ronan, 1981; Ronan and Northcutt, 1981; Ronan, 1989); Chondrichthyans (Smeets and Timerick, 1981). Moreover, the normal ascending spinal projections to various brain centers were reported in the adult zebrafish (Becker et al., 1997); Himé salmon (Oka et al., 1986b); *Cyprinus carpio* (Xu et al., 1994); the teleost *Sebastiscus marmoratus* (Murakami and Ito, 1985); the teleost *Gnathonemus petersii* (Libouban and Szabo, 1975); dogfish (Hayle, 1973a); agnathans (Ronan and Northcutt, 1981, 1990); nurse shark (Ebbesson and Hodde, 1981). However, in goldfish, the fate of the ascending spinal projection, to our knowledge, was not reported

Most of the studies, dealing with the descending and ascending spinal projections in various animals, used either the degeneration techniques or horseradish peroxidase (HRP) method. However, with these techniques neither the detailed course and distribution of the spinal pathways, nor the terminal arbors of the nerve fibers were clearly defined. New avenues to investigate the spinal pathways have been opened by the recent introduction of Dextran amines, as anterograde and retrograde tracers. Dextran amines are readily taken up only by cut axons, do not leak out of intact profiles, lack any transcellular staining (Fritzsche and Wilm, 1990), and show a fast transport, long persistence in the axonal terminals (Fritzsche, 1993; Matz, 1995). Accordingly, the present study was planned to investigate and compare both normal and regenerated spinal projection patterns in the adult goldfish with special reference to their terminal arbors using the Biotinylated Dextran Amines (BDA). Therefore, the question of whether the restoration of function resulted from the formation of appropriate synapses by the regenerated axons with their targets, or whether that was due to adaptation following the formation of "new" projection patterns will be addressed, thus the specificity of the regenerated axons following spinal cord transection will be screened.

REVIEW OF LITERATURE

