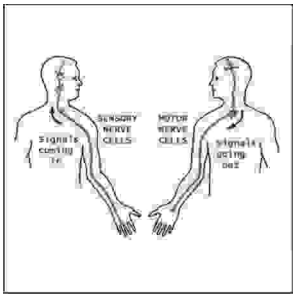
ORIGINAL PROF-1231



SCIATIC NERVE;
MOTOR AND SENSORY NEURONS OF THE RAT. A HORSERADISH PEROXIDASE STUDY

DR. ABDUL JABBAR
MBBS (DOW), M.Phil
Associate Professor of Anatomy
Bolan Medical College, Quetta

DR. ANJUM NAQVI
MBBS, M.Phil
Assistant Professor of Anatomy
BMSI, JPMC Karachi

DR MUMTAZ HAIDER
MBBS, M.Phil
Associate Professor of Physiology
Bolan Medical College, Quetta

SUMMERY ... Objective: To determine the number, size, somatotopy and segmental distribution of HRP labeled motor and sensory neurons forming sciatic nerve in albino rat by using HRP technique. To fined out the distribution of neurons in sciatic nerve in albino rat in spinal cord from L_3S_1. The average number, size and segmental distribution of motor and sensory neurons were localized by HRP method of tracing neuronal connections. The motor neurons forming SCN ranged 10-60 microns and extended between the caudal part of L3 and rostral part of SI spinal segment. They occupied PPL, PL, C and aL subgroups. The peak frequency distribution of motor neurons was observed in L4-L6 spinal segment in SCN. The labeled sensory neurons whose peripheral process run in SCN were localized in L3-S1 ipsilateral Dorsal Root Ganglia (DRG). No somatotopic organization of the cells was found in the DRG. The cells were distributed throughout the ganglia without forming groups. The somal diameters of sensory neurons forming SCN measured between 14-58 microns.

INTRODUCTION

Researchers using both physiological and anatomical techniques have determined the locations of motor and sensory neurons whose axons form the spinal nerves, the major efferent link connecting the central nervous system to the muscles. But majority of these researchers have used a combination of Golgi staining methods and chromatolytic techniques, following either nerve crush injury or nerve section or limb amputations[1]. Recent anatomical and physiological observations, however, have indicated that the anatomical descriptions obtained from the above methods were incomplete in certain respects[2].

Neuroanatomy experienced a revolution in the beginning of the 1970s with the development of powerful

techniques based on axonal transport of tracer substances such as proteins, radio-labelled amino acids and horseradish peroxidase (HRP)[3]. This technique is based upon the uptake of HRP by axons and its subsequent retrograde transport to the neuronal perikaryon[4], 1978). Uptake of HRP macromolecules takes place throughout the membranes of neurons, including dendrites, perikarya axons and their terminals by the process of pinocytosis[5]. Cutting vagus nerve of monkey and applying horseradish peroxidase to its proximal cut end produced more labelled cells in the dorsal motor nucleus than when HRP was applied to intact nerve. Since then, the horseradish peroxidase neurohistochemistry has become the major tool to localize motor, sensory and autonomic neurons[6]. The present study is designed to determine the number, size, somatotopy and segmental distribution of HRP labeled motor and sensory neurons forming sciatic nerve in albino rat by using HRP technique.

MATERIALS AND METHODS

Animals used in this Study

Twenty five adult male albino rats, weighing between one hundred and two hundreds were used in this study. Animals were anaesthetize with ether vapours and maintained with intra-peritoneal administration of 3.5% chloral hydrate solution in distilled water in a dose of 300mg/kg body weight. The Sciatic nerve (SCN) was exposed along with the tibial and common peroneal nerves in the popliteal fossa. The SCN was cut tangentially at the level of mid thigh before its division into tibial and common peroneal nerves, to obtain maximum tracer-nerve contact area. HRP crystals (Sigma type-vi) were applied with fine probe to the proximal cut end of nerve at frequent intervals of about 10 minutes for 3 to 4 hours. At the end of HRP application, the wound was closed in layers. After this procedure the animals were allowed to survive for a period varying between 72 to 96 hours. The variable distance which the tracer travels and the versatile uptake by axons has necessarily made the survival periods variable[7]. For perfusion and fixation, the animals were re-anaesthetized after a suitable survival period. The perfusion and fixation was done[4].

REMOVAL AND CUTTING OF LUMBAR SPINAL SEGMENTS AND DORSAL ROOT GANGLIA (DRG).

The lumbar portion of spinal cord and dorsal root ganglia (DRG) between I2 and S2 were removed from the animal immediately after perfusion and were transferred to labelled specimen bottles containing 30% sucrose in 0.1 M phosphate buffer, PH 7.4.

The tissues removed were stored at 4 C for 12-48 hours[6]. Under dissecting microscope, the relevant segments of the excised lumbo-sacral portion of the spinal cord were identified. Each segment between suprajacent and subjacent dorsal roots, was directly transferred to the freezing microtome and transverse sections of the spinal cord and DRG were cut in a scrial order rostrocaudally at the thickness of 40 um. The cut sections of the spinal cord segments and DRG were collected in a comparatmentalised plastic tray having mesh glued on its under surface. The tray was placed in an incubation vessel containing 200ml of 0.1 M phosphate buffer at PH 7.4. the cut sections were kept at 4 C in 0.1 M phosphate buffer for 6 to 12 hours before enzymatic reaction.

DEMONSTRATION, COUNTING AND MEASUREMENT OF MOTOR AND SENSORY NEURONS.

Histochemical demonstration of HRP was done according to the method of Mesulam protocol. The total number of labelled motor and sensory neurons forming sciatic nerve (SCN) were counted in all the animals studied at magnification of X100- X400. Measurement of somal size of labeled motor and sensory neurons was done in all sections containing labeled neurons[8].

OBSERVATIONS AND RESULTS

HRP-Labeled Motor Neurons Forming Sciatic Nerve (SCN) in Adult Rat.

Following application of HRP crystals to the proximal cut end of Sciatic nerve (SCN), an intense labeling of the soma and dendrites of motor neurons forming this nerve in the ventral horn of the spinal cord was observed.

The HRP-TMB reacting product was visualized as intra

cytoplasmic coarse granules of dark blue color within labelled SCN neurons. The labeled motor neurons appeared round or polyhedral in shape and were of various sizes intermingled with each other without any special distribution pattern. Most of the small sized cells displayed intense labeling. In heavily labeled cells, the nucleus, in most cases, could not be identified (Fig-1).

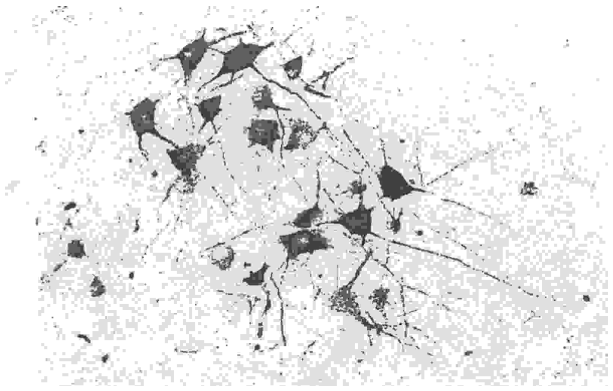


Fig-1. Photomicrograph of 40 microns thick section of the ventral horn of spinal cord at L3-level showing soma (s), dendrites (d) and axon (a) of HRP- labelled motor neurons following application of HRP to the proximal cut end of the sciatic nerve. Section counter-stained with neutral red. X 200.

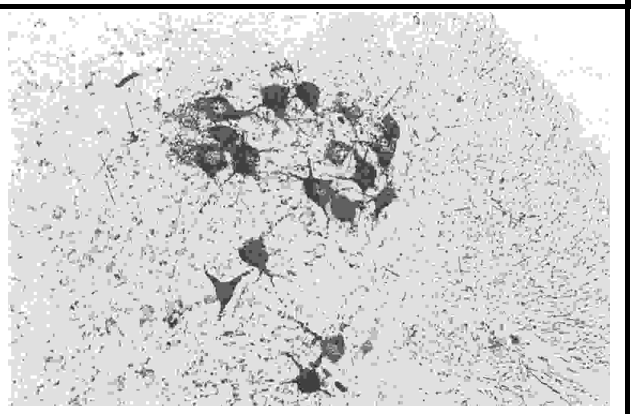


Fig-2. Photomicrograph of 40 microns thick transverse section of the ventral horn of spinal cord at L5-level showing HRP-labelled sciatic nerve motor neurons in PPL, PL, C and aL groups following application of HRP to the proximal cut end of the sciatic nerve. Section counter-stained with natural red. X 200.

SIZE SPECTRUM AND PERCENTAGE FREQUENCY DISTRIBUTION OF SCIATIC NERVE (SCN) MOTOR NEURONS.

The size spectrum and percentage frequency distribution of various sizes of motor neurons is shown in table-I. The motor neurons forming sciatic nerve measured between 9 and 60 microns. The percentage of sciatic motor neurons measuring more than 25 microns was 73.68. The peak frequency distribution occurred in neurons measuring between 20 and 42 microns. The labelled SCN motor neurons occur in Post-posterolateral (PPL), Posterolateral (PL), Central (C)and Anterolateral (aL) subgroups, (Fig-2). They extend between the caudal part of L3 segment and the rostral part of S1 segment.

They start to appear in the PPL and PL subgroups in the caudal part of L3 segment and then, in the C and aL subgroups in the rostral part of L4 segments. Labelled motor neurons of SCN in PPL and PL subgroups extend up to the caudal part of L6 segment. when S1 segment is also labelled, the labelled motor neurons then occupy only the PPL subgroup. Labelled motor neurons in the central (C) and aL, subgroups appear in the rostral part of L4 segment and extend only to the rostral part of L6 segment.

Labelled motor neurons are least dense in the caudal part of L3 segment, the caudal part of L6 segment, and the rostral part of S1 segment and are most dense in the intermediate parts, (Fig-3). The segment distribution of motor neurons of sciatic nerve is shown in Fig-4.

HRP-LABELLED SENSORY NEURONS FORMING SCIATIC NERVE IN ADULT ALBINO RAT.

After application of HRP crystals to the proximal cut end of sciatic nerve (SCN) in adult albino rats, sensory rats, sensory neurons in the ipsilateral dorsal root ganglia (DRG) L3 to S1 were labelled. The peak frequency distribution was observed in L4, L5 and L6 DRG. The segmental distribution of DRG cells of sciatic nerve is shown in (Fig-4). The perikarya of sensory neurons filled with HRP reaction product were of oval or round shape.

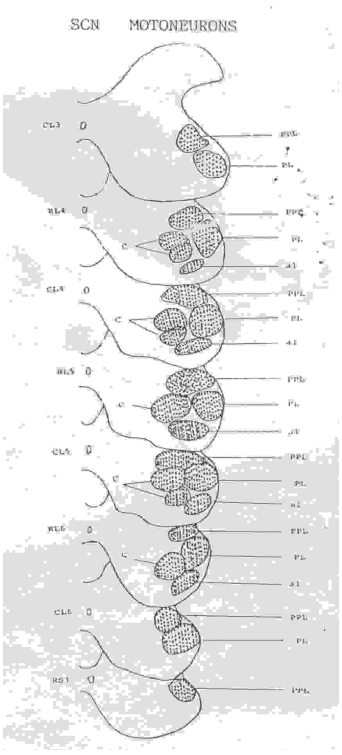


Fig-3. Camera lucida drawing showing topographic organization of motor neurons in spinal cord segments L3-S1 forming sciatic nerve in adult albino rat. R and C indicate representative camera lucida drawings of the rostral and caudal parts of the relevant segment. The dots are randomly placed.

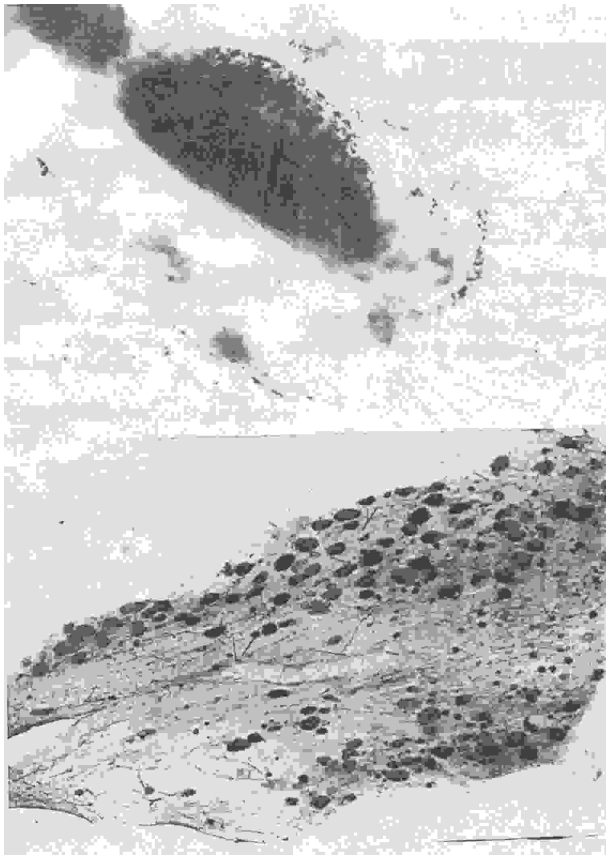


Fig-4,5. Photomicrograph of 40 microns thick longitudinal section of the L4 dorsal root ganglion (DRG) showing HRP-labelled sensory neurons of sciatic nerve, following application of HRP to the proximal cut end of the sciatic nerve. Section counter stained with neutral red. X 100.

The labelled neurons of different sizes were distributed throughout dorsal root ganglia without any apparent clustering (Fig-5).

Some of cells were, located within central part of the ganglion. This finding indicates that there is no somatotopic organization within dorsal root ganglion. The size spectrum and frequency distribution of various sizes of dorsal root ganglion cells are shown in table-II. The size spectrum ranged between 14-58 microns with the majority of labelled neurons being in the range of 22-38 microns.

DISCUSSION

MOTOR NEURONS

The localization of motor and sensory neurons whose axons are present in sciatic nerve (SCN) in adult albino rat by horseradish peroxidase (HRP) method provides information on (1) the number and size of theses neurons, and (2) their segmental distribution and somatotopy. HRP was applied unilaterally to the proximal cut end of sciatic nerve (SCN) and results were studied bilaterally. Labelled neurons were present only ipsilaterally in all animals without labelling any neurons on the contra lateral side in the spinal cord and dorsal root ganglia. This finding is incomplete agreement with

the findings of Ha et al[9] who injected HRP into anterior muscle and found labelled motor neurons in the ipsilateral ventral horn of the spinal cord and labelled sensory neurons in ipsilateral DRG L3-L5.

In present study, labelled SCN motor neurons extend from the caudal part of L3 segment to the rostral part of SI segment. This is in agreement with Yamamoto et al[10] who after intra neural injection of 0.1% Raccinus Communis Agglutinins (RCA 60) in the sciatic nerve of rat observed diffuse chromatolysis of motor neurons between the segmental levels of L3 to L6. The motor neurons forming the SCN, labelled with retrograde uptake of HRP applied to the proximal cut end of the nerve, were located in the lateral part of the ventral horn of spinal cord . This finding is in agreement with the results of Maqbool et al[11], who also reported that the common peroneal nerve motor neurons in monkey or located in the lateral part of the ventral horn. Labelled motor neurons of SCN in the ventral horn of the ventral horn of the spinal cord are arranged in four groups.

These are PPL, PL, C and al, this is in agreement with the observations of Janjua and Leong[12], who observed that HRP labelled motor neurons of Tibial nerve occur in PPL, PL, C and aL subgroups. The average soma diameters of motor neurons forming SCN are between 10 and 60 microns. This corresponds of the values reported by Maqbool et al[11]. The total number of labelled motor neurons are shown in table-III.

SENSORY NEURONS

Following the application of HRP crystals to the proximal cut end of SCN, HRP reaction product was present in the perikarya of ipsilateral L3-S1 DRG cells as well as in their axons. This finding is in good agreement with the findings of Mesulam and Brushart[13] who observed labelling of DRG cells perikarya as well as of their central and peripheral processes after applying HRP to the sciatic nerve of rat. No HRP granules were observed in the nuclei of the DRG cells. This observation is in agreement with the observation of Ellison and Clark[14] who, injecting HRP into cats and guinea pigs, observed that there were no HRP granules within the nuclei of DRG cells. No somatotopic organization within the DRG was observed. The labelled DRG cells were scattered throughout the DRG.

This finding is in conformity with the observations of Peyronnard and Charron[15] who, after applying 20% HRP solution to the proximal cut end of the sural nerve of adult male albino rats for three hours, observed that HRP labelled cells were scattered throughout the DRG with no apparent clustering. The average somal diameter of 12-58 microns corresponds to the findings of Peyronnard and Charron[15] who observed average somal diameters of 10-62 microns for sensory neurons forming the sural nerve in rat. The findings in the present study are not in agreement with Janjua and Leong[6] who observed average somal diameters of 12-78 microns for sensory neurons forming CPN in macaque monkeys. This difference in somal diameters may be attributed to species difference.

Table-I. Table showing the percentage frequency distribution of various diameters of HRP labelled Sciatic Nerve (SCN) motor neurons in adult albino rats.

Diameters of HRP-labelled motor neurons in microns.	Percentage frequency distribution of motor neurons forming SCN in various segment of spinal cord.					Mean %age frequency distribution of SCN motor neurons.
	L3	L4	L5	L6	S1	
10	0.04	_	_	_	_	0.04
12	0.04	_	_	_	0.08	0.12
14	_	0.16	0.32	0.04	_	0.52

16	0.08	0.40	0.60	0.16	0.04	1.28
18	0.08	0.88	1.24	0.40	0.08	2.68
20	0.28	1.94	1.48	0.64	0.16	4.50
22	0.38	1.94	2.22	1.08	0.20	5.82
24	0.48	2.10	2.70	1.39	0.16	6.83
26	0.80	2.66	2.34	1.47	0.12	7.39
28	0.72	2.22	2.42	2.10	0.04	7.50
30	0.56	2.47	3.06	2.94	0.08	9.38
32	0.48	2.86	2.54	2.58	_	8.46
34	1.04	2.46	2.22	2.07	0.04	7.83
36	0.72	2.54	2.14	1.51	0.04	6.95
38	1.12	2.03	2.22	1.32	_	6.69
40	0.80	1.71	2.14	1.04	_	5.69
42	0.84	1.60	2.22	0.60	0.04	5.30
44	0.60	0.16	1.90	0.56	_	4.22
46	0.40	0.56	1.24	0.48	_	2.68
48	0.16	0.64	1.32	0.24	_	2.36
50	0.04	0.40	0.68	0.12	_	1.24
52	0.08	0.32	0.48	_	_	0.88
54	0.08	0.24	0.20	0.12	_	0.64
56	0.04	0.08	0.12	0.04	_	0.28
58	0.24	0.04	0.24	0.04	_	0.56
60	0.04	0.04	0.08	_	_	0.16

Table-II. Table showing the percentage frequency distribution of various diameters of HRP labelled Sciatic Nerve (SCN) Sensory Neurons in adult albino rat.

Diameters of HRP-Labelled Sensory neurons In microns.	Percentage frequency distribution of neurons forming SCN at various segmental levels.					Mean percentage frequency distribution of SCN motor neurons.
	L3	L4	L5	L6	S1	

10	_	_	_	_	_	_
12	_	_	_	_	_	_
14	0.09	_	_	_	0.03	0.12
16	0.09	_	_	0.15	0.06	0.30
18	0.03	0.51	0.09	0.54	0.15	1.32
20	0.66	0.50	0.60	1.02	0.53	3.31
22	0.57	1.32	1.23	1.44	0.85	5.41
24	0.66	2.60	2.96	1.89	1.32	9.43
26	0.73	3.80	3.12	3.18	1.95	12.78
28	1.44	4.05	3.65	2.92	1.32	13.38
30	1.07	3.20	3.46	4.05	1.16	12.94
32	1.25	3.68	3.05	2.64	0.59	11.12
34	1.61	1.98	2.58	2.04	0.76	8.97
36	1.26	1.85	1.89	1.10	0.59	6.69
38	1.20	0.88	1.38	0.79	0.28	4.53
40	1.10	0.69	1.44	0.41	0.09	3.73
42	0.63	0.54	0.73	0.28	0.12	2.30
44	0.59	0.18	0.53	0.18	0.06	1.54
46	0.32	0.06	0.24	0.15	0.03	0.80
48	0.16	_	0.12	_	_	0.28
50	0.15	0.06	0.15	0.03	_	0.39
52	0.06	0.09	0.12	_	_	0.27
54	0.09	_	0.03	_	_	0.12
56	_	_	0.06	0.03	_	0.09
58	0.06	_	0.03	_	_	0.09

Table-III. Total counts and segmental distribution of Motor and Sensory Neurons whose axons are present in Sciatic Nerve (SCN) in different adult Albino Rats.

S. No	Neurons Forming Nerve.	Total Number of labelled Neurons	Segmental distribution of labelled neurons					
			L2	L3	L4	L5	L6	S1
1	2	3	4	5	6	7	8	9
R1	Motor	1813 (13.51%)	_	325 (17.93%)	383 (21.12%)	459 (25.31%)	503 (27.74%)	143 (07.88%)
	Sensory	10318 (76.91%)	_	1773 (17.18%)	2002 (19.40%)	2322 (22.50%)	2203 (21.35%)	2018 (19.56%)
R3 B	Motor	2083 (15.32%)	_	353 (16.95%)	427 (20.50%)	497 (23.85%)	509 (24.43%)	297 (14.25%)
	Sensory	9879 (72.69%)	_	1854 (18.76%)	2148 (21.74%)	2235 (22.62%)	2440 (24.70%)	1202 (12.16%)
R7 A	Motor	1539 (11.18%)	_	279 (18.12%)	358 (23.26%)	372 (24.17%)	417 (27.09%)	113 (7.34%)
	Sensory	10750 (78.15%)	_	2091 (19.45%)	2167 (20.15%)	2316 (21.54%)	2379 (22.13%)	1797 (16.71%)
R9 B	Motor	1623 (16.58%)	_	327 (20.15%)	356 (21.93%)	424 (26.12%)	516 (31.79%)	_
	Sensory	7099 (72.54%)	_	1478 (20.81%)	1771 (24.95%)	1916 (26.98%)	1934 (27.24%)	_
R11	Motor	1315 (13.49%)	_	262 (19.92%)	321 (24.41%)	352 (26.76%)	380 (28.89%)	_
	Sensory	7556 (77.56%)	_	1544 (20.43%)	1811 (23.96%)	2087 (27.62%)	2114 (27.98%)	_
R13 B	Motor	1874 (16.18%)	_	387 (20.65%)	415 (22.14%)	438 (23.37%)	469 (25.02%)	165 (8.81%)
	Sensory	8712 (75.22%)	_	1473 (16.90%)	1735 (19.92%)	1796 (20.61%)	1821 (20.90%)	1887 (21.65%)
R14 A	Motor	2402 (18.45%)	_	451 (18.77%)	497 (20.69%)	557 (23.18%)	603 (25.10%)	294 (12.24%)
	Sensory	9318 (71.59%)	_	1683 (18.06%)	1837 (19.71%)	2018 (21.65%)	2071 (22.23%)	1709 (18.34%)
R15 A	Motor	1710 (20.30%)	_	336 (19.64%)	407 (23.80%)	538 (31.46%)	429 (25.08%)	_
	Sensory	5684 (67.49%)	_	1211 (21.30%)	1364 (23.99%)	1507 (26.51%)	1602 (28.18%)	_

R16 A	Motor	1345 (11.29%)	_	129 (9.59%)	327 (24.31%)	405 (30.11%)	359 (26.69%)	125 (9.29%)
	Sensory	7907 (71.92%)	_	1782 (22.53%)	1865 (23.58%)	2106 (26.63%)	2154 (27.24%)	_
R18 B	Motor	975 (17.96%)	_	194 (19.89%)	271 (27.79%)	298 (30.50%)	212 (21.74%)	_
	Sensory	3895 (71.75%)	_	807 (20.71%)	894 (22.95%)	1085 (27.85%)	1109 (28.47%)	_
R22	Motor	2371 (17.58%)	_	397 (16.74%)	516 (21.76%)	582 (24.54%)	640 (26.99%)	236 (9.95%)
	Sensory	9548 (70.81%)	_	1889 (19.78%)	1913 (20.03%)	1962 (20.54%)	2086 (21.85%)	1698 (17.78%)

REFERENCES

1. Adams, J.C. and Warr, W.B. **Origin of axons in the cats acoustic stria determined by injection of Horscradish peroxidase into severed tracts.** J. Comp. Neurol, 1976; 170: 107-121.

2. Light, A.R. and Perl, E.R. **Differential termination of large diameter and small diameter primary afferent fibres in the spinal dorsal grey matter as indicated by labelling with horseradish peroxidase.** Neurosis. Lett., 1977; 6:59-63.

3. Winer, J.A. **A review of the status of the horseradish peroxidase method in neuroanatomy.** Biobehav. Rev., 1977; 1(1): 45-54.

4. Rosene, D.L and Mesulam, M.M. **Fixation variables in horseradish peroxidase neurohistochemistry. J.** Histochem. Cytochem., 1978; 26 (1): 28-39.

5. Krishnan, N. And Singer, M. **Penetration of peroxidase into peripheral nerve fibers,** Am. J. Anat., 1973; 136: 1-14.

6. Janjua, M.Z. and Leong, S.K. **Sensory, motor and sympathetic neurons forming the common peroneal and tibial nerves in the macaque monkey.** J. Anat.,1987; 153: 63-76.

7. Furstman, L., Saporta, S. And Kruger, L. **Retrograde axonal transport of horseradish peroxidase in sensory nerves and ganglion cells of the rat.** Brain Res., 1975; 84: 320-324.

8. Burke, R.E., Strick, P. L., Kanda, K., Kim, C.C. and Walmsley, B. **Anatomy of medical gastrocnemius ans Soleus motor nuclei in cat spinal cord.** J. Neurophysiol., 1977; 40; 667-680.

9. Ha, H. Kao, T. and Tan, E.C. **Muscle sensory neurons in the spinal ganglia in the rat determined by the retrograde transport of HRP. Exp.** Neurol., 1988; 70: 438-445.

10. Yamamoto, T., Iwasaki Y. and Konno, H. **Experimental sensory ganglionectomy by way of suicide axoplasmic transport.** J. Neurosurg, 1984; 60: 10-114.

11. Maqbol, A., Athar, Z. and Janjua, M.Z. **Study of motor units innervated by Common Peroneal Nerve in monkey.** Prof, 1996; 151: 1-8.

12. Janjua, M.Z. and Leong S.K. **Organization of neurons forming the femoral, sciatic, common peroneal and tibial nerves in rats and mokeys.** Brain Res., 1984; 310: 311-323.

13. Mesulam, M.M. and Brushart, T,M. **Transganglionic and anterograde transport of horseradish peroxidase across dorsal root ganglia.** Neurosci., 1979; 4: 1107-1117.

14. Ellison P.J. and Clark, G.M. **Retrograde axonal transport of HRP in peripheral autonomic nerves.** J. Comp. Neurol., 1995; 161:103-114.

15. Peyronnard, J.M. and Charron, L. **Motor and sensory neurons of the rat sural nerve.** Muscle and Nerve, 1982; 5: 654-660.