LOCALIZATION OF THE MOTOR NEURON SOMATA OF THE MEDIAN NERVE IN ALBINO RAT

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FINAL REPORT FOR
SHORT TERM PROJECT : 304/PPSP/ 6131285
(from June 2003- June 2005)

Tuan Hj. Abd Halim bin Othman
Setiausaha,
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Pusat Pengajian Sains Perubatan
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Sir.

LAPORAN AKHIR PENYELIDIKAN
'LOCALIZATION OF THE MOTOR NEURON SOMATA OF THE MEDIAN NERVE IN ALBINO RAT'

Here with I am submitting the final report of my short term project.
(No. Acc. 304/PPSP/6131285) From June 2003 – June 2005.

Thanking you
Your's sincerely.

B. S. Rathna.

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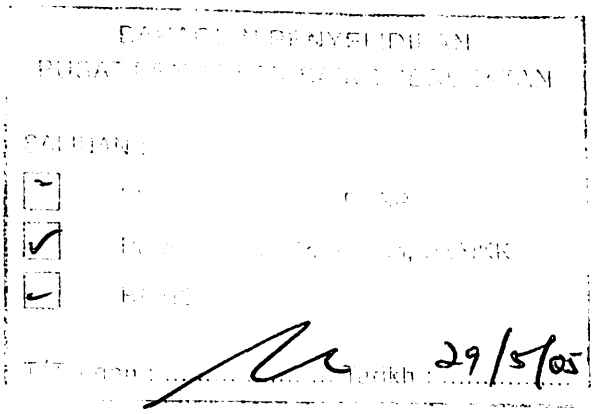

Semua laporan kemajuan dan laporan akhir yang dikemukakan kepada Bahagian Penyelidikan dan Pembangunan perlu terlebih dahulu disampaikan untuk penelitian dan perakuan Jawatankuasa Penyelidikan di Pusat Pengajian.

USM R&D/JP-04

LAPORAN AKHIR PROJEK PENYELIDIKAN R&D JANGKA PENDEK

A. *MAKLUMAT AM*

Tajuk Projek: LOCALIZATION OF THE MOTOR NEURON SOMATA OF THE MEDIAN NERVE IN ALBINO RAT.

Tajuk Program:

Tarikh Mula: 1st April 2003 - 2004

Nama Penyelidik Utama: DR. B.S. RATHNA
(*berserta No. K/P*) E 3227186

Nama Penyelidik Lain: 1). PROF. MADYA MUZAMMIL ULLAH
(*berserta No. K/P*) B - 2100127

2). PROF..MADYA OTHMAN MANSOR
I.D. NO. 490518-02-5195

B. PENCAPAIAN PROJEK:

(*Sila tandakan [/] pada kotak yang bersesuaian dan terangkan secara ringkas di dalam ruang di bawah ini. Sekiranya perlu, sila gunakan kertas yang berasingan*)

Penemuan asli/peningkatan pengetahuan

As a result of this study, findings were as follows.

1. The motor neuron somata of median nerve in Albino rat are located in the 6th, 7th, 8th, cervical and 1st tjoracic segments of spinal cord .

2. In 6th, and 7th, cervical and 1st thoracic segments these somata were located in the dorsolateral (DL) cell column,

   whereas in 8th cervical and 1st thoracic segments these somata were located in dorsolateral (DL) and retrodorsolateral (RDL) cell columns

3. The motor neuron somata of the median nerve supplying the muscles of hand are located only in the retrodorsal (RDL) cell column of ventral grey horn of 8th cervical and 1st thoracic segments of spinal cord.

☐ **Rekaan atau perkembangan produk baru,**
(*Sila beri penjelasan/makluman agar mudah dikomputerkan*)

(1) NA

(2)

(3)

☐ **Mengembangkan proses atau teknik baru,**
(*Sila beri penjelasan/makluman agar mudah dikomputerkan*)

(1) NA

(2)

(3)

☐ **Memperbaiki/meningkatkan produk/proses/teknik yang sedia ada**

(1) NA

(2)

(3)

C. PEMINDAHAN TEKNOLOGI

☐ Berjaya memindahkan teknologi.

Nama Klien: (1)
(Nyatakan nama penerima pemindahan teknologi ini dan sama ada daripada pihak swasta ataupun sektor awam)
(2)
(3)

☐ Berpotensi untuk pemindahan teknologi.
(Nyatakan jenis klien yang mungkin berminat)
NIL

D. KOMERSIALISASI

☐ Berjaya dikomersialkan.

Nama Klien: (1) NA

(2)

☐ Berpotensi untuk dikomersialkan.
(*Nyatakan jenis klien yang mungkin berminat*)

E. PERKHIDMATAN PERUNDINGAN BERBANGKIT DARIPADA PROJEK
(*Klien dan jenis perundingan*)

(1) _____

(2) _____

(3) _____

(4) _____

F. PATEN/SIJIL INOVASI UTILITI
(*Nyatakan nombor dan tarikh pendaftaran paten. Sekiranya paten/sijil inovasi utiliti telah dipohon tetapi masih belum didaftarkan, sila berikan nombor dan tarikh fail paten*).

(1) _____

(2) _____

(3) _____

G. PENERBITAN HASIL DARIPADA PROJEK

(i) LAPORAN/KERTAS PERSIDANGAN ATAU SEMINAR

(1) Will be presenting in 52nd National conference of the Anatomical society of India in 2004 Dec.

(2) _____

(3) _____

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(4) _____

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(5) _____

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(ii) PENERBITAN SAINTIFIK

(1) _____

_____

(2) _____

_____

H. <u>HUBUNGAN DENGAN PENYELIDIK LAIN</u>
(*sama ada dengan institusi tempatan ataupun di luar negara*)

(1) _____

_____

I. <u>SUMBANGAN KEWANGAN DARI PIHAK LUAR</u>
(*Nyatakan nama agensi dan nilai atau peralatan yang telah diberi*)

(1) _____

(2) _____

(3) _____

J. <u>PELAJAR IJAZAH LANJUTAN</u>

Nama Pelajar

Sarjana _____

_____

_____

Ph.D _____

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K. MAKLUMAT LAIN YANG BERKAITAN

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_____

14/09/2004 — B. S. Rahim

Tarikh — **Tandatangan**

Professor Zabidi [illegible] Hussin
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TANDATANGAN PENGERUSI
JAWATANKUASA PENYELIDIKAN
PUSAT PENGAJIAN

Fn. My doc geran j/pendek

ABSTRACT

Localization of the motor neuron somata of the median nerve in Albino rat.

Authors : B.S. Rathna, Muzammil Ullah, Othman Mansor

Institution : School of Medical sciences, University Sains Malaysia, Kubang Kerian.

Introduction:
The position of the median motornuclei and also the rostral and caudal limits of the location of motor neuron somata of median nerve in the spinal cord has been investigated

Methodology:
Twelve Albino rats (Sprague – Dawley) of either sex were used in the study and divided into two groups, Gp1 & Gp11 .
GROUP-1:The animals of this group further divided into two subgroups (Gp1A and Gp1B) Under general anaesthesia (Nembutal sodium at a dose of 30 mg / kg. weight intraperitonealy), the right median nerve was exposed in the axilla, cut and a part of it removed to prevent reunion.In other group the right median nerve was exposed just above the wrist before it gives any branch and a portion removed to prevent reunion . The left side acted as control.The animals were sacrificed 3 – 5 weeks after operation, circulation was flushed with normal saline and perfused with 10 % formal saline at a pressure of 125mm Hg. The 5^{th},6^{th}, 7^{th}, 8^{th}, cervical and 1^{st}, and 2^{nd}, thoracic segments of the spinal cord were removed by dorsal approach, separated from each other and embedded in paraffin.Their serial sections were cut at 40 micrometers and stained with thionine for nissl granules.The sections were examined microscopically to identify the chromatolysed neuron somata. From the the transverse serial sections a reconstruction of the cell column was made.
GROUP – 11: In this group , the motor neuron somata was localized by using the horseradish peroxidase (HRP).The right median nerve was cut in the axilla under general anaesthesia (like above) and its proximal stump will be put in a small container filled with HRP (30%) for 90 minutes.After 48-72 hours of survival, the animals were re-anaesthetized and perfused transcardially with saline followed by fixative (o.5% paraformaldehyde, 1.25% glutaraldehyde, Ph 7.2) and fixative containing sucrose (30%). The 5^{th}, 6^{th}, 7^{th}, 8^{th}, cervical and 1^{st} and 2^{nd} thoracic segments of the spinal cord were removed and transverse frozen sections were cut at 60 microns with a cryostat treated with tetramethyl benzidine .The sections were examined microscopically for tracer positive somata and their locations.from the serial transverse sections a reconstruction of cell columns was made.

Results:

In those animals in which median nerve cut in the axilla: Chromatolysed neuron were observed in the 6^{th} ,7^{th} ,& 8^{th} cervical &1^{st} thoracic segments of the spinal cord.

Those animals in which median nerve was cut at wrist: chromatolysed neuron were observed in the 8^{th} cervical &1^{st} thoracic segments of the spinal cord.

Horseradish peroxidase(HRP) technique: HRP was applied to the proximal cut end of median nerve in the axilla.HRP-labeled cells seen in 6^{th} 7^{th} 8^{th} and 1^{st} thoracic segments of the spinal cord.

HRP was applied to the proximal cut end of median nerve at the wrist. HRP- labeled cells were observed in 8^{th} and 1^{st} thoracic segments of the spinal cord.

Conclusions:

The motor neuron somata of median nerve in Albino rat are located in the 6^{th} 7^{th} 8^{th} and 1^{st} thoracic segments of spinal cord.

In 6^{th} and 7^{th} segments neurons were located in the dorsolateral(DL) and retrodorsolateral (RDL) cell columns of the ventral grey horn of spinal cord.

Median nerve supplying the hand muscles are located only in the retrodorsal (RDL) cell column of ventral grey horn of 8^{th} cervical and 1^{st} thoracic segments of spinal cord.

Localization of the motor neuron somata of the Median nerve in Albino rat.

INTRODUCTION

The motor neuron somata of the median nerve supplying the forearm muscles and the hand arise in the cervical and upper thoracic segments of the spinal cord. There are many conflicting views regarding the rostral and caudal limits of the location of these neurons and also their location in the ventral grey horn of the spinal cord. The median nerve motor nucleus is located in C5-T1 spinal segments in wobbler mice by Pollin (1990). Some investigators(Mutai,Shibata, and Suzuki, 1986) located these neurons in the dorsolateral and retrodorsal nucleus of ventral grey horn at levels from the cranial tip of C7 to the cranial third of T2. they also observed that the motor neuron supplying the proximal muscles of the upper limb were more caudally situated, whereas those supplying the caudal muscles were situated more caudally.According to (jenny and Inukai 1983) motor columns innervating the forearm muscles with similar actions on the hand appear to overlap in the anterior horn, also motorneurons controlling hand movement are located in C8 and T1 segments. To investigate the motor neuron somata of the median nerve and also rostral and caudal limits of the location of motor neuron somata of median nerve in the spinal cord of Albino rat.

MATERIALS AND METHODS

Twelve Albino rats (Sprague-Dawley) of either sex were used in this study . The animals were divided into two groups of 6 animals each, group –1 and group-11.
Group-1: The animals of these group were divided into two subgroups.
(Table 1). Under general anaesthesia (Nembutal sodium, at a dose of 30 mg per kg, intraperitoneally) and aseptic condition. In gp-1A the trunk of the right median nerve was exposed in the axilla and a portion removed to prevent reunion. In gp-1B the right median nerve was exposed above the wrist before it gives any motor branch for the hand and a portion removed to prevent reunion.
After 2-4 weeks the animals were killed, and the circulation flushed with normal saline and perfused with 10 % formal saline through the left ventricle of heart at a pressure of 120 mm of mercury. Immediately after perfusion the remaining portion of the median nerve will be examined after thorough dissection to ensure that the nerve had been cut at the desired level.
The 5^{th}, 6^{th}, 7^{th}, and 8^{th}, cervical and the 1^{st} and 2^{nd} thoracic segments of the spinal cord were removed, using a dorsal approach. These segments were embedded in paraffin wax and serial transverse sections cut at a thickness of 40 Micrometers (microns).All sections were affixed to slides and stained with thionine. They were examined microscopically to identify the cell columns containing the chromatolysed cells and for comparisons to be made between the experimental right side with the control left side.
Group-11: Remaining 6 animals were divided into two sub groups(table 2), the median motor neuron cell bodies will be localized using the horseradish peroxidase (HRP) technique(Friet al., 1982).In gp-11A the right median nerve was cut in the axilla under general anaesthesia (Nembutal sodium, 30 mg per kg body weight), and its proximal stump was immediately put in a container filled with HRP 30 % where it will remain for

90 minutes. In gp-11B the right median nerve was cut just above the wrist, and its proximal stump was put in a container filled with HRP (30 %) where it will remain for 90 minutes.
After 42- 48 hours of survival , the animals of both groups were re-anaesthetized and perfused with normal saline followed by fixative (0.5% paraformaldehyde, 1.25% glutaraldehyde, pH 7.2) and fixative containing sucrose (30%). The 5^{th}, 6^{th}, 7^{th}, 8^{th},cervical segments and 1^{st}, and 2^{nd} thoracic segments of the spinal cord were removed by a dorsal approach, and there serial transverse frozen sections were cut at 60 micrometer with a cryostat they are treated with tetramethyl benzidine (Mesulam, 1978). The sections were examined for tracer positive neuron somata and their locations was recorded , reconstructions of cell columns showing tracer positive cells was made.

RESULTS (OBSERVATIONS)

(A) RETROGRADE DEGENERATION TECHNIQUE.

(a) In those animals in which the median nerve was cut in the axilla:

- Chromatolysed meuron somata were observed in the 6^{th} , 7^{th},and 8^{th}, and 1^{st} thoracic segments of spinal cord on the right experimental side. No chromatolysis was observed on the control left side.(Fig, 1.)
- In 6^{th} and 7^{th} cervical segments, the chromatolysed neuron somata were located in the dorsolateral cell column (DL), whereas in 8^{th} cervical and 1^{st} thoracic segments these somata were located in the dorsolateral(DL) and retrodorsal (RDL) cell columns of ventral grey horn of spinal cord. (Fig 1.a)

(b) In those animals in which the median nerve was cut at the wrist:

- Chromatolysed neuron somata were observed in the 8^{th} cervical and 1^{st} thoracic segments of the spinal cord on the right experimental side. No chromatolysis was observed on the control left side.(Fig, 3)
- In both 8^{th} cervical and 1^{st} thoracic segments, the chromatolysed neuron somata were located only in the retrodorsolateral (RDL) cell column of ventral grey horn. (Fig 1.b)

(B) HORSERADISH PEROXIDASE (HRP) TECHNIQUE.

(a) In those animals in which HRP was applied th the proximal cut end of median nerve in the axilla:

- HRP- labeled neuron somata were observed in the 6^{th} , 7^{th},8^{th}, cervical and 1^{st},thoracic segments of spinal cord on the right experimental side. No such neuron somata were observed on the control side.
- In 6^{th} and 7^{th} cervical segments, the HRP- labeled neuron somata were located in the region of dorsolateral cell column (DL), whereas in 8^{th} cervical and 1^{st} thoracic segments these somata

were located in the region of retrodorsolateral (RDL) cell columns of ventral grey horn of spinal cord.

(b) In those animals in which the HRP was applied to the proximal cut end of median nerve at the wrist.

- HRP-labeled neuron somata were observed in the 8th and 1st thoracic segments of spinal cord on the right experimental side. No such neuron somata were observed on the control side.
- In both 8th cervical and 1st thoracic segments, the HRP- labeled neuron somata were located only in the region of retrodorsolateral (RDL) cell column of ventral grey horn.

Table 1.GROUP – 1 Sex, weight, days after operation.

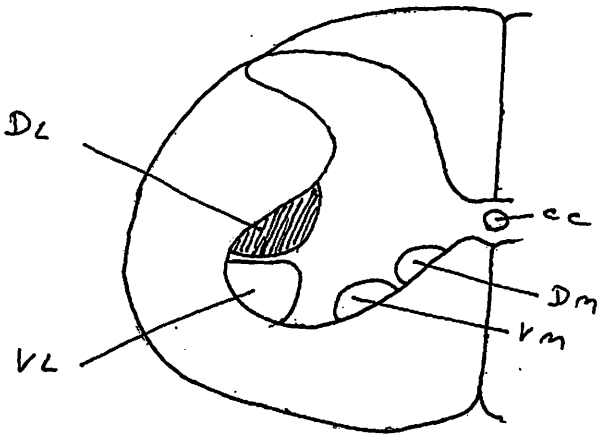
Gp -1A			
No.	Sex	Weight (gm)	Days after operation
1	Fe	350	21
2	M	300	25
3	M	375	24
Gp-1B			
1	M	300	28
2	M	350	24
3	M	300	24

Table 2. GROUP-11 Sex, weight, .

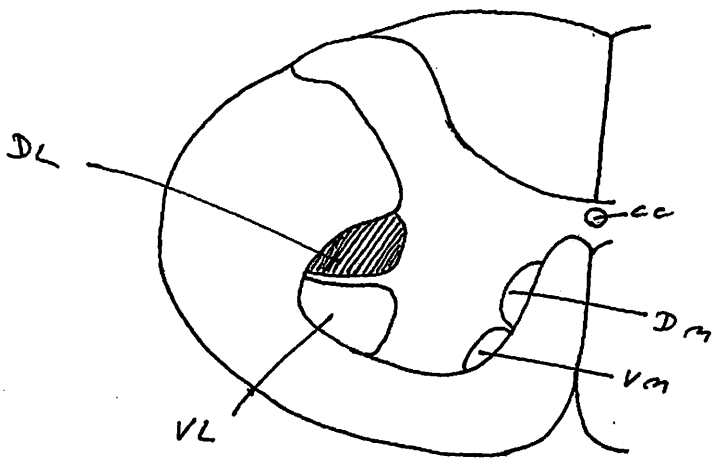
Gp -11A		
No.	Sex	Weight (gm)
1	M	350
2	M	375
3	Fe	325
Gp –11B		
1	Fe	300
2	M	350
3	M	375

ulnar nerve cut in axilla

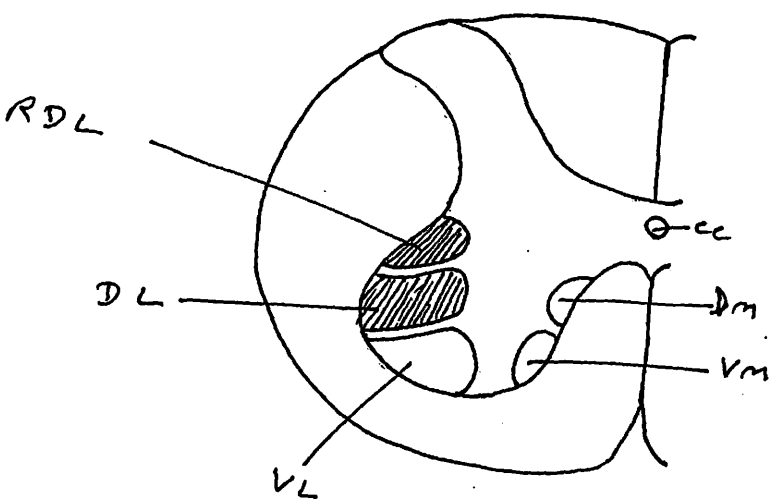
(Fig 1.a)



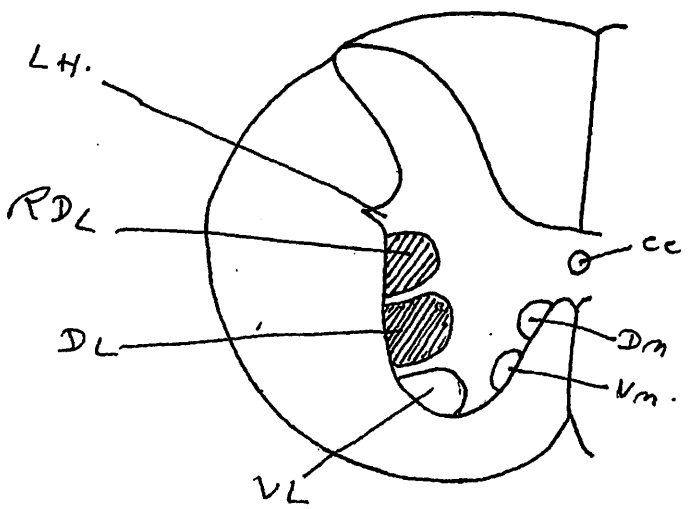
C-6



C-7

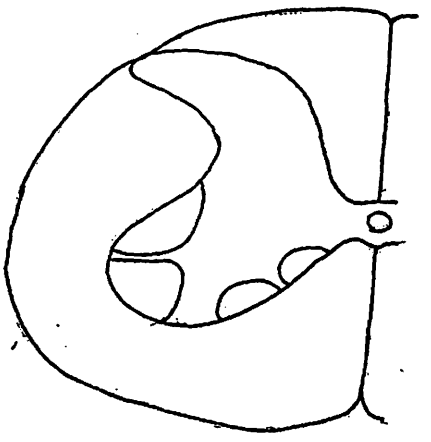


C-8

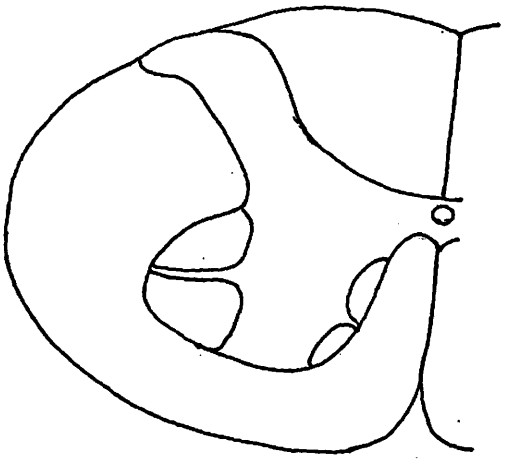


T-1

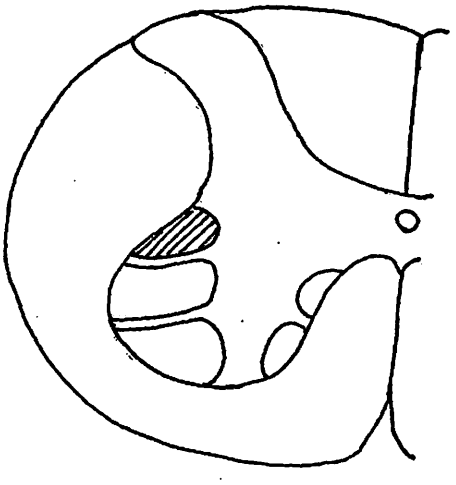
Ulnar nerve cut at the wrist (Fig 1:b)



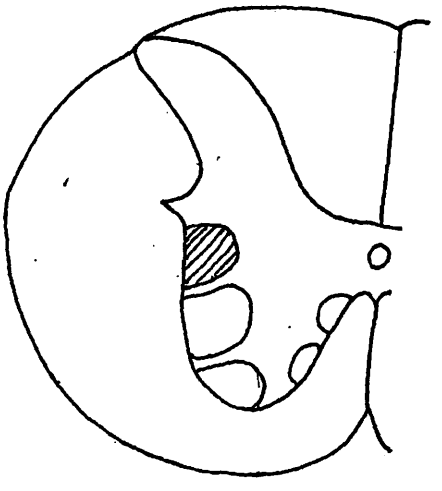
C-6



C-7



C-8



T-1

Fig, 1. Cervical 8th ,segment

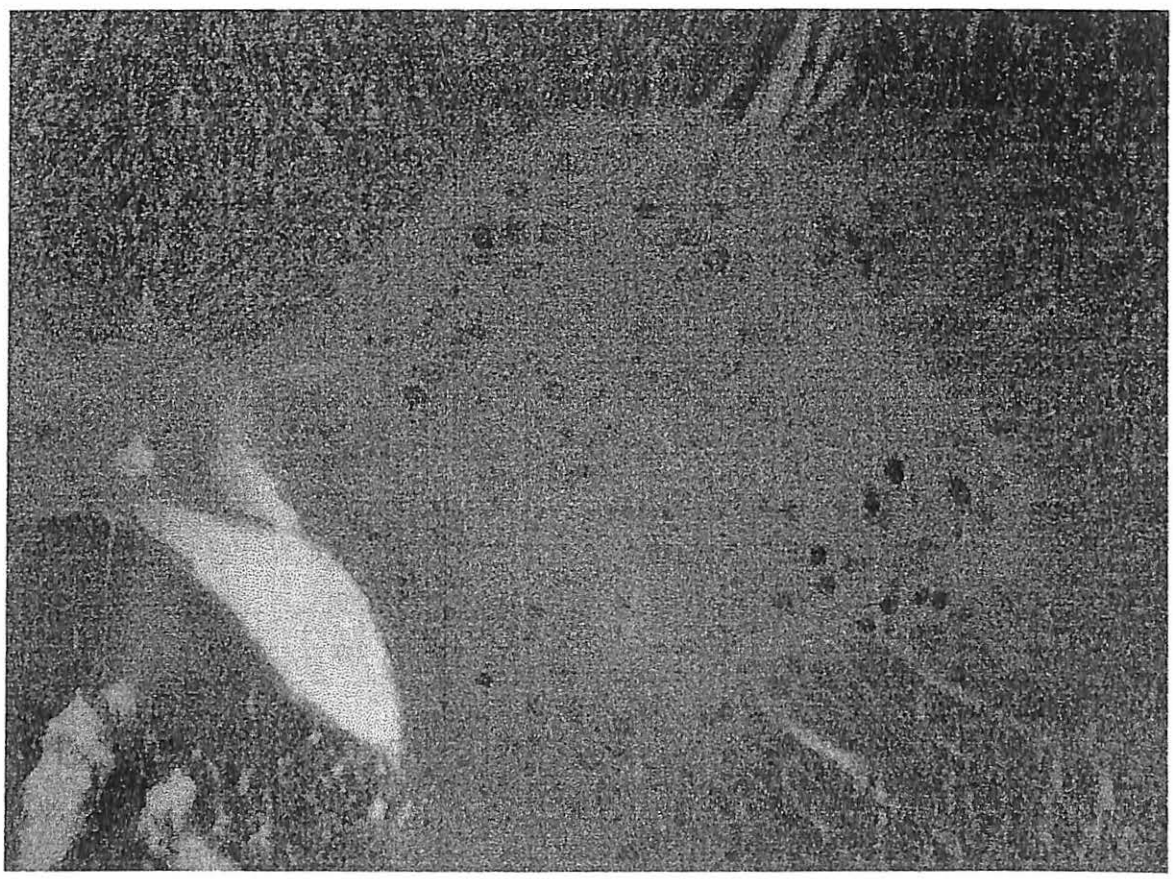


Fig. 2. Thoracic 1st segment (HRP- labeed cell)

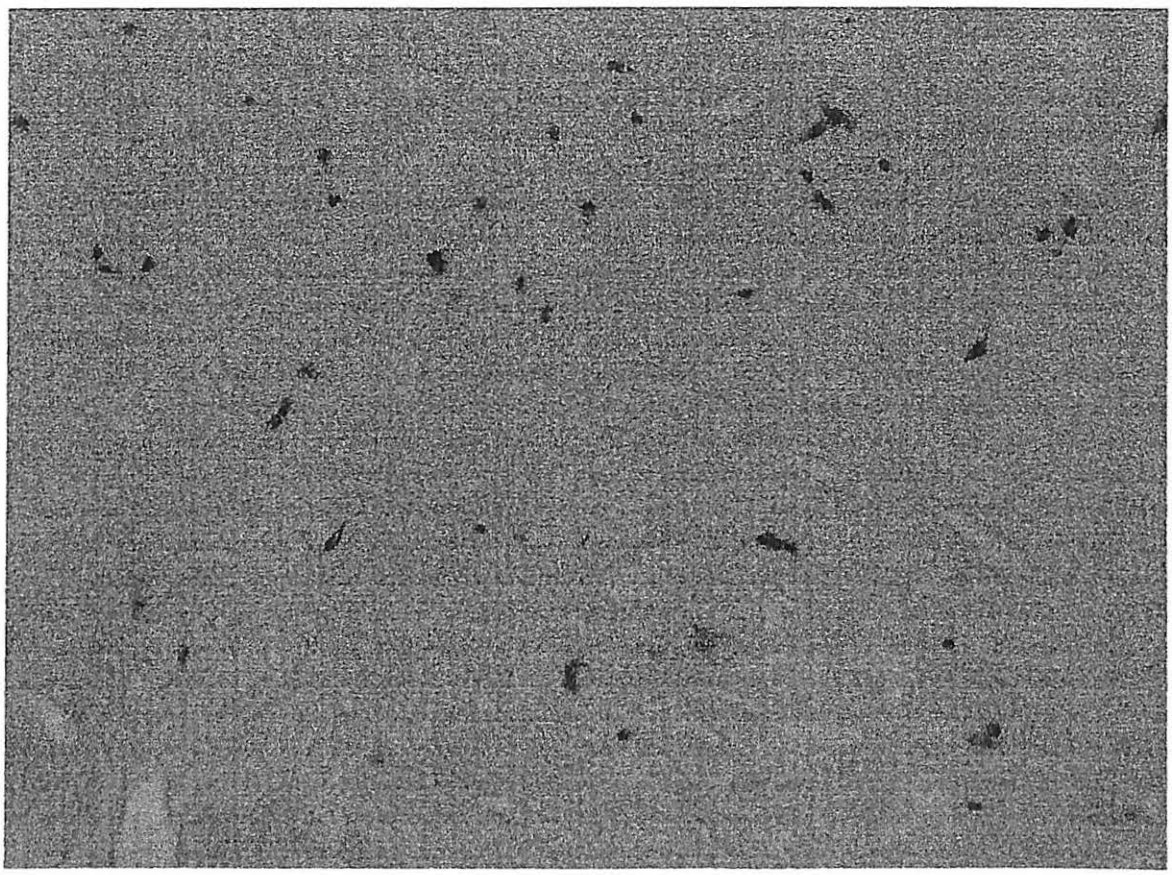
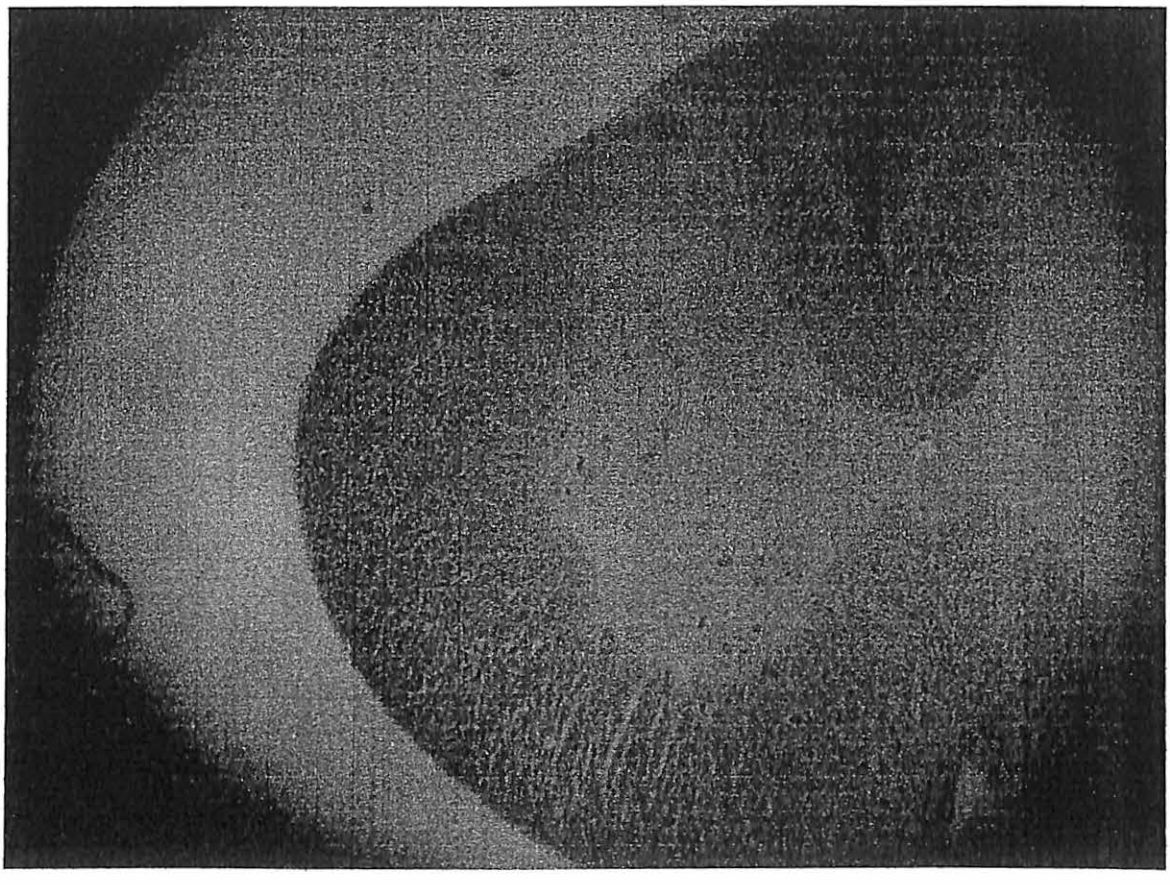


fig. 3. Thoracic 1st segment.



DISCUSSION

In man the median nerve arise from the ventral rami of the cervical 5, 6, 7, 8, and Thoracic 1st, segments .In our study median nerve somata were observed in the C6 – T1 thoracic segments of the spinal cord. According to Scarisbrick, Haase(1990) median motorneurons were found in C6-T1 segments of the spinal cord in rats. But Pollin, Mchanwell,(1990) located in the C5-T1 spinal segments. In our study 6th and 7th cervical segments neuron somata were observed in dorsolateral column, and 8th and 1st thoracic segments were located in dorsolateral (DL) and retrodorsolateral (RDL). Mutai, Shibata, (1986) found HRP – labeled cells in dorsolateral and retrodorsal nucleus of the ventral horn at levels from the cranial tip of C7 to the cranial third of T2 segments. According to Oka, Ohtani, Satou (1989) along the longitudinal axis of the spinal cord, motorneurons innervating the flexor side muscles were located in the more rostral part of the spinal cord, whereas those innervating the extensor muscles were located in the more caudal

part of the spinal cord. Thus, motorneurons innervating forearm muscles were well organized somatotopically not only in the transverse plane, but also along the longitudinal axis of the spinal cord. Median nerve supplying the hand muscles were located more caudally in the 8th cervical and 1st thoracic segments , they are present only in the retrodorsal (RDL) column of ventral grey horn of the spinal cord. Fritz, Illert, Reech (1982) used horseradish peroxidase (HRP) technique to investigate the location of the motor nuclei of the median and ulnar nerves. Each nucleus is described its longitudinal extent along the rostro-caudal spinal cord axis and by its dorso-ventral and medio-lateral position within the ventral horn, they also demonstrates the complete overlap of two different nuclei. Jenny and Inukai (1983) observed that the motor columns innervating the forearm muscles by the median nerve and ulnar nerve with similar action on the hand appear to overlap in the ventral grey horn . They further observed that the motor neurons controlling the hand muscles were located in C8 and T1.According to Iwamoto, Haber, Dixon, Gonyea (1980) the motor neuron somata of flexor carpi radialis supplied by the median nerve are located in the spinal cord segments C6-T1. Saito (1986) observed that the motor neuron neuron somata of the median nerve were present in three discrete fusiform cell columns within the dorsolateral part of the ventral grey horn of spinal cord extending from C6- T1 segmnets.

CONCLUSIONS

As a result of this study, the conclusions drawn were as follows:

1. The motor neuron somata of median nerve in Albino rat are located in the 6th, 7th, 8th, and 1st, thoracic segments of spinal cord.
2. In 6th, 7th, cervical segments, these motor neuron somata were located in the dorsolateral (DL) cell column,
   Whereas in 8th, cervical and 1st thoracic segments these somata were located in the dorsolateral (DL) and retrodorsolateral (RDL) cell columns of the ventral grey horn of spinal cord.
3. The motor neuron somata of the median nerve supplying the muscles of hand are located only in the retrodorsal (RDL) cell column of ventral grey horn of 8th cervical and 1st thoracic segments of spinal cord.

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