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AN HRP STUDY OF LOCALIZATION AND TOPOGRAPHICAL MAPPING OF THE MOTONEURONS SUPPLYING THE RAT SERRATUS ANTERIOR MUSCLE *

B. JAMEIE[1**], H. MATSUMOTO[2] AND S. KITAMURA[2]

[1] Department of Anatomy, Cellular & Molecular Research Center, Neuroscience Division, Iran University of Medical Sciences, Hemmat Express Way, Tehran, I. R. of Iran, E-Mail: melika2@iums.ac.ir
[2] Department of Oral Anatomy, School of Dentistry, University of Tokushima, Tokushima, Japan

Abstract – Shoulder movements are a complex motor act, controlled by many muscles attached to the scapula and the vertebral column. These muscles are innervating by motoneurons (Mns) located in the ventral horn of the spinal cord. Motoneurons of the ventral horn are arranged such that several groups of Mns can be distinguished in a transverse section through the spinal cord. The anatomical or functional implication of these groups is still unknown. The present research was designed to determine the location and topographical mapping of Mns to the rat serratus anterior (SA) muscle.

Thirty young adult male rats weighing 180-250g were used in this study. The location and distribution of the Mns in the rat cervical spinal cord to the serratus anterior (SA) muscle of the rat have been studied using horseradish peroxidase (HRP) intramuscular injection and the application of a HRP nerve dipping method to the long thoracic nerve. Following the intramuscular injection of HRP and also a nerve dipping for the long thoracic nerve, labeled motoneurons were identified ipsilaterally to the side of HRP application forming two main cell columns extended rostrocaudally in the ventral horn of both sides. The column G5 extended longitudinally from the caudal half of the fifth (C5) to the rostral half of the seventh (C7) cervical segments in group five. The column G7, located mainly in the rostral half of the C7 in group seven. The serratus anterior labeled motoneurons showed a rostrocaudal somatotopic distribution in each of the columns G5 and G7.

These results suggest that there is a specific functional role for motoneurons, and also that motoneurons are arranged in the form of a motor pool with a musculotopic pattern of distribution corresponding to the embryonic origin and anatomical position of the muscles they innervate to.

Keywords – Serratus anterior motoneurons, localization, topographical mapping, HRP

1. INTRODUCTION

Motoneurons are the final common pathway in the motor system. Anatomical studies of the spinal cord have shown that the motoneurons are arranged in longitudinal columns, each supplying defined muscle groups [1, 2, 3]. Physiological studies also indicated that not only motoneurons, but also the projections of muscle efferents are somatotopically organized in the ventral horn [4, 5]. The population of motoneurons innervating a particular muscle called a motor pool, which projects onto muscles in a topographical manner [1]. In its broader meaning topography refers to the ordered projection of consecutive ventral roots onto muscles associated with a limb or the trunk. Most previous studies of topography have centered on groups of limb muscles, but more recently attention

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**Corresponding author

has focused on the topographical projection of neurons onto a single muscle [6-9]. When a single muscle is considered, topography has a more restricted meaning. In this context, topography means that the motoneuron pool maps systematically over the surface of a muscle or its component [9]. If the muscle is compartmentalized, then there is an ordered mapping of motoneurons within those compartments [6].

There is some anatomical and physiological evidence for spatial organization within the discrete, longitudinal motor columns which supply individual muscles [1, 3, 7, 10, 11]. The studies of early investigators were particularly important because they provided an atlas of the ventral horn. The combined work of Rexed and Romanes showed that the cells supplying muscles of forelimbs and hind limbs have a topographical relationship in which the location of the cell in the ventral horn can be related to the location of its muscles in the periphery [12, 13]. Their classification is broad for the study of the topographical relationship between the localization of motoneurons and distribution of muscles. Kitamura et al studied cytoarchitectonically the localization of motoneurons in the ventral horn of the rat cervical spinal cord, divided the motoneurons into three nuclei including medial, ventrolateral and dorsolateral nuclei which are, in turn, subdivided to eleven groups. Medial nucleus is divided into Group 1, 2 and 3, the ventrolateral nucleus is divided into Group 4, 5, 6 and 7 and the dorsolateral nucleus is divided into Group 8, 9, 10 and eleven [14]. (Fig. 1)

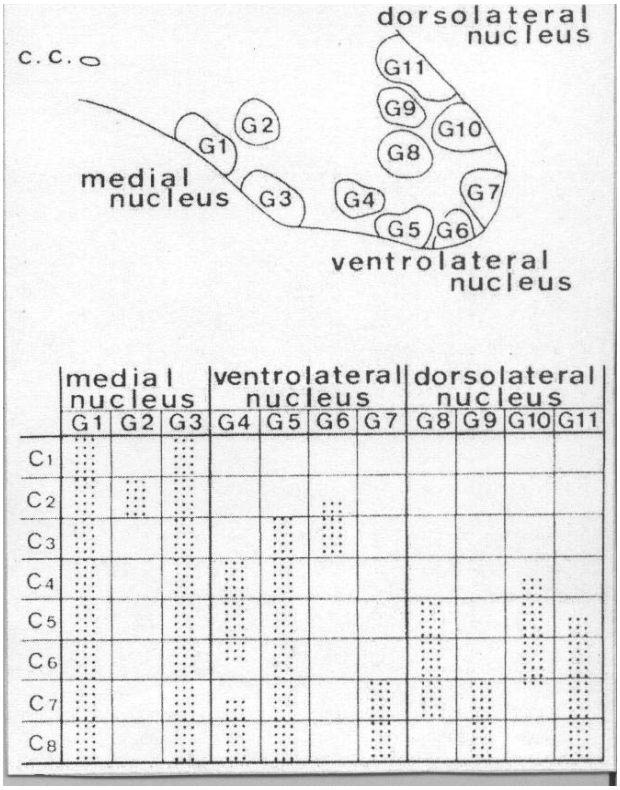

Fig. 1. The ventral horn cell groups based on Kitamura's classification: a. Drawing to show the position of the cell groups in the cross section of the left ventral horn, b. Diagram to show their approximate extent in the cervical spinal cord. The medial nucleus includes three cell groups; group 1 (G1), group 2 (G2) and group 3 (G3). The ventrolateral one includes four cell groups; group 4 (G4), group 5 (G5), group 6 (G6) and group 7 (G7). The dorsolateral nucleus includes four cell groups; group 8 (G8), group 9 (G9), group 10 (G10) and group 11 (G11). "c.c" indicates the central canal

Even though it was demonstrated that motoneurons located in the groups innervate certain muscles [15, 16], detailed spatial organization has generally been assumed to be absent. The question that arises is to what degree the peripheral innervation is matched with motor pool distribution. Therefore, in the present research, horseradish peroxidase (HRP) has been used as a retrograde tracer to determine the location and topographical organization of motoneurons to the serratus anterior muscle in the rat, based on the classification of Kitamura et al [14].

2. MATERIALS AND METHODS

Thirty young adult male Sprague-Dawely rats weighing 180-250g were used in this study. The animals were maintained on 14:10 hour lighting with food and water ad libitum. The animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (35mg/kg). Under local sterility with 70% ethanol, an incision was made through the shaved skin along the midline of the neck and thoracic region, in order to expose the muscle and its nerve. The skin and pectoralis muscles of both sides were reflected, and then the serratus anterior and its nerve, the long thoracic nerve, were exposed. The right side received an HRP intramuscular injection and the left, the nerve dipping method [17]. Five different points of the serratus anterior muscle were injected with 15µl of 30-50% HRP solution in physiological saline by using a 10 µl microsyringe. For each injection, a 10 µl microsyringe needle was inserted into the muscle distally and gently advanced through the muscle to a point near its origin. Particular care was taken to minimize damage to the fascia and epimysium, as well as preserve the blood and nerve supply. The tracer was then continuously injected into the muscle as the needle was slowly withdrawn. As the needle exited the muscle, a drop of special glue was immediately applied to the needle hole to prevent tracer leakage from this site. After the muscle injections were completed, the area was gently swabbed with cotton to test for tracer leakage and washed with distilled water. For the long thoracic nerve of the opposite side, after reflecting the pectoralis and serratus anterior muscles, the nerve was proximally exposed and cut. The nerve stump was put in a hollow waxed pool filled with 15µl of 30-50% HRP solution and immediately covered by low melting wax. To prevent the leakage of HRP, the muscles around the nerve were covered by low melting wax and excessive HRP was removed by several washes of distilled water. After 30 minutes, remaining HRP and wax on the left side were removed, and the operation site was washed and sutured.

Following an 18-24 hour period for retrograde transport of the tracer, the animals were deeply reanestethized (Pentobarbital, 50 mg/kg, i.p.) and transcardially perfused with 300ml of heparinized saline solution, followed by 350 ml of cold fixative (1.0% paraformaldehyde, 1.25% glutaraldehyde in 0.1M sodium phosphate buffer, pH 7.4) and 300 ml of cold 10% sucrose in 0.1M sodium phosphate buffer, pH 7.4. The brain and cervical spinal cord were removed and placed in 10% sucrose solution at 4°C overnight. They were transferred through graded sucrose solution (10%, 20% and 30%) in 0.1M sodium phosphate buffer, pH 7.4. The right dorsal surface of the cervical spinal cord was marked with a notch for orientation. Serial sections of 50µ thickness of the cervical spinal cord were cut by using a freezing microtome and immersed in 0.1M phosphate buffer, pH 7.4 and floated in 0.1M phosphate buffer, pH 7.4, then reacted with 3.3', 5.5'-tetrametylbenzidine (TMB) and 0.03% hydrogen peroxidase. The sections were mounted onto coated slides, counterstaining with 1% neutral red and coverslipped [18]. HRP- labeled and non-labeled neurons were observed and counted by a light microscope. "Labeled motoneurons somata" were defined as those cells which contained a nucleus completely surrounded by intracytoplasmic granules of HRP reaction products. The

identification of motoneuron groups in rat cervical spinal cords was based on Kitamura classification [14].

3. RESULTS

a) General observation

HRP-labeled cells were marked by granularity of the HRP products. The staining ranged in all preparations from excellent to light. Excellently stained cells were distinguished by HRP granules and by processes labeled for a distance from the cell body. (Fig. 2)

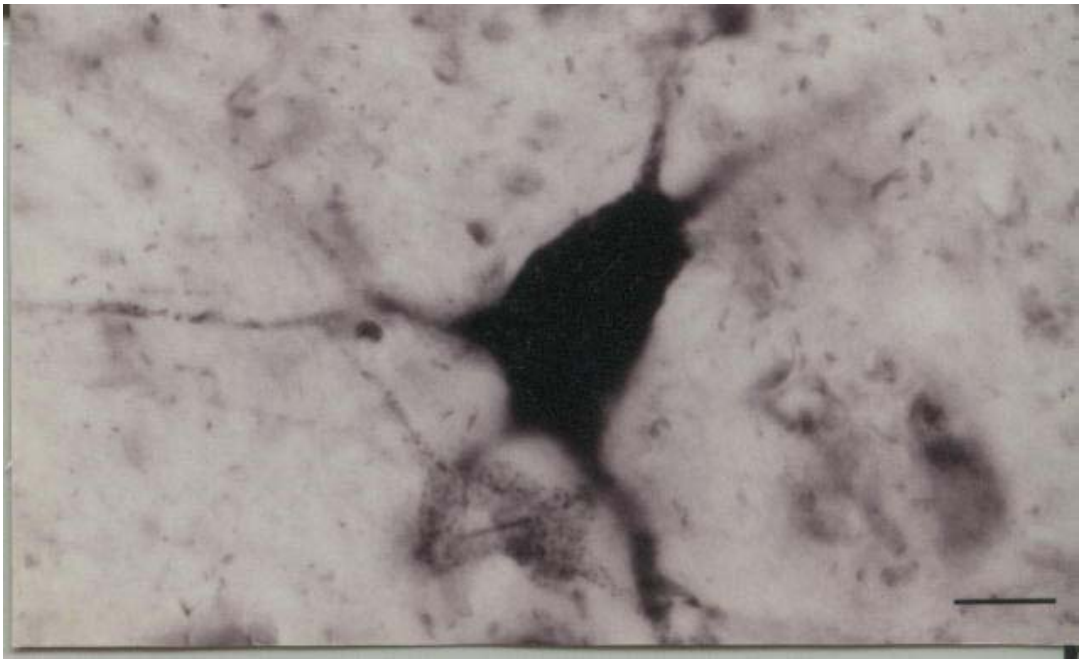

Fig. 2. A typical motoneuron labeled with HRP. Scale bar=50 μ

b) Distribution of labeled motoneurons following injection of HRP into serratus anterior muscle

The serratus anterior motoneurons were labeled in the ventral horn ipsilaterally. Labeled neurons were found in G1, G3, G4, G5, G7, G8 and G10 motor cell groups based on Kitamura's classification [14]. Labeled motoneurons with a high appearance were found in two cell columns including G5 and G7, and extended rostrocaudally. In G5, the labeled motoneurons distributed with a high appearance from the caudal half of C5 to the rostral half of C7. Labeled neurons in G7 were distributed from the caudal half of C6 to the rostral half of C8. These neurons showed a high appearance at the level of the rostral half of C7. (Fig. 3 A*-C* Fig. 4 A*-B*)

c) Distribution of labeled motoneurons following application HRP to cut nerve end

Labeled motoneurons were found ipsilaterally in G1, G3, G5, G7, G8 and G10 motor cell groups. Motoneurons in G1, G3, G6, G8 and G10 showed low appearance. For G5 and G7 the labeled motoneurons showed the same appearance as for intramuscular injection. (Fig. 3 A-C Fig. 4 A-B)

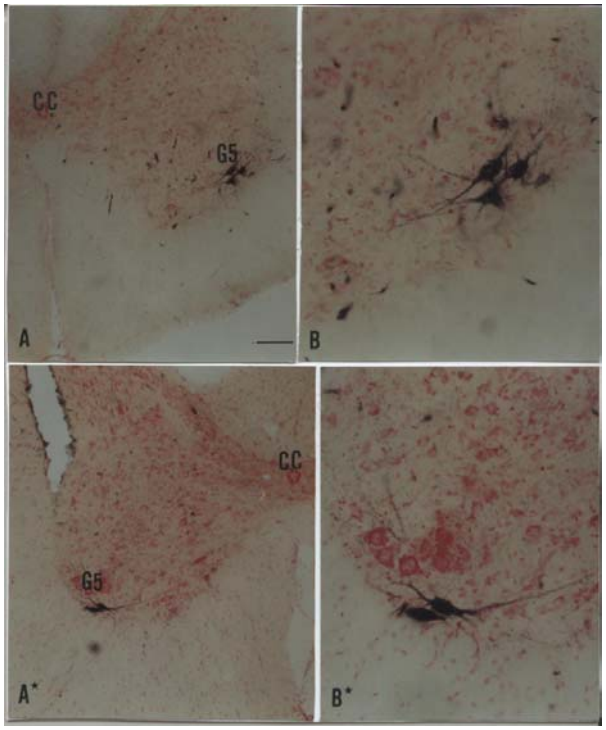

Fig. 3. Photomicrograph of the labeled motoneurons. A & B; Labeled motoneurons in G5 after nerve dipping method to the left side. A*& B*; Labeled motoneurons in G5 after intramuscular injection to the right side. Scale bar A, A* =240 μ, B, B*=110 μ

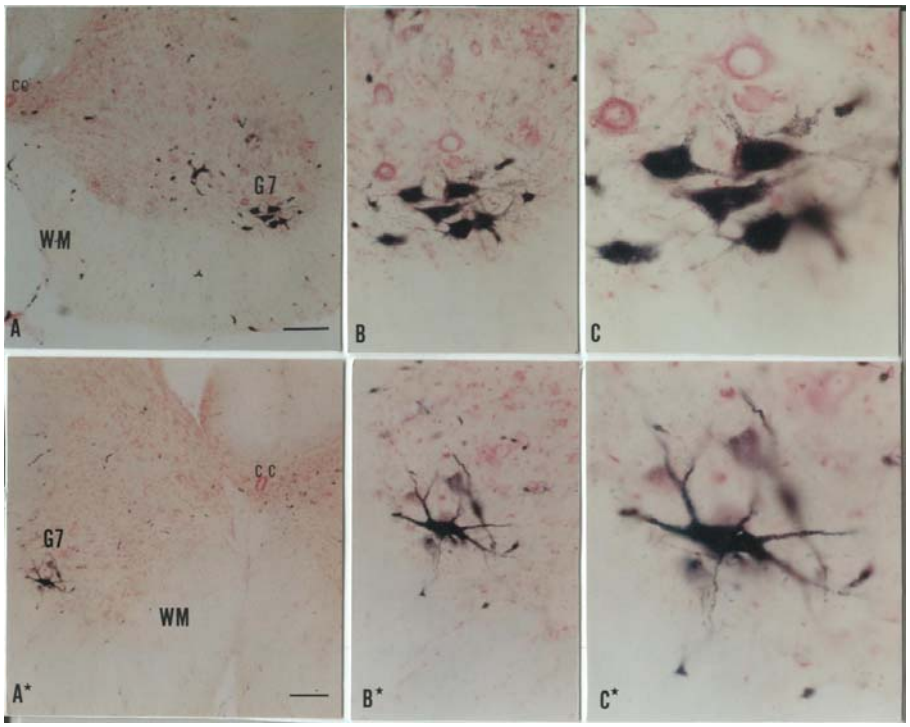

Fig. 4. Photomicrograph of the labeled motoneurons. A, B & C; Labeled motoneurons in G7 after nerve dipping method to the left side. A*, B *, & C *; Labeled motoneurons in G7 after intramuscular injection to the right side. Scale bar A, A*=240 μ, B, B*=110 μ, C, C*=50μ

d) Distribution of commonly labeled neurons followed by two methods

It was found that certain motoneurons with a high appearance in G5 and G7 were labeled by both methods. In G5, the labeled motoneurons distributed from the caudal half of C5 to the rostral half of C7, and in G7 the labeled cells were seen located mainly in the rostral half of C7. The rostrocaudal distribution of main motoneurons to the serratus anterior muscle shifted from a medial to a lateral position in ventrolateral nucleus (Fig. 5).

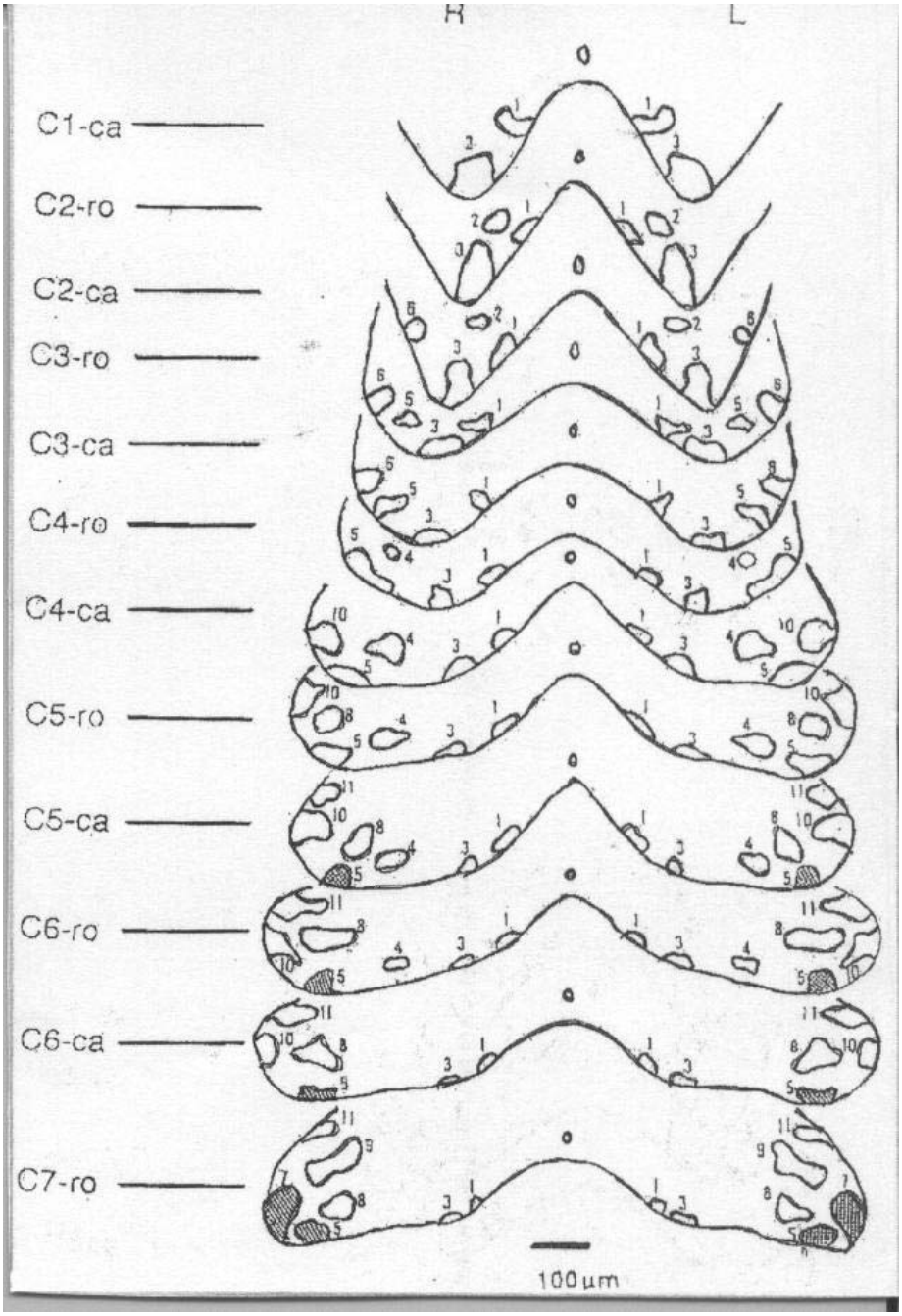

Fig. 5. Distribution of commonly labeled serratus anterior motoneurons followed by two methods

4. DISCUSSION

a) Methodological Considerations

Two different methods of HRP tracing have been used in the present experiment to determine the localization of motoneurons in the rat cervical spinal cord. The first is a conventional technique which has been previously used to map motoneuron columns of the motor system, including the tongue [19], jaw [20], extraocular muscles [21], hindlimb musculature [22], forelimb musculature [23], diaphragm and phrenic nerve [24], pelvic muscles [25], abdominal muscles [26], laryngeal muscles [27] and orbicularis oculi [11]. However, because the HRP solution readily diffuses throughout extracellular spaces, and even through perimysial vascular boundaries, one may concern when using an intramuscular injection, HRP can spread into an adjacent area. Hasse & Hrycyshyn pointed out that the muscular fascia sheets are important in preventing the diffusion of HRP [28]. The injection of HRP into muscles was found to produce labeling of motoneurons, presumably due to diffusion of HRP into adjacent muscles. For any of the HRP preparations, the majority of labeled cells formed a continuous body of cells and never found labeled cells in upper cervical and thoracic segments. However, in a few preparations some labeled cells also appeared variously outside the main body of the labeled columns. Attempts were made to eliminate these latter cells, thought to be due to the peripheral spread of the HRP. Neither varying HRP amount and injection volume, nor the ablating muscles were completely effective in reducing the numbers of labeled cells outside the main body of labeled motoneurons without also reducing the numbers of labeled cells lying within the main body. Indeed, similar injections often resulted in quite different cell totals. It should be remarked that the rats were mobile within hours after HRP application, so it is possible that the movements of the animals promoted leakage of HRP from the injected muscles. Concerning the method of nerve dipping, De Vito et al have shown that somata of vagal efferent fibers can be identified in the dorsal motor nucleus of the vagus following exposure of cut nerve end to HRP [29]. It seems from the present work and that of Richmond et al., and Gottschall et al., applying HRP to a cut nerve end and avoiding the severance of other motor nerves avoids the problem of HRP diffusion and also provides very selective labeling [17, 30]. For the examination of individual motoneuron pools, the technique of cut nerve exposure was therefore used [17]. This method is easily applied for cats, but it is necessary to carefully treat rats, because the nerves of the rats were fine and short for surgical operations. However, each labeling method has its own advantages and disadvantages. Therefore, the two mentioned methods were used to determine the localization of rat cervical motoneurons. It is reasonable that commonly labeled neurons by both methods show "true localization of motoneurons or motor pool" of certain muscles.

b) Consideration of Results

According to anatomical description, the serratus anterior muscle is located in the anterolateral chest wall and innervated by a long thoracic nerve from sixth, seventh and eighth cervical nerves. There are few reports concerning the localization of the serratus anterior motoneurons. The localization of the motoneurons supplying the serratus anterior muscle in the rat is studied by using HRP methods which have been previously adopted for the identification of motoneurons in a wide range of situations [19, 28, 31], and suggested that the serratus anterior motoneurons form two mainly independent cell columns, including G5 and G7.

The G5 belongs to the ventrolateral nucleus of the classification considered in this study and corresponds to the ventromedial nucleus of Rexed and the nucleus anteromedialis described by

Okamoto and Deura [12, 14, 32]. With respect to the function of this group, Okamato and Deura described that the motoneurons in this group supply the long and short back muscles. The G7 also belongs to the ventrolateral nucleus of Kitamura's [14] classification, located more lateral than G5 [28]. The ventrolateral nucleus includes a Mns cluster supplying rhomboideus, and the cervical portion of the trapezius and diaphragm. That cluster, corresponding to the rostral G5, may be an motoneuron pool responsible for the dorsal division of the cervical plexus. This hypothesis has been strengthened by the finding that the rostral G5 continues caudally to a motoneuron pool for the serratus anterior muscle which is innervated by a more caudal nerve of the same category as the nerves to the rhomboideus and trapezius. However, the coexistence of those Mns implies a close antigenic relationship between these muscles and serratus anterior reported by others [33]. Thus, innervation could be a reliable factor in the determination of muscles ontogeny and homology.

Based on these findings and the previous studies by other researchers, the following two points are concluded. First, motoneurons are somatotopically arranged along the ventral horn margins so that the trunk, the superficial back and chest, the shoulder, arm and hand are represented in that order from medially to ventrolaterally and then dorsolaterally. Therefore, the limb motoneurons are exclusively lateral in position and the neurons of the axial muscles are medial. Also the branching patterns of the cervical nerves such as bifurcation into dorsal and ventral rami and the stratification of the ventral rami have some type of relationship with the motoneuron distribution in the spinal cord.

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