NOTE *Physiology*

Distribution of Dendrites from Longissimus Lumborum Motoneurons Stained Intracellularly with Biocytin in Adult Cats

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ABSTRACT. The three-dimensional distribution of dendrites from motoneurons innervating longissimus lumborum (Long Motoneurons) in the L4 spinal segment was examined in the adult cat using intracellular staining techniques. Long Motoneurons were electrophysiologically identified, stained with injection of biocytin and reconstructed from serial histological sections. Somas of Long Motoneurons were mainly located in the lateral-ventral area of the ventral horn. The dendritic distribution followed an orderly pattern in all motoneurons examined. Long Motoneurons showed a multi-directional distribution of dendrites, and the dendritic distribution pattern varied depending on the motoneuron. All studied motoneurons distributed dendrites from the spine into the white matter. The most significant morphological characteristic of the Long Motoneurons was the variation in their dendritic distribution. No relationship was observed between the effects of peripheral afferent inputs from the hindlimb and morphological characteristics of motoneurons.

KEY WORDS: biocytin, feline, motoneurons, postsynaptic potentials, trunk muscles.

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The trunk is the main mass of the body in most animals and is located in the center of the body. The center of gravity (COG) is located in the trunk during most types of movement and in most postures. The position of the COG is influenced by movements, and the movements of all body parts are controlled by the activity of trunk muscles. Neuronal control of trunk muscles during locomotion has been investigated [20, 23]. The erector spinae control body balance by increasing spinal column stiffness and/or inducing inward movements from the lateral side during locomotion. Macpherson *et al.* [15, 16] reported the activity of neuronal control of trunk muscles in a standing position. Somatosensory information from different body parts is very important for controlling trunk muscle activities. Experiments using intracellular recording techniques demonstrate that the trunk muscle motoneurons have strong effects on muscle and cutaneous afferent inputs from mechanical receptors in different body parts [21, 22, 24, 25].

A motoneuron is composed of dendrites, a cell body, and axons. The morphological characteristics of motoneurons are considered to reflect their functional characteristics. Morphological studies are very important for understanding the functional aspects of motoneurons. Previous studies have demonstrated the morphological characteristics of spinal motoneurons innervating the hindlimbs, the tail, and the cervical muscles [8, 14, 18], and there may be a close relationship between dendritic distribution and the soma location of motoneurons [2, 8, 9]. Holstege *et al.* [11] reported that the soma location of motoneurons innervates trunk muscles. However, the morphological characteristics of trunk muscle motoneurons have not been characterized. The purpose of the present study is to demonstrate the morphological characteristics of motoneurons innervating longissimus lumborum (Long Motoneurons), one of the main erector spinae muscles, using intracellular staining with biocytin. The Long Motoneurons in L3 and L4 are strongly influenced by afferent inputs from the hindlimbs [21, 24]. In addition, the relationship between the morphological characteristics of motoneurons and the effects of stimulating the hindlimb muscles and cutaneous nerves were investigated.

Experiments were performed on 10 adult cats of both sexes, weighing between 3.2 and 4.5 kg, in accordance with the Guide for the Care and Use of Laboratory Animals of Yamaguchi University. The animals were anesthetized with halothane (3–5%). Under anesthesia, the animals were decerebrated by passing a spatula rostroventrally from a line approximately 1 mm rostral to the superior colliculus and excising the tissue rostral to the transection. A laminectomy was performed from L1 to L5. The nerves innervating longissimus lumborum (Long Motoneurons) originated from the L4 spinal segments and muscles (Q, quadriceps femoris; PBSt, posterior biceps femoris and semitendinosus; GS, medial and lateral gastrocnemius). The cutaneous nerves (Sur, sural cutaneous; SPc, superior peroneal cutaneous; Tib, tibialis) innervating the hindlimb were isolated from the surrounding tissue and mounted on bipolar stimulating electrodes. The animal was fixed in a stereotaxic frame, paralyzed with pancuronium bromide (0.4 mg/kg/hr) and artificially ventilated. The end-tidal CO_2 concentration was monitored and maintained at approximately 4.0% by adjusting the respiratory rate and/or tidal volume. Rectal temper-

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ature was monitored and maintained at approximately 37°C with a heat lamp. Arterial blood pressure was monitored through a cannula inserted into the common carotid artery, and the mean blood pressure was maintained above 80 mmHg throughout the experiment. Intracellular recordings were made with glass microelectrodes (2.5–3.0 Mohm) containing 3% biocytin (Sigma-Aldrich, St. Louis, MO, U.S.A.) in 0.05 M Tris buffer and 0.3 M KCl [12]. The Long Motoneurons were identified by the presence of an action potential after stimulation at L4. After identification, the effects of stimulation of various hindlimb nerves at different stimulus intensities were recorded. Biocytin was injected using depolarizing current pulses (5–15 nA) for 15–30 min. One to three neurons from each cat were injected intracellularly with biocytin. The cats were perfused through the heart with 1 *l* of Ringer solution, followed by 2 *l* of 1% formaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), 2–5 hr after the last intracellular injection of biocytin. The spinal cord was removed and postfixed in the same fixative for 2 hr at room temperature. Horizontal serial sections were cut from spinal segments L3–S1 (50- or 100-μm thick) and incubated overnight in avidin-horseradish peroxidase (Sigma-Aldrich). All incubations were performed at room temperature. Biocytin-filled neurons were revealed with a nickel-intensified diaminobenzidine reaction that resulted in a black reaction product. The biocytin-filled neurons of serial sections were photographed (Imager M1; Carl Zeiss, Oberkochen, Germany) and duplicated on the same sheet of paper. The final magnification of these images was 500×. Dendritic profiles for each section were drawn, and their exit points from the sections were labeled. The drawing of the adjacent section was then superimposed. Part of the dendritic tree was photographed using a 100× oil-immersion objective. Diameters were measured using an Imager M1 microscope (Carl Zeiss). Dendritic length was calculated according to the method described by Rose [18].

Stable intracellular recordings were obtained from 27 Long Motoneurons. Fourteen of these cells were not sufficiently stained to trace the dendrites to the distal end. To minimize the loss of dendrites due to poor staining, only those 10 motoneurons whose distal dendritic and fine dendritic branches were stained with an intensity almost equal to that of proximal dendritic trees were selected for detailed study. Most Long Motoneurons were located in the lateral area of the ventral horn, although some were scattered in the medial regions of the ventral horn. Holstege *et al.* [11] reported the distribution of long motoneurons in the ventral nucleus.

Figure 1 shows two Long Motoneurons in a horizontal section. The panel under each motoneuron indicates the pattern of postsynaptic potentials (PSPs) after electrical stimulation of hindlimb muscles and cutaneous nerves at 5 times the threshold (T). A Long motoneuron (No. 4) distributed dendrites in the rostrocaudal and medial-lateral directions

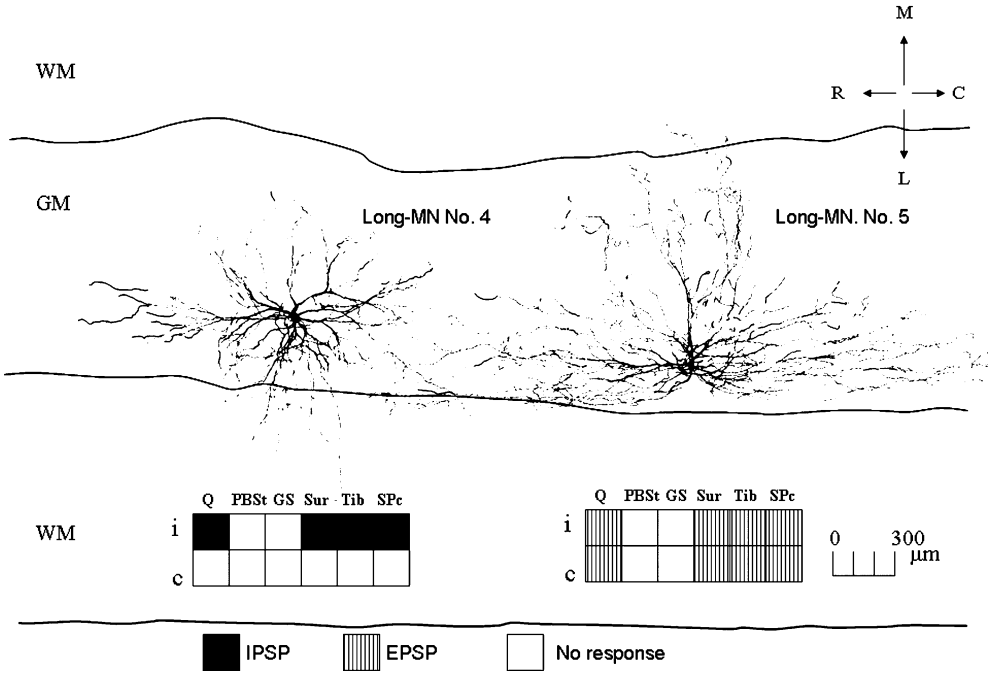

Fig. 1. Reconstruction of 2 Long Motoneurons in the horizontal plane. The effects of electrical stimulation on the hindlimb muscle and cutaneous nerves are shown in the lower panel. Note the Long Motoneuron somas located in the lateral area of the ventral horn and the differing dendritic distribution patterns among the motoneurons. WM, white matter; GM, gray matter; R, rostral; C, caudal; M, medial; L, lateral; i, ipsilateral; c, contralateral; Q, quadriceps femoris; PBSt, posterior biceps femoris and semitendinosus; GS, gastrocnemius; Sur, sural; Tib, tibialis; SPc, superior cutaneous.

and showed IPSPs after stimulation of hindlimb muscles and cutaneous afferent nerves on the ipsilateral side. Long motoneuron No. 4 distributed dendrites in the lateral direction out of the gray matter. Another Long motoneuron (No. 5) distributed dendrites mainly in the rostrocaudal and medial directions. This motoneuron showed EPSPs after stimulation of Q and cutaneous nerves on both sides. The alignment, branching, direction, and extension of dendrites differed among the Long Motoneurons. Furthermore, the effects of stimulation of hindlimb muscles and cutaneous nerves also varied among the Long Motoneurons. Table 1 shows the morphological characteristics of 13 Long Motoneurons, including the area of the cell body, the average diameter of the cell body, the number of primary dendrites, the extension of dendrites in different directions (rostrocaudal, medial-lateral, dorsoventral), the total length of dendrites and the length of dendrites in the white matter. The average diameter of the cell body ranged from 38.0 to 61.3 μm. Six to twelve primary dendrites originated from each cell body. The dendrites spread omnidirectionally. The average rostral extent was 991 μm, and the average caudal extent was 942 μm. The total rostral-caudal length varied from 1,254 to 3,133 μm. The average medial extent was 690 μm, and the average lateral extent was 572 μm. The total medial-lateral length varied from 804 to 1,792 μm. The average dorsal extent was 835 μm, and the average ventral extent was 665 μm. The total dorsal-ventral length varied from 800 to 2,600 μm. Dendrites were distributed not only in the gray matter but also in the white matter. Dendrites in the white matter possessed spine-like nodes in 8 out of 13 motoneurons. The total dendritic length ranged from 11,918 to 58,199 μm. The rate of dendrites in white matter varied between 2.2 and 43.1%. The morphological characteristics of the Long Motoneurons varied widely.

The relationship between the effects of hindlimb nerve stimulation and the morphological characteristics of motoneurons was examined. Table 2 shows the types of PSPs occurring after electrical stimulation of muscles and cutane-

Table 1. Morphological characteristics of Long Motoneurons

Neuron	Indicators of parameter										
No.	(1)	(2)	(3)	(4)*1	(4)*2	(4)*3	(4)*4	(4)*5	(4)*6	(5)	(6)
1	1,853	48.6	10	1,053	906	600	893	300	1,050	58,199.7	1,655.9
2	2,895	61.3	8	1,680	1,453	1,133	200	750	600	34,013.9	3,415.9
3	2,578	57.3	9	1,343	1,493	746	866	700	800	41,520.7	4,224.8
4	1,712	46.7	6	720	704	824	248	600	200	11,918.9	5,141.9
5	2,561	57.1	10	900	942	900	283	800	400	23,834.5	519.1
6	2,358	54.8	12	880	544	600	312	300	600	24,145.8	3,200.4
7	1,114	37.7	7	1,272	1,111	556	1,056	900	600	13,638.7	2,116.6
8	1,280	40.4	6	560	1,030	1,008	784	1,600	1,000	31,668.9	6,706.5
9	1,137	38.0	9	705	549	123	694	1,800	800	15,658.0	2,006.6
10	2,252	53.6	9	800	690	416	388	600	600	44,000.7	3,324.8
11	1,812	49.7									
12	2,501	56.4									
13	2,300	52.8									

(1) Soma area (μm^2); (2) average diameter (μm); (3) number of primary dendrites; (4)*1 (μm), (4)*2 (μm), (4)*3 (μm), (4)*4 (μm), (4)*5 (μm) and (4)*6 (μm), dendritic distribution ranges in the rostral, caudal, medial, lateral, dorsal and ventral directions from the nucleus; (5) total dendritic length (μm); (6) dendritic length in white matter (μm).

Table 2. PSP pattern after stimulation of hindlimb muscle and cutaneous nerves at 5T

No.	iQ	cQ	iPBSt	cPBSt	iGS	cGS	iSur	cSur	iTib	cTib	iSPc	cSPC
1	I	N	N	N	I	N	I	N	I	N	I	N
2	E	E	N	N	N	N	E	E	E	E	E	E
3	E	E	I	N	E	E	E	E	E	E	E	E
4	E	E	N	N	N	N	E	E	E	E	E	E
5	E	N	N	N	I	N	E	N	I	I	I	I
6	E	E	I	I	E	E	E	E	E	E	E	E
7	E	N	N	N	N	N	N	N	N	N	N	N
8	N	N	N	N	N	N	E	N	E	N	N	N
9	E	N	N	N	E	N	N	N	N	N	N	N
10	I	N	N	N	N	N	N	N	N	N	N	N
11	E	N	N	N	N	N	E	N	E	N	E	N
12	E	N	N	N	N	N	E	N	E	N	E	E
13	E	E	I	N	I	N	I	N	I	N	I	N

E: EPSP. I: IPSP. N: No response.

ous nerves with a low threshold (1.2–5 T) in each motoneuron. All recorded motoneurons showed responses to the peripheral inputs from the hindlimbs. The motoneurons were divided into 2 groups to study the relationship between morphological characteristics and responsiveness to afferent inputs from the hindlimbs. Group 1 motoneurons predominantly showed EPSPs or IPSPs; Group 2 motoneurons showed PSPs after stimulation from muscles of either both sides or one side. Statistically significant relationships were not observed ($P<0.05$).

The motoneurons described in the present study may represent only a small subpopulation of Long Motoneurons. A possibility for bias exists because we only recorded the large cells among the Long Motoneurons. The different hindlimb and neck muscles contain different types of muscle fibers, and the sizes of hindlimbs and neck muscle motoneurons vary [1, 3–5, 13, 17]. Carlson [6, 7] noted that Long Motoneurons include different types of motor units but mainly consist of fast units (Group 2 muscle fibers; more than 90%). The innervation ratio of each type of Long motoneuron has not been reported, though Carlson [6] seems to indicate that the Long motoneuron pool predominantly consists of larger motoneurons. This finding and the present experiment indicate that the diameters of the cell body of the studied Long Motoneurons were around 50 μm, corresponding to motoneurons innervating fast muscle fibers. Kernell and Zwaagstra [13] reported that the average soma diameter of slow and fast motoneurons in transverse sections was 39.5 ± 5.1 and 54.7 ± 6.9 μm, respectively. However, Rose [18] reported that motoneurons innervating BC and CM had a fusiform cell body, and that the cell body size in horizontal and sagittal sections ranged from 53 to 90 μm (mean, 75 μm). The average diameter of the Long Motoneurons in the present study was smaller than that of BC and CM motoneurons.

Dendrites of Long Motoneurons seem to be distributed multidirectionally and varied among motoneurons (Fig. 1, Table 1). The hindlimb and neck motoneurons located in the central part of the ventral horn have wide-ranging dendritic trees that spread over much of the ventral horn [2, 8, 9]. Rose [18] demonstrated the characteristics of the dendritic distribution of neck muscle motoneurons (biventer cervicis, complexus) of the rostrocaudal dendritic collection. Long rostrocaudal dendritic extensions are a common feature of lumbosacral motoneurons located in the dorsolateral part of the ventral horn [10, 14]. The relationship between the dendritic distribution pattern and the motoneuron soma location reported in cervical and lumbosacral motoneurons was not observed in the Long Motoneurons. Rose [18] and Vanner and Rose [19] showed that neck muscles receiving multisegmental innervations, such as erector spinae, extend their dendrites predominantly in the rostrocaudal direction. Long Motoneurons receive muscle and cutaneous afferent inputs from different body parts including the tail, the hindlimbs, the forelimbs and the trunk, at different levels [21, 22, 24, 25]. This might reflect a tendency for the dendritic extension range to be the greatest in the rostrocaudal direction. The distribution of dendrites in the white matter of the spinal cord was observed in all of the Long Motoneurons that were studied. This suggests that Long Motoneurons received inputs from the descending pathways. The most important role of the longissimus is to control COG, for which the inputs from the muscle and cutaneous afferents from various parts of the body and descending pathways are essential. The morphological characteristics of the Long Motoneurons shown in the present experiments could correspond to the functional characteristics of Long Motoneurons.

No significant relationship was observed between the morphological characteristics and the effects of hindlimb peripheral afferent inputs; however, there was a trend for the motoneurons that predominantly showed EPSPs to have greater dendritic distributions in the rostral direction and for the motoneurons receiving synaptic inputs from the hindlimb afferents from both sides to have a greater somatic area. Further study is therefore required to elucidate the relationship between morphological characteristics and function.

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