Rat motor neuron plasticity induced by dorsal rhizotomy

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Abstract

The control of peripheral structural plasticity of motor neurons by primary sensory neurons was studied in rat extensor digitorum longus (EDL) muscle. Polyinnervation of muscle fibers, sprouting and the motor neuron peripheral field size following L4 dorsal root cutting were evaluated using three different approaches: intracellular recording of end plate potentials, histochemical demonstration of sprouting and polyinnervation and in vivo recording of nerve-evoked twitch. Nodal sprouting was found in rhizotomized rats but not in controls and consistently muscle polyinnervation appeared. The muscle portion innervated by L3 ventral root was relatively reduced and that innervated by L5 was relatively enlarged: a trend to caudal shift of muscle innervation arose in rhizotomized rats. A control of motor neuron plasticity by primary sensory neurons is suggested. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

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During neonatal motor innervation development, the growing motor fibers sprout within the muscle and produce a redundant number of nerve endings with respect to the number of muscle fibers and therefore several nerve endings originating from the same or from different neurons converge on the same muscle fiber (polyinnervation). Afterwards, all nerve endings are withdrawn but one and the adult innervation ratio, one nerve ending per muscle fiber, is reached [11]. Plastic processes in motor innervation, that is sprouting and polyinnervation, occur again in the adult in different experimental and pathological conditions [3,5,8,15,16]. Sprouting may be induced by an altered activity of muscle that might release some trophic factors [9] or, alternatively, by nerve degeneration products [7]. There are suggestions that these local peripheral factors may be not the sole regulatory factors, but that the activity of the synapses afferent to motor neuron soma may affect neurite growth and arborization [5,6]. Primary sensory neurons project to motor neuron both directly and through interneurons.

The role of primary sensory neurons in controlling peripheral structural plasticity of motor neurons has been studied.

Male Sprague–Dawley rats were divided into two groups: controls and rhizotomized. Surgery was performed on rats deeply anesthetized with an intraperitoneal injection of sodium pentothal (45 mg/kg body weight). In 1-month-old rats hemilaminectomy was carried out at lumbar level, dura was opened to expose spinal roots and left fourth lumbar dorsal root (L4) was cut. A tract of dorsal root at least 2 mm long was removed to prevent nerve regeneration. Rats were killed 60 days after surgery by an overdose of sodium pentothal intracardially injected and it was verified that stumps of root were not reconnected. Age-matched rats which did not undergo any surgery were used as controls.

Motor innervation of extensor digitorum longus (EDL) muscle was studied by three different approaches. (1) To study muscle polyinnervation, intracellular recordings of excitatory post-synaptic potentials were carried out. Briefly, the left EDL muscle, together with a tract of peroneus nerve, was removed and put into a Ringer's solution. A mixture of O_2 (95%) and CO_2 (5%) was bubbled into the bath. Muscle cells of the anteromedial face were impaled with an intracellular micropipette filled with 3 M KCl (tip resistance 10–20 MΩ). 10^{-7}–10^{-6} g/ml (+)-tubocurarine was added to the bath, adjusting the concentration to prevent muscle action potentials. Excitatory post-synaptic potentials were evoked

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by peroneus nerve stimulation and polyinnervated muscle cells were recognized; muscle cells were regarded as multiply innervated when multiple or discretely larger postsynaptic potentials appeared when stimulus strength was increased. At least 60 cells per muscle were impaled. (2) The presence of sprouting and of polyinnervated muscle cells was demonstrated on muscle slices also by a histochemical technique (Fig. 1). EDL muscle was quickly removed and fixed for 30 min in formalin (10% v/v in Tris buffer, pH 7.2). The muscle was cut using a cryostat in 50-μm thick sections. A combined staining for the simultaneous demonstration of motor nerve endings and endplate areas was used. This technique employs 5-bromoindoxyl acetate as a substrate for acetylcholinesterase and silver-gold impregnation for nerve terminals. The occurrence of terminal and nodal sprouts was observed. Sprout arising where axon loses the myelin sheath near the endplate was considered 'terminal sprout'; sprout leaving the parent axon from node of Ranvier was regarded as 'nodal sprout' [2]. (3) In vivo tension recording experiments were carried out on rats deeply anestethized by sodium pentothal intraperitoneally injected [5]. EDL muscle and ventral roots were exposed and the distal tendon was connected to a tension transducer. EDL muscle contractions were obtained by stimulating L3, L4 or L5 ventral roots, peroneus nerve and, finally, by directly stimulating EDL muscle in order to evaluate the contribution of each ventral root to muscle contraction. Twitch amplitude, latency from the stimulus to the beginning of contraction, rise time and half decay time were measured.

In intracellular recording experiments, no muscle cell of control rats ($n = 6$) was found to be multiply innervated. In rats with L4 dorsal rhizotomy $15.8 \pm 7.13\%$ ($n = 4$) of muscle cells were polyinnervated (Student's t-test: $P < 0.05$).

Results obtained from histochemistry were consistent with electrophysiological data. Multiple innervation was found in some muscle cells of rhizotomized rats, but not in controls. No terminal sprouts were found both in control and in rhizotomized rats. Sprouts arising from nodes of Ranvier (nodal sprouts) were found only in rhizotomized rats (Fig. 1). Sprouts were thin and scarcely myelinated or, alternatively, similar to the parent axons, possibly depending on the birth time; sometimes more than one sprout was seen to arise from the same node of Ranvier.

In vivo tension recording showed that direct and peroneus nerve-evoked EDL muscle contraction of rhizotomized rats was similar to those of control rats in all the parameters considered, showing that neither muscle atrophy nor innervation loss occurred. When twitch was evoked by stimulating L4 ventral root, an increase in latency was observed in rhizotomized rats; latency of twitch evoked by L3 and L5 ventral roots were not significantly different from those of controls, but in one animal the twitch obtained by L3 ventral root stimulation showed a very long latency (15 ms) and a similar result was obtained by L5 ventral root stimulation
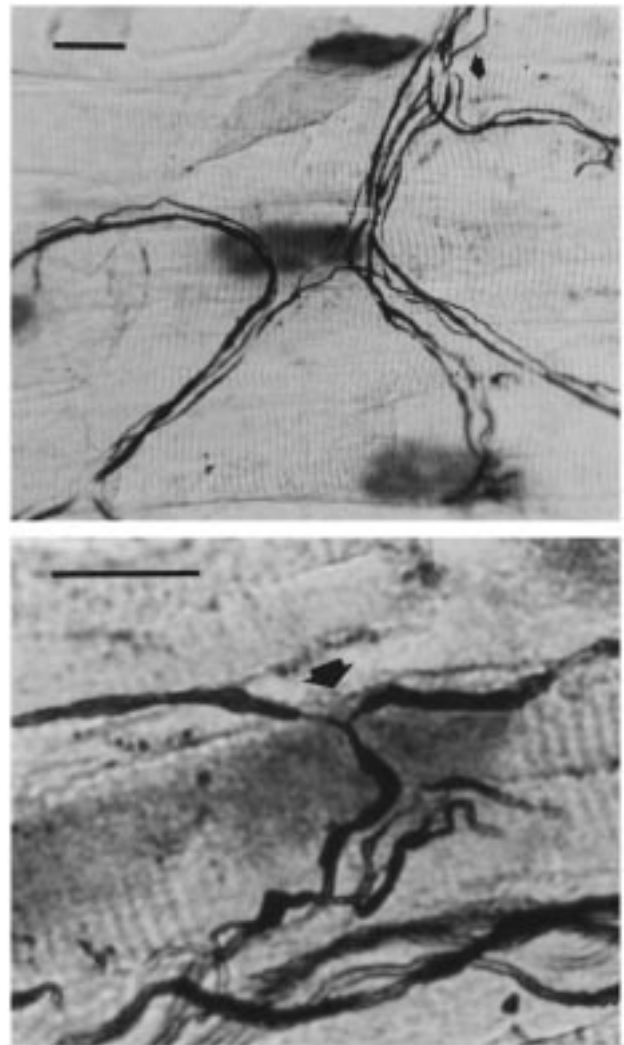

Fig. 1. Sections of extensor digitorum longus muscle of rhizotomized rat; neuromuscular junctions and axons were stained by a histochemical technique. Nodal sprouts are visible (arrows). Scale bar, 20 μm.

(20 ms) of another rat. The duration of twitch obtained by L5 ventral root stimulation was markedly increased in rhizotomized animals, while the durations of twitches evoked by L3 and L4 ventral roots were similar to that of controls (Table 1). The relative contribution of each root to muscle contraction expressed as (tension evoked by ventral root stimulation)/(tension evoked by peroneus nerve stimulation) ratio was determined. When this ratio was normalized to the contralateral (unoperated) side, a decrease in relative contribution of L3 and an increase in the relative contribution of L5 ventral roots were evident in rhizotomized rats (Fig. 2).

The present findings show that dorsal rhizotomy induces rearrangement of muscle innervation. The rearrangement consists in nodal sprouting by motor axons and consequent polyinnervation of muscle fibers. When sprouting occurs, a slowing in the duration of twitch may be expected due to the

Table 1
Time course of EDL muscle contractions[a]

	Latency	Rise time	Half decay time	Total duration*
Controls				
L3	8.1 ± 0.75	23.79 ± 1.58	15.23 ± 1.08	39.02 ± 2.32
	$n = 5$	$n = 6$	$n = 6$	$n = 6$
L4	6.1 ± 0.08	27.54 ± 1.45	12.19 ± 0.52	39.73 ± 1.72
	$n = 9$	$n = 9$	$n = 9$	$n = 9$
L5	6.5 ± 0.20	14.50 ± 4.99	8.87 ± 2.35	23.37 ± 7.04
	$n = 4$	$n = 4$	$n = 4$	$n = 4$
Rhizotomized				
L3	11.2 ± 1.90	26.08 ± 2.89	19.44 ± 2.12	47.53 ± 3.63
	$n = 4$	$n = 5$	$n = 5$	$n = 5$
L4	7.0 ± 0.25***	29.86 ± 1.71	17.69 ± 3.74	47.33 ± 4.70
	$n = 9$	$n = 9$	$n = 9$	$n = 9$
L5	9.7 ± 1.52	27.61 ± 2.42**	21.29 ± 3.76	50.32 ± 6.21**
	$n = 8$	$n = 7$	$n = 7$	$n = 7$

[a] All parameters are expressed in milliseconds. *Sum of rise time and half decay time. Student's t-test: **$P < 0.05$ and ***$P < 0.005$ with respect to the corresponding controls.

presumptive low conduction rate of sprouts and to the consequent late components of contraction. Consistently, the duration of twitch evoked by L5 ventral root was slower than that of controls. The duration of twitch evoked by L3 and by L4 ventral roots was not significantly different from that of the controls. This suggests that the caudal motor neurons might be more susceptible in giving place to sprouting following dorsal rhizotomy, possibly enlarging their projection field by sprouting. This suggestion, together with the decrease of contribution of L3 ventral root, indicates a trend toward a caudal shift of motor innervation following dorsal rhizotomy. An analogous enlargement of the peripheral projection field of caudal segments innervating EDL muscle has been found to spontaneously occur in non-operated rats with increasing age [4]. Therefore, dorsal rhizotomy seems to make a spontaneously occurring age-related plastic process faster.

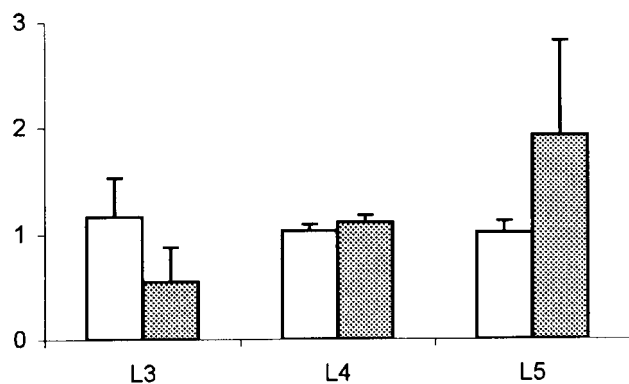How may dorsal rhizotomy induce plasticity at the

Fig. 2. Relative contribution of each single ventral root (L3, L4 and L5) to muscle contraction (see text). White columns: controls; gray columns: rhizotomized. Two-way ANOVA test shows that rhizotomy differently affects the contribution of the three ventral roots (interaction: $F = 5.03$; $P < 0.025$).

periphery of motor neuron? Two possible speculative views may be proposed at the peripheral or central level, respectively. First, the loss of spinal afferents is likely to alter the activity pattern of motor neurons that are directly or indirectly connected to them. If motor neuron activity changes, muscle activity also changes. Changes in electrical and/or mechanical muscle activity are known to induce sprouting and polyinnervation, probably through paracrine interactions [9,10]. This has been demonstrated by several authors and is probably also true in the present case.

However local peripheral interaction is not sufficient to explain the different behavior of the three spinal segments. In fact no distinction is possible at the periphery among neurons with a different spinal location. Therefore we propose that a central direct mechanism controlling motor neuron plasticity by primary sensory afferents is also involved. This idea is supported by three considerations. (1) The synaptic input onto motor neurons is likely to change substantially following dorsal rhizotomy because of loss of sensory-motor direct synapses, due to change in the activity pattern of interneurons interposed between primary sensory neuron spinal nerve endings and motor neurons and because of intraspinal lesion-induced plasticity [14,17]. (2) Many synapses onto motor neurons are glutamatergic and, in particular, synapses mediating the monosynaptic reflex [12]. (3) Glutamate receptor activation was shown to control structural neuron plasticity both in vitro [6] and in vivo [13], possibly through intracellular $[Ca^{2+}]$ transients [1]. A central mechanism allows an individual and, in principle, differentiated control of motor neuron plasticity.

[1] Cambray-Deakin, M.A. and Burgoyne, R.D., Intracellular Ca^{2+} and N-methyl-D-aspartate stimulated neuritogenesis in rat cerebellar granule cell culture. Dev. Brain Res., 66 (1992) 25–32.

[2] Cuppini, R., Cecchini, T., Ciaroni, S., Ambrogini, P. and Del Grande, P., Nodal and terminal sprouting by regenerating

nerve in vitamin E-deficient rats. J. Neurol. Sci., 117 (1993) 61–67.

[3] Cuppini, R., Age-related changes in maturation of regenerated motor innervation. J. Neurol. Sci., 114 (1993) 99–103.

[4] Cuppini, R. and Ambrogini, P., Developmental changes in innervation of rat extensor digitorum longus muscle. Mech. Ageing Dev., 92 (1996) 211–225.

[5] Cuppini, R., Ambrogini, P. and Sartini, S., Enlargement of motor neuron peripheral field following partial denervation with or without dorsal rhizotomy. Neuroscience, 84 (1998) 151–161.

[6] Cuppini, R., Sartini, S., Ambrogini, P., Falcieri, E., Maltarello, M.C. and Gallo, G., Control of neuron outgrowth by NMDA receptors. J. Submicr. Cytol. Pathol., 31 (1999) 31–40.

[7] Diaz, J. and Pecot-Dechavassine, M., Nerve sprouting induced by a piece of peripheral nerve placed over a normally innervated frog muscle. J. Physiol., 421 (1990) 123–133.

[8] Dixon, R.W. and Harris, J.B., Nerve terminal damage by beta-bungarotoxin: its clinical significance. Am. J. Pathol., 154 (1999) 447–455.

[9] Funakoshi, H., Belluardo, N., Arenas, E., Yamamoto, Y., Casabona, A., Persson, H. and Ibanez, C.F., Muscle-derived neurotrophin-4 as an activity dependent trophic signal for adult motor neurons. Science, 268 (1995) 1495–1499.

[10] Herrera, A.A. and Werle, M.J., Mechanisms of elimination, remodeling and competition at frog neuromuscular junction. J. Neurobiol., 21 (1990) 73–98.

[11] Jansen, J.K.S. and Fladby, T., The perinatal reorganization of the innervation of skeletal muscle in mammals. Prog. Neurobiol., 34 (1990) 39–90.

[12] Jiang, Z.G., Shen, E. and Dun, N.J., Excitatory and inhibitory transmission from dorsal root afferents to neonate rat motor neurons in vitro. Brain Res., 535 (1990) 110–118.

[13] Kaczmarek, L., Kossut, M. and Skangel Kramska, J., Glutamate receptors in cortical plasticity: molecular and cellular biology. Physiol. Rev., 77 (1997) 217–255.

[14] Murrey, M. and Goldberger, M.E., Replacement of synaptic terminals in lamina II and clarke's nucleus after unilateral lumbosacral dorsal rhizotomy in adult cats. J. Neurosci., 6 (1986) 3205–3217.

[15] Pachter, B.R. and Spielholz, N.I., Tenotomy-induced motor end plate alterations in rat soleus muscle. Anat. Rec., 228 (1990) 104–108.

[16] Tsujimoto, T., Umemiya, M. and Kuno, M., Terminal sprouting is not responsible for enhanced transmitter release at disused neuromuscular junction of the rat. J. Neurosci., 10 (1990) 2059–2065.

[17] Wang, D.-S., Goldberger, M.E. and Murray, M., Plasticity of spinal system after unilateral lumbosacral dorsal rhizotomy in the adult rat. J. Comp. Neurol., 304 (1991) 555–568.