Morphofunctional Basis for Recovery of Locomotor Movements in Rats with Completely Crossed Spinal Cord

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Treadmill training of spinalized rats creates conditions for early appearance of rhythmic locomotor movements of the hind limbs. Recovery of the movements was paralleled by an appropriate structural organization of neurons in the anterior horns of the distal compartment of the spinal cord.

Key Words: *rat; spinal cord; injury; treadmill training; motor neurons*

The aim of this study was to evaluate the possibility of locomotion recovery after complete crossing of the spinal cord (spinalization). The hypothesis on the possibility of activation of rehabilitation of the locomotor function is based on the concept about the existence of a step movement generator in the spinal cord. The presence of this generator is confirmed by the data demonstrating that application of neurochemical agents onto the rostral and caudal compartments of the lumbar portion of the spinal cord in rats induces false locomotion [3]. Moreover, rhythmic locomotor-like activity was recorded in the preparations of the spinal cord and brain stem of newborn rats [12].

In recent years treadmill training was used for locomotion recovery in chronic partially or completely spinalized cats [11].

Experiments performed by Edgerton *et al.* [7] are most close to our studies by their contents and purposes; these scientists showed that the locomotor function and body weight support function were restored in rats after complete crossing of the spinal cord in the middle thoracic segment due to treadmill training. In these studies 5-day-old rats were spinalized, and in this case rehabilitation could be due to natural regenerative potentialities of a growing organism. It is known that sprouting is extremely limited in damaged adult spinal cord because of glial microenvironment of the mature brain and inhibitory effects of the molecules connected to the myelin [2]. Some anatomical and functional connections of damaged spinal cord can be partially restored by neurotransplantation or drugs blocking the neuronal process growth inhibitors [1,5, 6,10]. Modern scientists consider that the reserves of the spinal cord are sufficiently high: voluntary movements of the limbs can be to a certain measure restored even by just 10% of the descending spinal tract fibers left after the injury [4]. However, the mechanisms of plasticity in the damaged spinal cord are little studied. The medical and social significance of these studies prompted us to clear out the role of long training in the recovery of the hind paw movements in spinalized rats and the morphofunctional changes in the spinal cord associated with this recovery.

MATERIALS AND METHODS

Experiments were carried out on adult female Sprague-Dawley rats (200-220 g) under narcosis (Nembutal 40 mg/kg intraperitoneally). After dissection of soft tissues, laminectomy at the level of thoracic vertebrae 9-11 (Th 9-11) was carried out on the dorsal side of the spinal cord. The spinal cord was completely cros-

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sed. Bleeding was arrested and the wound was sutured. The animals were placed into warmed cages (1 per cage). On days 1-5 postoperation the rats were injected with gentamicin twice daily (40 mg/kg intramuscularly) and the urinary bladder was emptied by pressing the abdominal wall. Later these measures were carried out if necessary. The animals were kept with free access to water and food.

Experimental group consisted of 7 animals, which started treadmill (band velocity about 9 m/sec) training 1 day after the operation. The training was carried out 5 times a week during the first half of the day. The duration of training sessions increased from 30 sec (during the first days) to 10 min (usually starting from the 3rd week postoperation). During the first days after spinalization the animals were supported at the base of the tail. Controls (*n*=5) were not trained after spinalization.

Rats of both groups were weekly tested over the course of the entire experiment (usually 9 weeks): the animals were weighed, the length of voluntary (without support) running on the treadmill was measured, and locomotor activity was evaluated. The motor activity of the hind limbs (each paw separately) was evaluated. The recovery of motor activity was scored using 5-point scale (0 points: absolutely immobilized relaxed paws; 1 point: movements of both limbs during stimulation of the perineum; 2 points: immobile paw with the femur constantly drawn to the trunk; 3 points: the femur is drawn from the trunk, some movements of all joints; 4 points: rhythmic step-like movements of both limbs after stimulation of the sole or tail base; 5 points: jumping movements during running on the treadmill).

Morphological study was carried out 9 weeks after spinalization of experimental and control animals. After anesthesia with ether vapor, the thoracic and lumbar portions of the spinal cord were isolated from experimental and control animals, fixed in Bouin's fluid, and embedded in paraffin. Longitudinal and transverse 10-μ sections were made and stained with toluidine blue (by Niessle's method), the cicatrix, distal and proximal ends of the crossed spinal cord were examined under a photooptic microscope.

The results were obtained on animals in which the spinal cord was crossed at the Th 9-11 level (accor-

TABLE 1. Time Course of Recovery of Motor Functions of Hind Paws in Different Periods after Spinalization in Rats

Group, period of observation	Activity of hind paws, points											
	left						right					
	0	1	2	3	4	5	0	1	2	3	4	5
Control (*n*=5)												
week 1	5							5				
week 2	5						5					
week 3	3	1	1				3	1	1			
week 4	2	1	1	1			3	1	1			
week 5	2	1	1				3	1				
week 6	2		2				2		2			
week 7	1	1	1	1			1	1	1	1		
week 8			3	1				1	2	1		
week 9			3			1		2	1	1		
Experiment (*n*=7)												
week 1	6	1					6	1				
week 2*	2	1	4				2	1	4			
week 3**	2		2	2	1		2	1	2	1	1	
week 4**	1	1		2	1		1		1	2	1	
week 5***			1	1	2		1			1	2	
week 6***			1		2	1	1			1	2	
week 9****			1		2				1		1	1
week 8****			1		1	1			1		1	1
week 9			1		1	1			1		1	1

Note. *n*: number of animals. **p*<0.01, ***p*<0.02, ****p*<0.05, **p*<0.1 compared to the control.

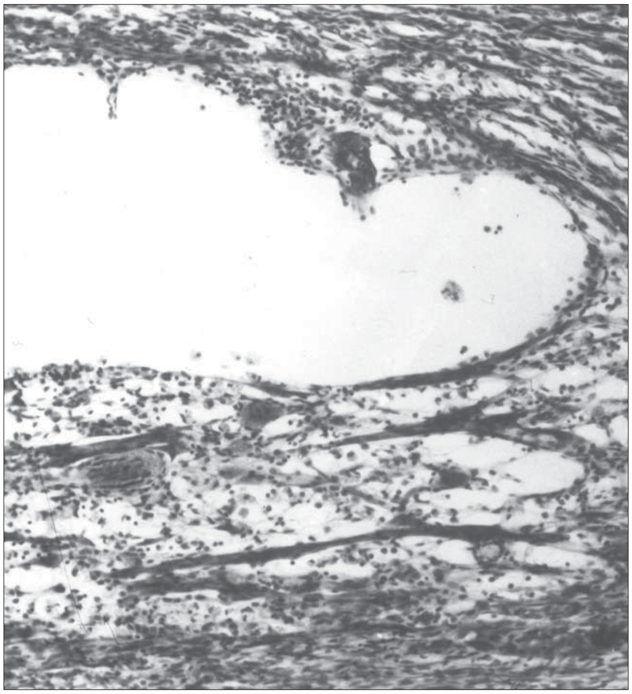

Fig. 1. Cicatrix at the site of spinal cord crossing 9 weeks after surgery, ×200.

ding to morphological data) and the length of the cicatrix was at least one segment, which fully confirmed isolation of the distal portion of the spinal cord.

The results were statistically processed using bilateral Mann—Whitney's *U* test.

RESULTS

The animals were weighed for evaluation of their physical status. The weights of experimental rats were greater, which seemed to be due to rapid increment of muscle weight because of training.

Locomotor activity of experimental rats was higher than in the controls during the entire period of observation.

Movements of the hind paws were observed in both groups (Table 1), but the velocity and volume of motor activity recovery differed. The differences between the groups were significant during weeks 2-8 after spinalization. In the controls the hind paws remained immobile (0 points) until week 7 after spinalization, *vs.* only until week 4 in the experimental group. The types of movements were limited in control rats: reflectory movements of viscerosomatic nature of both limbs after stimulation of the perineum (1 point); activation of the femoral flexors of the hind paw drawn to the trunk (2 points). Separate voluntary movements of all joints of the hind paws (3 points) were observed in only 1 rat during 2 last weeks of observation. The same rat exhibited jumping movements of the left paw during treadmill testing (5 points) during the last week of the experiment. In experimental rats the same types of movements appeared earlier and were sooner replaced by more complex types. Coordinated step-wise movements of both limbs (4 points) were observed only in rats trained on the treadmill. The body supporting function was not restored in rats of both groups.

A coarse glio-connective tissue cicatrix formed at the site of spinal cord crossing in all rats of both groups 9 weeks after the intervention (Fig. 1). This cicatrix occupied at least 1 segment of the spinal cord and consisted of Schwann cells, astrocytes of numerous fibroblasts, and collagen fibers with cavities and

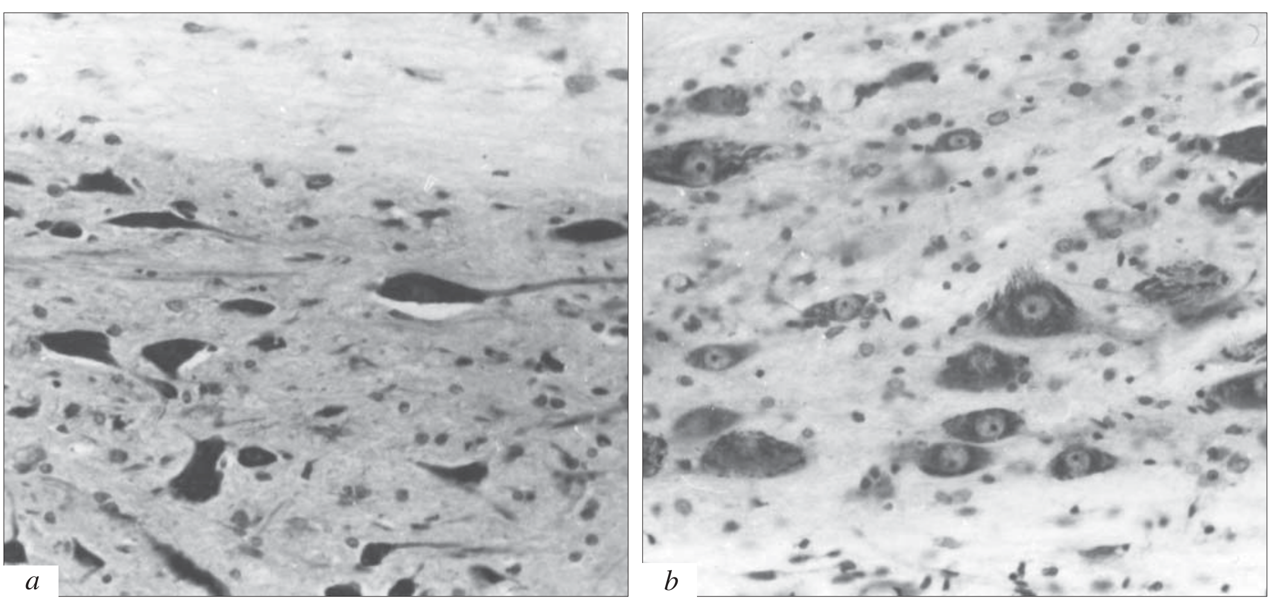

Fig. 2. Neurons of the anterior horns 9 weeks after crossing, ×200. *a*) no treadmill training (control group); *b*) after treadmill training (experimental group).

cysts of different size seen in them. Accumulations of lymphocytes were observed in some preparations.

Morphological study of the distal portion of crossed spinal cord showed elongated narrowed motoneurons with angular contour and long processes in untrained control animals (Fig. 2, *a*). Normally round convex contours of the neurons became angular. The cells drastically shrank. Their nuclei and cytoplasm were hyperchromatophilic with poorly discernible nuclei because of intense staining of the cell in the majority of cases, and in some cases the nuclei were not seen. Such a morphological picture is considered as a sign of cell shrinking and sclerosis. Neurons with pronounced sclerosis were considered not viable, but cells in the last stage of shrinkage can be present in tissue for a long time.

In experimental animals the neurons of the spinal anterior horns remained viable. They were much larger than in controls. The cell contours were round, typical of spinal neurons in intact rats. The nuclei were clear, situated in the center of the cells, and well seen. The Niessle substance in the cytoplasm was more rough than normally, and some of its granules were not seen (Fig. 2, *b*).

Our data indicate that the distal portion of the spinal cord possesses mechanisms capable of inducing the locomotor activity after injury. Treadmill training promotes recovery of the locomotor functions of the isolated distal portion of the spinal cord, which is in line with morphologically intact structure of its motoneurons. It seems that this phenomenon can be observed only after complete isolation of the distal end of the spinal cord, as recent studies on rats demonstrated that regular training after partial damage of the spinal cord did not modify its locomotor activity [8]. Locomotor movements in the hind paws are poorly restored without regulatory flow of afferent pulsation in the distal part of the spine. Presumably, motor training is associated with afferent stimulation of the isolated portion of the spine through activation of the proprioceptive and tactile systems. The general motor activity in trained rats was higher than in controls, which can be explained, among other things, by the production of some neuropeptides regulating the functional and morphological adaptation [9].

Hence, our findings prove that prolonged treadmill training of spinalized rats creates conditions for restoration of the hind paw movements and ensure the preservation of the structural organization of motoneurons in the distal segment of the spinal cord.

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