Effect of Locomotor Activity on Ultrastructure of Cerebellar Neurons, Neurological Disturbances, and Survival of Krushinsky–Molodkina Rats with Hemorrhagic Stroke

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We studied the effect of locomotor activity on the ultrastructure of cerebellar neurons, neurological disturbances, and survival rate in Krushinsky–Molodkina rats during the development of hemorrhagic induced by acoustic stress. In animals with high spontaneous locomotor activity, severe edema of cerebellar neurons (resulting in the destruction of surrounding structures) and swelling of the synapses (terminals of mossy fibers on granule cell dendrites) were observed. By contrast, the areas of intracerebral, subdural, and subarachnoid hemorrhages were lower in rats under conditions of forced rest.

Key Words: *cerebellum; spontaneous locomotor activity; neuron; nitric oxide; sodium nitrite*

The brain requires constant blood flow for the supply with oxygen and glucose. The impairment or local arrest of blood circulation is followed by severe brain injury (strokes) [2]. Experimental studies on the model of strokes have been performed over the past decades. This approach is suitable for evaluation of biochemical, biophysical, and morphological characteristics of the brain [1,3-5,14]. We have previously studied the effect of glutamate (Glu) and NO-generating compound ($NaNO_2$) on the ultrastructure of frog cerebellar neurons (stroke model) [11-13]. The present work on Krushinsky–Molodkina rats with the genetic predisposition to audiogenic epilepsy and hemorrhagic strokes is a continuation of studies on stroke models [3-7,12,13,15].

Hemorrhagic stroke (intracerebral hemorrhage) usually develops under conditions of BP elevation or essential hypertension. This state results from vascular rupture against the background of disturbed autoregulation of cerebral blood flow. Neurological disorders are typical of strokes [2,3-9]. Patients and experimental animals with strokes are characterized by the increase in spontaneous locomotor activity (SLA). Activation of blood flow and increased shear stress under conditions of SLA can contribute to an increase in the content of endothelium-derived relaxation factor (nitric oxide, NO) and decrease in BP [1,2,8]. Thus, increased content of NO in the endothelium can be a favorable factor, while an increase in NO content in the brain is an undesirable event.

Here we studied the ultrastructure of cerebellar neurons, neurological disturbances, and survival of Krushinsky–Molodkina rats with hemorrhagic stroke caused by acoustic stress.

MATERIALS AND METHODS

Experiments were performed on 77 male Krushinsky–Molodkina rats aging 4-4.5 months. Control group 1 consisted of intact animals (n=13) that were not exposed to acoustic stress. Control group 2 animals (n=32) were exposed to acoustic stress under

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forced rest (FR) conditions in Plexiglas tubes. They heard sounds due to special holes for the ears, but could not move freely. Group 3 animals (n=32) with SLA could move freely in the cage during acoustic stress.

Acoustic stimulation was delivered as described elsewhere [5]. The animals were initially exposed to the influence of a strong electric bell (110-115 dB) for 1.5 min. Then a series of strong and weak acoustic signals (80 dB, duration 10 sec) was delivered at 10-sec intervals. This exposure lasted for 15 min and was followed by a 3-min interval. In the follow-up period, the rats were subjected to strong acoustic stimulation for 1 min. The latency and severity of seizure in animals of the control and treatment groups were evaluated during acoustic stimulation. These parameters characterize the excitability of CNS. Moreover, we studied the survival rate and severity of locomotor disorders in animals during acoustic stress. The severity of disorders was classified as follows: mild disorders (insignificant disturbances of muscle tone not limiting animal's movement); moderate disorders (paresis of the limbs, particularly of the hindlimbs, that makes the movement difficult); and severe disorders (loss of the ability to move). The area of subdural and visible subarachnoid hemorrhages was measured with a binocular micrometer [4-7]. The presence or absence of intraventricular hemorrhages was estimated in transverse brain slices (1-2 mm in thickness) [4]. All experiments were performed in winter. The animals were decapitated in accordance with the "Rules of Studies on Experimental Animals" (USSR Ministry of Health, Order No. 775, 12.08.1977).

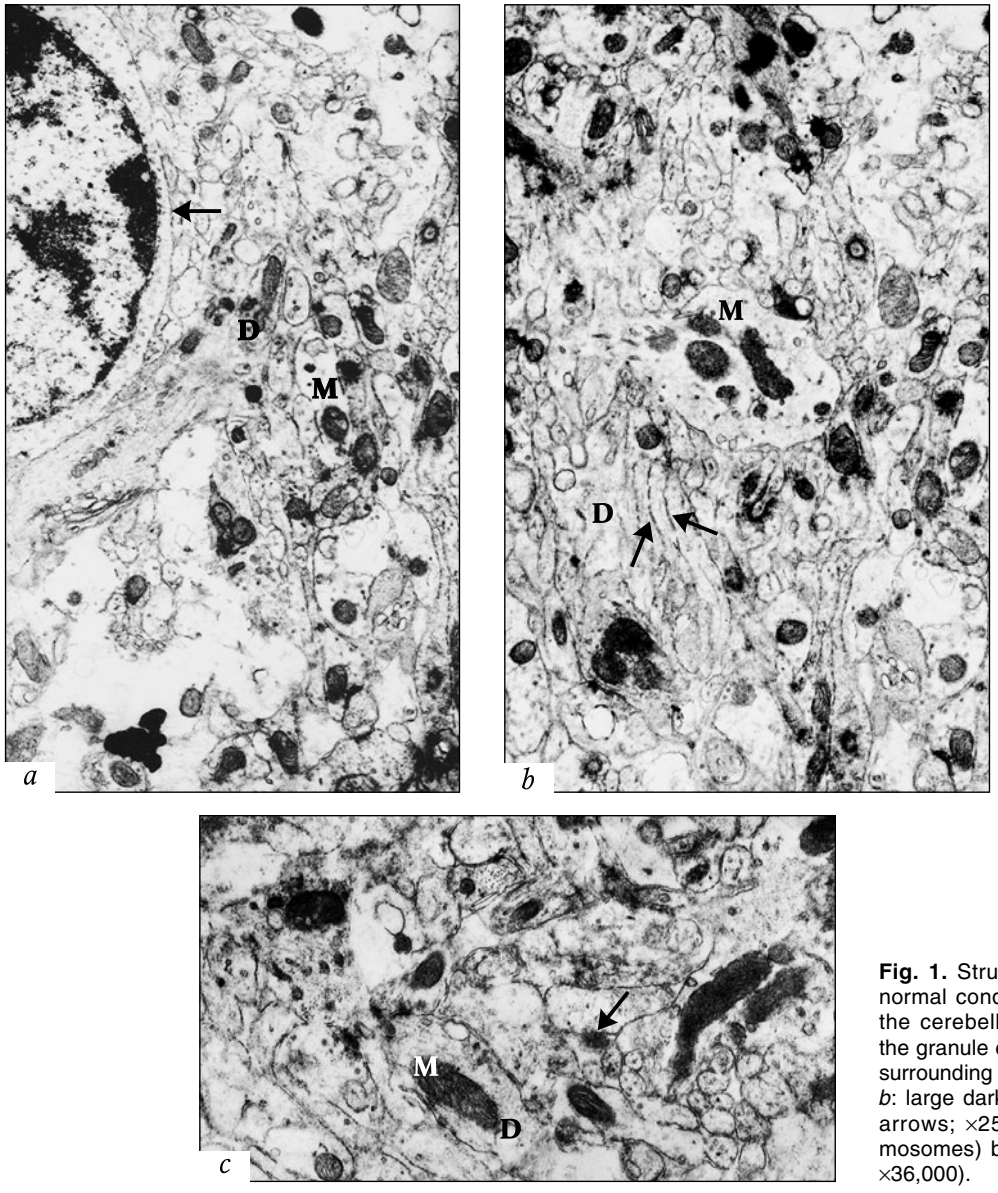

Fig. 1. Structure of the rat cerebellum under normal conditions. *a*: lower molecular layer of the cerebellum (supragranular layer): part of the granule cell and part of the nucleus (arrow); surrounding dendrites of granule cells (×25,000). *b*: large dark mitochondria (M) in dendrites (D, arrows; ×25,000). *c*: cell-cell contacts (desmosomes) between the dendritic walls (arrow, ×36,000).

The samples for electron microscopy were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (0.2% tannin and 0.3% sucrose, pH 7.2-7.4) at 4°C for 1 h and postfixed in 1% OsO_4 in the same buffer for 1 h [10]. The samples were dehydrated with ethyl alcohol in increasing concentrations, absolute alcohol, and acetone and embedded into a mixture of Epon and araldite. The sections were contrasted with uranyl acetate and lead citrate and examined under a JEM-100SX electron microscope (accelerating voltage 90 kV). The results were analyzed by Wilcoxon test and Fisher's test.

RESULTS

We examined the supragranular layer of the cerebellum. Cerebellar neurons in intact Krushinsky–Molodkina rats were well preserved (Fig. 1) despite enhanced NO synthesis (in comparison with Wistar and outbred rats).

Swelling of the cerebellar dendrites and increase in the diameter of these structures (228.2±13.8 nm, by 1.4 times; $p<0.001$ compared to the control) were observed in FR animals after acoustic stress (Fig. 2, *a*). A swollen dendrite that comes in contact with the ter-

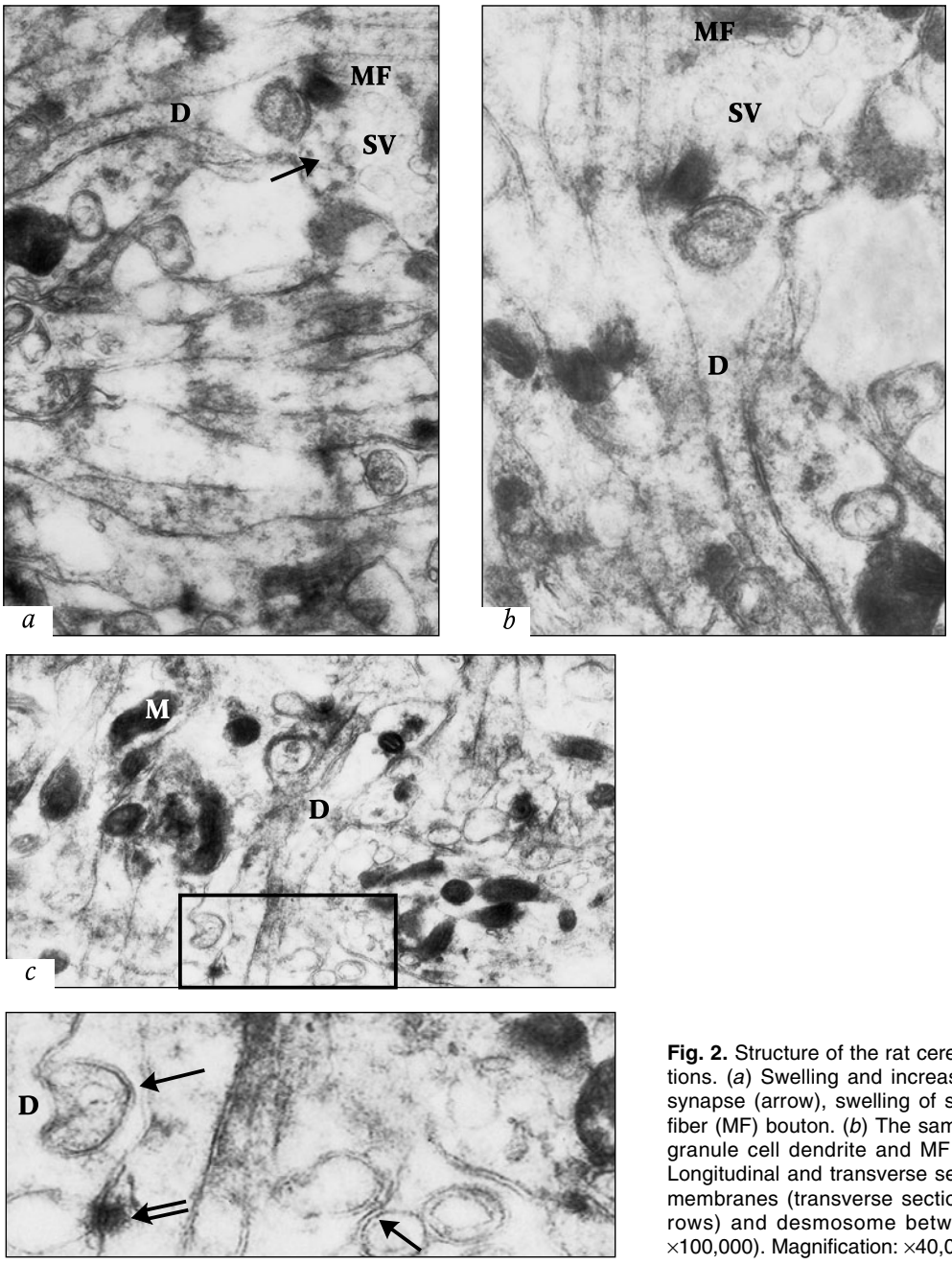

Fig. 2. Structure of the rat cerebellum after stroke under FR conditions. (*a*) Swelling and increase in the diameter of dendrites (D); synapse (arrow), swelling of synaptic vesicles (SV) in the mossy fiber (MF) bouton. (*b*) The same synapse at greater magnification: granule cell dendrite and MF bouton; SP appear as ghosts. (*c*) Longitudinal and transverse sections of dendrites. Insert: dendritic membranes (transverse section); well-defined dense deposit (arrows) and desmosome between two dendrites (double arrow, ×100,000). Magnification: ×40,000 (*a*), ×80,000 (*b*), and ×50,000 (*c*).

TABLE 1. Effect of SLA and FR on the Incidence of Neurological Disorders, Mortality Rate, Area of Hemorrhages, and Incidence of Hemorrhages in Krushinsky–Molodkina Rats during Acoustic Stress

Condition	Incidence of motor disorders, %			Mortality rate, %	Latency, sec	Hematoma, mm^2	Incidence of intraventricular hemorrhages, %
	mild	severe	heavy				
SLA (n=32)	3.1±3.1	25.0±7.7	71.9±7.9	18.8±6.9	3.8±0.3	57.30±14.09	100
FR (n=32)	87.5±5.8	12.5±5.8	0	0	N.d.	10.3±3.9	46.2±10.0
Significance	p<0.001	N.d.	p<0.001	p<0.01	—	p<0.01	p<0.001

Note. N.d., not determined.

minal (bouton) of the mossy fiber is well distinguished on the longitudinal section. Synaptic vesicles are significantly enlarged (114 *vs*. 40-50 nm in the control). These structures look like ghosts (Fig. 2, *a*, *c*, insert). Besides swelling, the presence of a dense deposit on the inner side of the dendritic membrane was typical of these animals. It is well defined on longitudinal sections (Fig. 2, *c*, insert). According to modern notions [14], the appearance of deposit on the membrane is related to disturbances in autophagy that plays the major role in elimination of protein aggregates and organelles from neurons after cerebral ischemia.

Ultrastructural changes in the cerebellum were most significant in animals with SLA during acoustic stress. These movements were usually accompanied by running and jumping, which required increased energy supply. An injury to mossy fiber boutons and destruction of nervous structures in the cerebellum were well defined in the supragranular layer (Fig. 3). Dendritic damage in rats made it impossible to measure the diameter of these structures (not shown in Table 1).

Studying the severity of neurological disorders showed that the degree of stress-induced changes in animals exposed to acoustic stress under FR conditions is lower than in rats from the SLA group (Table 1). For example, FR animals were characterized by a low incidence of severe motor dysfunction (p<0.001). The majority of these rats had mild disturbances of movements (p<0.001). Moreover, the mortality rate of FR animals was significantly reduced (Table 1). The area of subdural and visible subarachnoid hemorrhages in FR rats was 5.6-fold lower than in control animals (Table 1). The incidence of intraventricular hemorrhages in FR rats was much lower than in SLA animals (p<0.001).

Intracerebral hemorrhage is a common type of hemorrhagic stroke that occurs more often in middle-age and elderly people, as well as in patients with hypertension and/or cerebral atherosclerosis. Our study was performed on adult rats with experimental hemorrhagic stroke. Adult animals were pre-tested

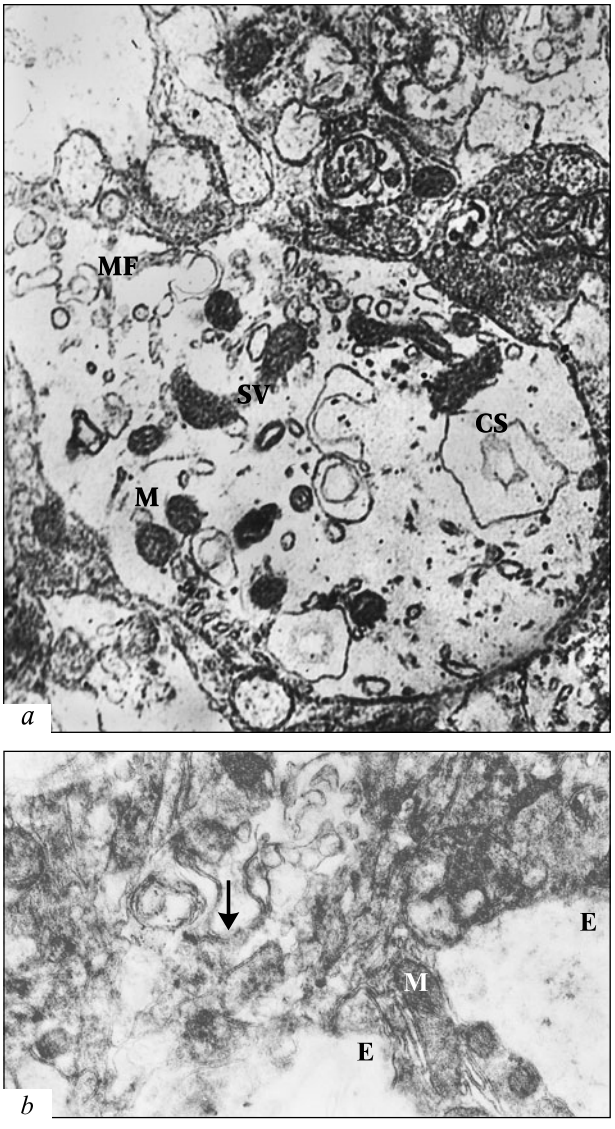

Fig. 3. Structure of the rat cerebellum after hemorrhagic stroke under SLA conditions. *a*: damaged bouton of the mossy fiber (MF); diffuse cytoplasmic structures (CS); synaptic vesicles (SV) compressed into a lump; destructed mitochondria (M, ×45,000). *b*: total destruction of the cerebellar nerve network due to edema, E); remaining damaged elements of the nerve network, cell membranes (arrow) and mitochondria (M, ×30,000).

for the severity of seizures (epilepsy). Further experiments were conducted on animals with the greatest severity of seizure activity (according to the standard 4-point scale). Previous studies showed that Krushinsky–Molodkina rats are characterized by epileptic attacks and hemorrhagic stroke under conditions of acoustic stress [5,7]. Seizure activity in these rats during hemorrhagic stroke serves as a factor that increases the severity of intracerebral hemorrhage. It is manifested in an increase in the area of subdural and subarachnoid hemorrhages. It should be emphasized that SLA contributes to severe edema of the cerebellar neuronal network, which destructs the surrounding structures. This state is also manifested in swelling of the synapses (terminals of mossy fibers on granule cell dendrites). By contrast, the area of intracerebral hemorrhage, subdural hemorrhages, and subarachnoid hemorrhages are reduced under FR conditions. However, the animals subjected to acoustic stress under FR conditions are characterized by dendritic swelling and 1.5-fold increase in the diameter of these structures (as compared to intact rats, 162.9±7.9 nm).

The influence of SLA on the course of hemorrhagic stroke, survival of rats with stroke, neurological disturbances in the brain, and ultrastructural changes in cerebellar neurons during acoustic stress is probably associated with an increase in NO concentration in the brain. Previous experiments revealed that selective inhibitors of inducible and neuronal NO synthases (aminoguanidine and 7-nitroindazole) significantly reduce the mortality rate, degree of motor disorders, and severity of intracranial hemorrhages due to audiogenic stress [5]. Our results suggest that the exposure of Krushinsky–Molodkina rats to acoustic stimulation under SLA conditions is accompanied by excess production of NO, which results in stress injury to the neurons. By contrast, the synthesis of excess NO is reduced under FR conditions.

We conclude that patients with hemorrhagic stroke should be treated with drugs, which decrease the synthesis of NO. Moreover, these patients should be maintained under rest conditions that appear to be similar to physiological sleep.

REFERENCES

1. L. M. Baider, V. P. Reutov, A. L. Krushinsky, *et al*., *Biofizika*, **54**, No. 5, 894-900 (2009).
2. C. P. Warlow, M. S. Dennis, J. van Gijn, *et al*., *Stroke. A Practical Guide to Management* [in Russian], St. Petersburg (1998).
3. V. B. Koshelev, A. L. Krushinsky, V. S. Kuzenkov, and V. P. Reutov, *Novosti Mediko-Biologicheskikh Nauk*, No. 1, 41-43 (2004).
4. A. L. Krushinsky, V. S. Kuzenkov, V. E. D'yakonova, and V. P. Reutov, *Byull. Eksp. Biol. Med*., **150**, No. 7, 38-41 (2010).
5. A. L. Krushinsky, V. P. Reutov, V. S. Kuzenkov, *et al*., *Izv. Ros. Akad. Nauk. Ser. Biol*., No. 3, 329-335 (2007).
6. A. L. Krushinsky, V. S. Kuzenkov, V. P. Reutov, *et al*., *Vestn. Mos. Gos. Univ. Ser. 16. Biol*., No. 1, 14-17 (2009).
7. V. S. Kuzenkov, V. P. Reutov, A. L. Krushinsky, *et al*., *Ibid*., No. 1, 3-7 (2010).
8. V. P. Reutov, V. S. Kuzenkov, A. L. Krushinsky, *et al*., *Vestn. Nat. Akad. Nauk Belarusi. Ser. Med. Biol. Nauk*, No. 1, 5-10 (2002).
9. V. P. Reutov, N. V. Samosudova, N. P. Larionova, and L. M. Chailakhyan, *Novosti Mediko-Biologicheskikh Nauk*, No. 1, 57-60 (2004).
10. V. P. Reutov, N. V. Samosudova, N. A. Filippova, *et al*., *Dokl. Ros. Akad. Nauk*, **426**, No. 3, 410-413 (2009).
11. N. V. Samosudova, V. P. Reutov, N. P. Larionova, and L. M. Chailakhyan, *Ibid*., **378**, No. 3, 417-420 (2001).
12. N. V. Samosudova, V. P. Reutov, N. P. Larionova, *et al*., *Tsitologiya*, **47**, No. 3, 214-219 (2005).
13. N. V. Samosudova, V. P. Reutov, N. P. Larionova, and L. M. Chailakhyan, *Morfologiya*, **131**, No. 2, 53-58 (2007).
14. Ch. Liu, Y. Gao, J. Barrett, and B. Hu, *J. Neurochem*., **115**, No. 1, 68-78 (2010).
15. V. P. Reutov, A. L. Krushinsky, V. S. Kuzenkov, *et al*., *Hypoxia Med. J.*, **12**, Nos. 3-4, 51 (2004).