2. R. A. Giniatullin, S. K. Bal'tser, E. E. Nikol'skii, and L. G. Magazanik, *Neirofiziologiya*, **18**, № 5, 645-654 (1986).
3. C. R. Anderson and C. F. Stevens, *J. Physiol. (London)*, **235**, 655-691 (1973).
4. J. B. Cohen and N. P. Strand, in: *Molecular Mechanisms of Desensitization to Signal Molecules*, Eds. T. M. Konjin *et al.*, Heidelberg (1987), pp. 257-273.
5. R. A. Giniatullin, Kh. S. Khamitov, R. Khazipov, *et al.*, *J. Physiol. (London)*, **412**, 113-122 (1989).
6. R. A. Giniatullin, R. N. Khazipov, T. I. Oranska, *et al.*, *J. Physiol. (London)*, **466**, 105-114 (1993).
7. R. A. Giniatullin, R. N. Khazipov, and F. Vyskocil, *Ibid.*, pp. 95-103.
8. T. Heidmann and J.-P. Changeux, *Europ. J. Biochem.*, **94**, 255-279 (1979).
9. B. Katz and R. Miledi, *J. Physiol. (London)*, **231**, 549-574 (1973).
10. B. Katz and S. Thesleff, *J. Physiol. (London)*, **138**, 63-80 (1957).
11. L. G. Magazanik, E. E. Nikolsky, and F. Vyskocil, *Europ. J. Pharmacol.*, **80**, 115-119 (1982).
12. K. L. Magleby and B. S. Pallotta, *J. Physiol. (London)*, **316**, 225-250 (1981).
13. L. G. Magazanik, V. A. Snetkov, R. A. Giniatullin, and R. N. Khazipov, *Neurosci. Lett.*, **113**, № 3, 281-285 (1990).
14. M. A. Maleque, C. Souccar, J. B. Cohen, and E. X. Albuquerque, *Molec. Pharmacol.*, **22**, 636-647 (1982).

On the Possibility of Ventral Root Fibers Undergoing Ephaptic Excitation under Conditions of Very Severe Spinal Hyperreflexia

E. A. Makii and I. A. Krayushkina

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, № 6, pp. 581-583, June, 1995
Original article submitted February 20, 1995

Monosynaptic discharges by ventral roots were studied in rats under conditions of pronounced spinal hyperreflexia 5 days after simultaneous transection of the sciatic nerve and spinal cord. In 40% of tests with such rats, an enhanced monosynaptic discharge of a ventral root was found to be followed by a synchronized and high-amplitude discharge similar in shape and amplitude to the response of the ventral root to electrical stimulation of its fibers. The threshold amplitude for elicitation of these extra discharges was close to the amplitude of the ventral root's monosynaptic discharges at which high-amplitude discharges occurred. It is concluded that when the excitability of spinal reflex arcs is excessively high, ephaptic transmission of excitation probably occurs in ventral roots from fibers involved in the enhanced reflex discharge to unexcited fibers.

Key Words: *spinal cord; hyperreflexia; ventral root; ephaptic excitation*

Excitability of spinal cord neurons in rats has been shown to be highest on days 3-5 after nerve transection [5,7]. After cordotomy, stably enhanced excitability of spinal cord reflex arcs was reported in rats 3 days postsurgery and persisted for a long time [4,6]. We assumed, therefore, that an operation combining nerve transection with cordotomy would lead 5 days later to the formation in the spinal cord of a focus of enhanced excitability exceeding that observed after separate nerve or spinal cord transection. Characteristics of the reflexes from the spinal cord after such a combined operation are of interest for studies designed to gain better insight into how a generator of pathologically enhanced excitation forms in the spinal cord [3].

Department of Normal Physiology, Medical Institute, Dnepropetrovsk, Ukraine. (Presented by G. N. Kryzhanovskii, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0006-0558$12.50

MATERIALS AND METHODS

To produce an animal model of very intensive spinal hyperreflexia, we transected in rats both the sciatic nerve (at the level of the middle third of the femur) and the spinal cord at the level of the lower thoracic segments (Fig. 1, *a*). Five days later, laminectomy in the lumbar region was performed under Hexenal anesthesia (5-7 mg/100 g body weight intraperitoneally), the dorsal and ventral roots in segment L_V were dissected out and cut, and recording and stimulating electrodes were placed on their central portions (Fig. 1, *a*). Then, 3-4 h after the laminectomy, the rats were administered dithylin in relaxing doses and transferred to artificial respiration. Evoked activity was recorded in two modifications. In one of these (Fig. 1, *a*), stimuli were applied to a dorsal root and reflex discharges were led off from the ventral root (VR); in the other (Fig. 2, *a*, *1*), stimuli were applied to a VR and total action potentials were led off from fibers of the same root with a ball-type electrode. The dorsal root was stimulated with 0.3 msec current pulses equal in strength to two thresholds required for eliciting action potentials from the dorsal surface of the spinal cord. For recording action potentials generated by VR fibers, the VR was stimulated with pulses of not more than 0.3 msec in duration and also equal (usually) to two thresholds. To determine the threshold for the generation of action potentials by VR fibers, 1.2 msec pulses were used (the need to use such pulses is explained below). The evoked bioelectrical activity was amplified with a UBM amplifier and photographed from the screen of a C-1-69 oscillograph. Other aspects of the procedure used in this acute experiment are described in our earlier publications [4,5]. Amplitudes of evoked responses and their thresholds upon VR stimulation were analyzed.

RESULTS

In the control series of tests, monosynaptic discharges (MD) from the VR were studied in acutely spinalized rats. These discharges had a mean amplitude of 2.34±0.22 mV (n=19; Fig. 1, *b*, *1* and *c*, *1*). Five days after just the spinal cord was cut, the mean amplitude of MD recorded from the VR was much higher (6.10±0.68 mV; n=24, p<0.01) than in the acutely spinalized rats (Fig. 1, *b*, *2* and *c*, *2*). Five days after both the nerve and spinal cord were cut, the amplitude of MD from the VR on the transected nerve side was 9.69±0.58 mV (n=28, p<0.05), i.e., still higher than in the chronically spinalized group (Fig. 1, *b*, *3* and *c*, *3*). In 12 (40%) of the 28 rats subjected to the combined operation, the evoked response had a second component characterized by an exceptionally high amplitude and synchronicity (Fig. 1, *b*, *4*). The mean amplitude of VR MD in these 12 rats was 13.06±0.96 mV, which is significantly higher (p<0.05) than the mean amplitude in this group (n=28). We hypothesized that the abnormally strong discharges following the VR MD under conditions of particularly pronounced hyperreflexia were a consequence of excitation of the VR fibers not participating in the reflex discharge. To check this, we conducted tests depicted schematically in Fig. 2, *a*, *1* and found that when the VR was stimulated with strong stimuli (those equal to 2 thresholds), the response recorded from this root, i.e., the total response of its fibers, was very similar in shape to the second component of the reflex response recorded under conditions of particularly pronounced hyperreflexia (cf. Fig. 2, *a*, *2* and Fig. *1*, *b*, *4*). The amplitudes of these two responses were almost equal. Thus, the second component that followed the VR MD in rats with a particularly pronounced hyperreflexia had a mean amplitude of 25.50±2.71 mV (n=12), whereas the mean amplitude of the total response by VR fi-

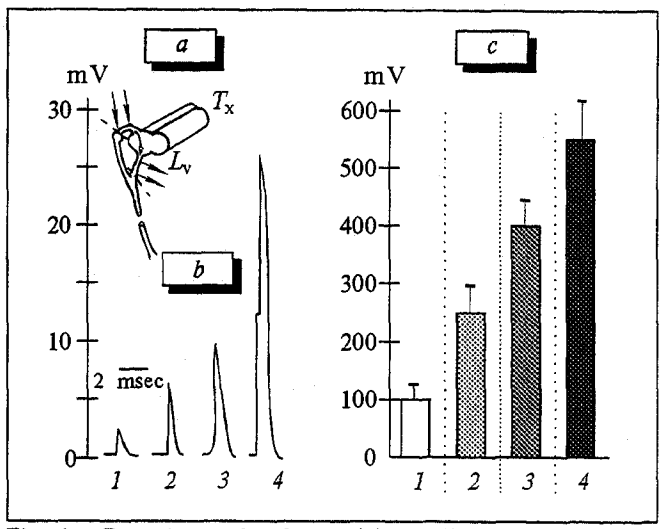

Fig. 1. Experimental scheme (*a*), oscillograms of reflex responses (*b*), and diagrams of mean amplitudes (*c*) of monosynaptic discharges (MD) recorded from a ventral root (VR) under different experimental conditions. *a*: the dotted line indicates the site of root transection in an acute test; the arrows directed toward the root denote stimulation, while those directed away from it denote recorded discharges. *b* and *c*: *1*) acutely spinalized rats (controls); *2*) chronically spinalized rats (5 days postsurgery); *3*) rats 5 days after the sciatic nerve and spinal cord were both cut; *4*) same as in *3*, but in rats with particularly strong MD by the VR. *b*: the reflex discharge was enhanced to the same degree in all cases (its initial part is not shown). *c*: the mean amplitude of MD by the VR in acutely spinalized rats was taken as 100%.

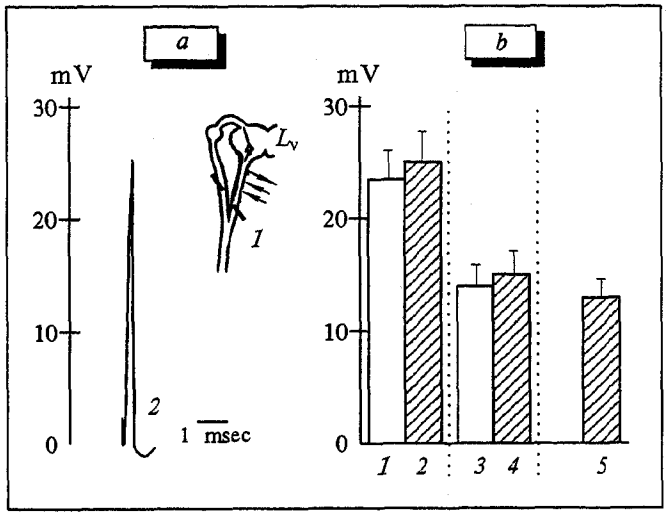

Fig. 2. Experimental scheme (*a*, *1*), oscillogram of total responses by ventral root (VR) fibers upon direct stimulation of the root (*a*, *2*), and diagrams showing mean amplitudes of these responses and mean threshold amplitudes for their occurrence (*b*). *a*: *1*) discharges led off from the central part of the VR with a ball–type electrode; *2*) stimulus of 0.3 msec – same enhancement as in Fig. 1, *b* (only the terminal portion of the stimulation artefact is shown). *b*: *1*) acutely spinalized rats; *2*) rats 5 days after both the sciatic nerve and spinal cord were cut; *3* and *4*) mean value of the threshold for eliciting a VR response with a 1.2 msec stimulus (same designations as for *1* and *2*); *5*) mean amplitude of the MD by the VR at which discharges of abnormally high amplitudes were observed.

bers in rats with transected sciatic nerve and spinal cord was 24.67±2.71 mV (n=12; p>0.05). It should be stressed that the corresponding mean amplitude in the control group was 22.90±3.76 mV (n=15) and did not significantly differ from that in the group with pronounced hyperreflexia (cf. *b*, *1* and *b*, *2* in Fig. 2). Of interest in this context are characteristics of the threshold for excitation of VR fibers, particularly its magnitude in comparison with that of VR MD at which abnormally strong discharges were recorded. To determine this threshold, we used 1.2 msec stimuli, given that the mean duration of VR MD in the group subjected to both sciatic nerve and spinal cord transection was 1.20±0.06 msec (n=25). With such stimulation, the threshold stimulus had an amplitude of 14.64±1.95 mV (n=14) in the control group and 16.77±2.90 mV (n=8) in the group that had undergone the combined operation (Fig. 2, *b*, *3* and *4*). A similar relationship was observed when the threshold current flowing through the stimulating electrodes was recorded (0.94±0.09 and 1.12±0.18 μA, respectively). Comparison of the amplitudes for threshold stimulation with the amplitude of VR MD after which abnormally strong discharges were recorded shows them to be very similar (cf. *b*, *3* and *4* and *b*, *5* in Fig. 2).

Thus, as this study shows, the occurrence in a VR of reflex discharges with amplitudes comparable to the threshold amplitude for exciting VR fibers may elicit a discharge from the VR fibers not participating in the reflex discharge. In this case, the reflex discharge itself appears to act as an electrical stimulus; its amplitude inside the VR is probably higher than that recorded in our study, since the discharge decreases in amplitude as it spreads electrotonically to the recording electrode [1]. In our view, such ephaptic propagation of excitation from excited to unexcited VR fibers, which is not possible under physiological conditions, is associated with the extremely high synchronicity of reflex discharges in VRs, for very high synchronicity is a *sine qua non* for ephaptic excitation of an axon [2]. Prerequisites for such synchronicity seem to be phenomena that do not normally occur, primarily electrical stimulation of the dorsal root such that all afferent group 1a fibers are excited at the same time. A second, and no less significant, factor is a substantial increase in the efficacy of synaptic transmission as a result of nerve and spinal cord transection. Other factors of importance appear to be the high excitability of type A-α fibers in the VRs and the very small scatter of conduction velocities in these fibers.

While negating the existence of ephaptic transmission in VRs under normal circumstances, we have demonstrated that such transmission may occur under pathological conditions. Presumably, this phenomenon may be involved in setting up a positive feedback whereby the generator of pathologically enhanced excitation is sustained in motor nuclei of the spinal cord, especially when the mechanism of spinal inhibition is shut off [3].

REFERENCES

1. D. S. Vorontsov, *General Electrophysiology* [in Russian], Moscow (1961).
2. A. M. Gutman, *Biophysics of Extracellular Brain Currents* [in Russian], Moscow (1980).
3. G. N. Kryzhanovskii, *Determinant Structures in Nervous System Pathology* [in Russian], Moscow (1980).
4. E. A. Makii, *Fiziol. Zh.*, **33**, № 6, 29 (1987).
5. E. A. Makii and P. I. Syabro, *Fiziol. Zh.*, **30**, № 2, 140 (1984).
6. T. C. Cope, S. G. Nelson, and L. M. Mendell, *J. Neurophysiol.*, **44**, № 1, 174 (1980).
7. Y. Miyata and H. Yasuda, *Neurosci. Res.*, **5**, № 4, 338 (1988).