
FROM PHYSIOLOGY OF SINGLE NEURONS TO PHYSIOLOGY OF MOVEMENTS

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That time coincided in the world neuroscience with the beginning and development of the era of microelectrode techniques, and Prof. Kostyuk was a pioneer in introducing these new experimental approaches in the Soviet Union and in opening up broad prospects in direct studies of intracellular electrical processes and cell-to-cell communication in the nervous systems. Principles of microelectrode techniques and first results of the studies of functional organization of the spinal cord obtained in the respective experiments were summarized by Prof. P.G. Kostyuk in the monographs "The two-neuronal reflex arc" (1959) and "Microelectrode technique" (1960), which became for many years the main manuals for research associates and postgraduate students working in the field of electrophysiology throughout the entire Soviet Union.

In early 1960ths, Prof. P.G. Kostyuk proposed to investigate the neuronal organization of the main descending systems of motor control (cortico-, rubro-, and

reticulo-spinal), and we were lucky to be involved in the research work at different stages of this project.

A general direction of these studies was certainly pioneering at that time, and some of our findings were fundamentally novel and original. We were really happy that these results were published in international leading scientific periodicals (Vasylenko, Kostyuk, 1968, 1979; Kostyuk, Piliavsky, 1969; Kostyukov *et al.*, 1976, and others) and then summarized in our candidate (Ph. D.) theses (1966, 1968, and 1974), and presented in several monographs, one of which, "The structure and function of the descending systems of the spinal cord", was written by Prof. P.G. Kostyuk in 1973.

Authors are greatly happy and very grateful to Prof. P.G. Kostyuk for the scientific guidance based on thorough and patient teaching for the smallest details of the theory of neurophysiology and experimental techniques, frank discussion of urgent unresolved general problems and particular questions in our field of studies, and direct participation in and valuable help for the experimental work (including surgery of animals and hours in front of an oscilloscope during the experiment itself). All of us, then young researchers, were greatly impressed by fantastic erudition of Platon Grygorovychin general physiology, neurophysiology, and neuromorphology.

Within the interval from the early 1960^{ths} to the late 1970^{ths}, a real explosion in the number of studies devoted to analysis of the structure and functional peculiarities of the main subdivisions of the CNS in mammals was observed. We were proud that our original experimental results and their interpretation provided certain contribution to the expanding knowledge of principles of the motor control.

In particular, microelectrode recording from spinal neurons under conditions of stimulation of the cortico-spinal pathways allowed us to identify a population of the spinal interneurons relatively selectively activated by volleys coming via pyramidal fibers, while primary afferent activity excited these interneurons to a much lesser extent.

This finding served as the reason for suggesting the existence of spinal interneuronal relay systems specialized on transmission of suprasegmental activities performed separately from transmission of peripheral afferent effects, a concept important for understanding general principles of the motor control. Further experiments showed that these interneuronal populations may provide integration of the influences coming to the segmental levels from different main supraspinal sources (cortical, rubral, reticular, and vestibular ones). These populations are separated topographically, they form a lateral and a ventromedial group; the former is activated by cortical, rubral, and some reticular sources, while the latter is connected mostly with reticulo- and vestibule-spinal fibers (Vasylenko *et al.*, 1972, Vasylenko and Kostyuk, 1979). Such interneurons possess rather specific relay properties, different in the "lateral" and "ventral" units. An important

feature of the abovementioned spinal interneurons was the following. These units were found to establish intersegmental connections; their axons project to the white matter funiculi and then re-enter the gray matter at a certain distance. Thus, such propriospinal intersegmental pathways should play an important role in spatial integration of descending and primary afferent signals in the course of motor control. A technique of selective stimulation of different propriospinal pathways (the idea was proposed by Prof. P.G. Kostyuk) opened possibilities to examine synaptic effects evoked by such activation in different groups of spinal motoneurons and to identify specific properties of these synaptic phenomena. Functional properties of different propriospinal systems (short and long, lateral and ventromedial) were combined with their structural description (morphological experiments were carried out in collaboration with Drs. V.A. Maiskyi and L.A. Savoskina). All these findings were summarized in the monograph by D.A. Vasylenko and P.G. Kostyuk "Intersegmental neuronal systems of the spinal cord" (1983).

In carnivores, the shortest pathways from the motor cortex and red nucleus to the flexor motoneurons are disynaptic; we demonstrated that the sustained rhythmic activity of these descending systems can form the basis for a "plateau of depolarization" in spinal motoneurons, thus increasing the effectiveness of the movement control. The fast- and slow-conducting cortico-bulbar neurons can induce monosynaptic excitation of most medullary reticulo-spinal neurons, a definite specialization of these connections does exist. Slow corticofugal fibers project predominantly to phasic reticulo-spinal neurons, while fast ones predominantly provide activation of tonic reticulo-spinal neurons (Piliavsky, 1975). After reports that many noradrenergic terminals in the spinal gray matter are produced by the coeruleo-spinal descending system (Kuyppers and Maisky, 1975), we showed that in spinal motoneurons long-term activation of the locus coeruleus evokes a considerable reduction of the inhibitory effects from flexor reflex afferents (Piliavskyi et al., 1982). It was first shown that the descending action of coeruleo-spinal projections does not include direct postsynaptic inhibition of motoneurons; instead, it strongly suppressed the efficiency of afferent transmission via the segmental interneuronal apparatus.

Progress in the studies of the function of calcium ions revealed that they are a key component of the chain of processes underlying transsynaptic transmission in neuromuscular connections and synapses between CNS neurons. The close relation between the generation potentials in the periaqueductal gray neurons and the spinal motoneurons was demonstrated in *in vitro* preparations. Testing of specific blockers allowed us to show inhibition of the function of calcium channels in the periaqueductal gray neurons which can be one of the mechanisms underlining the analgesic effect of a series of neurotropic agents after introduction of them in the substance.

Thus, calcium channel blockers can be regarded not only as substances which potentiate the action of known analgesics, but also as independent potential anal-

gesics (Yakhnitsa et al., 1996). Parallel changes in the amplitude and frequency of unitary PSP in spinal motoneurons under blockers of both high-threshold and low-threshold calcium channels observed while studying *in vitro* were considered a proof for both pre- and post-synaptic localization of N- and L- type channels and their potential role in formation of motor commands addressed to the skeletal muscles (Piliavskii et al., 1998).

The microelectrode technique allowed directly observing fine processes of transmission signals in the spinal cord and brain, and sometimes there appeared feelings that a little bit more time — and our efforts would be awarded by the creation of a complete detailed picture of functioning of the entire system of the motor control. Despite fundamental progress in this field of neuroscience, the initial euphoria accompanying those investigations step-by-step disappeared with time.

During the 1980^{ths}, we searched new approaches to study certain functional systems of the organism in more detailed and complex mode. A group of the former associates of the Dept. of general physiology of the CNS, who were especially interested in the studies of the motor control system, formed a "nucleus" of the today Dept. of physiology of movements headed by A.I. Kostyukov. We understood that adequate analysis of the motor control system is impossible without recording real movements and getting further detailed knowledge of the muscle behavior under conditions of continuously changing force interaction with the external environment.

Special electromagnet-driven mechanostimulators were developed in the Department; these devices could create arbitrary temporal profiles of the external force loading for the muscles of tested both humans and experimental animals. This technique allowed us to examine quantitatively the muscle dynamics, and these experiments resulted in the development of analytical non-linear model of the muscle contraction (Kostyukov, 1987). The model created possibilities to predict trajectories of changes in the muscle length under conditions of voluntary changes in the external loading and in the intensity of efferent activation. It has been found that muscle hysteresis determines a clear dependence of the muscle equilibrium length on the direction of the preceding movement, and the hysteresis-type uncertainties in the installing of equilibrium states of the muscles is a fundamental property of the motor system. This feature of muscle contraction provides the existence of hysteresis-related properties of most important proprioceptors providing feedback signals in the motor control system, namely muscle spindles and Golgi tendon organs. Due to these important properties of proprioceptors, the muscle hysteresis is not compensated at the level of the spinal cord reflexes.

Moreover, the incoming proprioceptive activity even intensifies muscle hysteresis *per se* which looks obviously undesirable from a functional point of view. This becomes especially clear after detailed analysis of the dynamic properties of

the stretch reflex, one of the most important reflex systems controlling muscle contractions in mammals (Kostyukov, 1998). As it was demonstrated, the movement-dependent uncertainty effects cannot be compensated at the spinal level; therefore, an "errorless" motor control needs special pattern of the descending activity coming from the "upper" centers of the motor system to motoneurons (Kostyukov 1998, 2007).

We elaborated a corresponding strategy to study central commands in humans during voluntary movements. Our approach is based on the performance by a tested object the stereotyped targeted movements produced under precise control of the external loads applied to the correspondent muscle groups. The processing of the EMG activity (full-wave rectification and averaging the standard tests) allow us to quantitatively estimate the efferent activity coming to the corresponding muscles; averaged EMGs also give information on the central commands related to a given movement program.

Basing on the results obtained in the Dept. of Physiology of Movements over last two decades, we offered to essentially revise the current theoretical concepts of the motor control in mammals. It was demonstrated that the so-called equilibrium point hypothesis, which began to be rather widely used in physiology after Feldman's papers (1966, 1986), should be crucially reconsidered. First of all, such a revision is related to the necessity of accounting for non-linear properties of the muscle and muscle proprioceptors, which lead to the appearance of the movement-dependent uncertainties in installing the equilibrium lengths of muscles. Basing on a detailed analysis of the muscle dynamics in the stretch reflex system, A.I. Kostyukov formulated a principle of the dynamic control of the equilibrium states in the motor system; these important results were presented in recently published monograph "Dynamical properties of the mammalian movement system" (Kostyukov, 2007).

Later on, research interests of D.A. Vasilenko and colleagues also shifted from examination of neurons constituting the systems of motor control toward analysis of phenomena *resulting* from the activity of these systems, i.e., movements themselves, and to general principles of the motor control. The roles of visual and kinesthetic control are compared by recording the accuracy of target positioning of the limb link in the course of such movements performed with and without visual feedback. The fundamental reason for the respective "blind-performance" errors, namely non-linear transducer characteristics of muscle stretch receptors (muscle spindles), has been identified (Mel'nichouk et al. 2003, 2007).

Then studies towards clarifying the central processes related to skeletal muscle fatigue began. A Fos protein-immunohistochemistry technique was used to reveal the expression of early proto-oncogene *c-fos* as a marker of neuronal activation. Lamellar disibutions of Fos- immunoreactive and nicotinamide adenine dinucleotide phosphate-diaphorase positive neurons activated after sustained muscle contractions were examined. It was found that fatigue of skeletal muscles

induced ipsilateral *c-fos* expression, but did not change the NADPH-d reactivity in the lumbar spinal cord.

The patterns of *c-fos* expression in the spinal cord after muscle fatigue and activation of nociceptive (vanilloid) receptors after intramuscular injection of capsaicin were found to be similar (Pilyavskii et al., 2005). The associated co-activation of glial cells during stimulation of muscle vanilloid receptors can reinforce local nociceptive processing (central sensitization) and, thereby, promote spreading of nociceptive signals to adjacent or even distant CNS regions. These data may be helpful for understanding the segmental mechanisms operating on the premotoneuronal level and producing depression of the motor output. These phenomena demonstrated the "wisdom of skeletal muscle" capable of preventing muscle deterioration under extensive motor experience. The data obtained were confirmed by direct intracellular investigation of muscle fatigue-related events in spinal motoneurons (see Kalesic et al., 2004; Kostyukov et al., 2005).

Finally, we would like also to emphasize that D.A. Vasilenko is now strictly involved in publishing the journal". This scientific periodical was founded by Prof. P.G. Kostyuk in 1969; thus, we celebrate now the 40th anniversary of the journal. Beginning from 1972, D.A. Vasilenko directly collaborated with the Editorial Board of *Neurophysiology*, and now he is one of two Co-Editor-in-Chiefs of the journal together with Prof. P.G. Kostyuk and also a scientific editor of the journal. In 1993, *Neurophysiology*, the first among periodicals of the National Academy of Sciences of Ukraine, was transformed in an international scientific journal and began to be published in two versions, Russian/Ukrainian and English ones. At present, the journal is published by a world-known corporation, Springer, and its full electronic version is presented in the Internet. We are proud that our duty is to make results of the studies of neuroscientists working in Ukraine and a number of other countries widely known for the international scientific community.

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Our progress in neurophysiology within the beginning period of our research work would be impossible without valuable friendly help of all our colleagues, first of all of Vilya Pyatigorskii (our "electronic boss") and of Kolya Preobrazhenskii and Yurii Lymanskyi (who worked in the neighboring rooms and examined brainstem neuronal mechanisms).

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REFERENCES

- Feldman AG, 1966. Functional tuning of nervous system with control of movement or maintenance of a steady posture: 3. Mechanomyographic analysis of execution by man of the simplest motor tasks. *Biophysics* 11: 667-675.
- Feldman AG, 1986. Once more on the equilibrium point hypothesis (λ -model) for motor control. *J Mot Behav* 18: 17-54.
- Kalezic I, Bugaychenko LA, Kostyukov AI, Pilyavskii AI, Ljubisavljevic M, Windhorst U, Johansson H, 2004. Fatigue-related depression of the feline monosynaptic gastrocnemius-soleus reflex. *J Physiol* 556: 283-296.
- Kostyuk PG, 1959. The two-neuronal reflex arc, Medgiz, Moscow, 256 p.
- Kostyuk PG, 1960. Microelectrode technique, Academy of Sciences of USSR Press, Kyiv, 127 p.
- Kostyuk PG, Pilyavsky AI, 1969. A possible direct interneuronal pathway from rubrospinal tract to motoneurons. *Brain Res* 14: 526-529.
- Kostyuk PG, 1973. The structure and function of the descending systems of the spinal cord, Nauka, Leningrad, 279 p.
- Kostyukov AI, Baev KV, Vasilenko DA, 1976. Regular pulse train transformation by monosynaptic connection — neurophysiological data and their treating with simple stochastic neuron model. *Biol Cybern* 24: 219-226.
- Kostyukov AI, 1987. Muscle dynamics: dependence of muscle length on changes in external load. *Biol Cybern* 56: 375-387.
- Kostyukov AI, 1998. Muscle hysteresis and movement control: a theoretical study. *Neuroscience* 83: 303-320.
- Kostyukov AI, Bugaychenko LA, Kalezic I, Pilyavskii AI, Windhorst U, Djupsjobacka M, 2005. Effects in feline gastrocnemius-soleus motoneurons induced by muscle fatigue. *Exp Brain Res* 163: 284-94.
- Kostyukov AI, 2007. Dynamical properties of the mammalian movement system, FADA, LTD, Kyiv, 199 p.
- Kuypers HGJM, Maisky VA, 1975. Retrograde axonal transport of horseradish peroxidase from spinal cord to brain stem cell groups in the cat. *Neurosci Lett* 1: 9-11.
- Mel'nichouk AP, Bulgakova NV, Vasilenko DA, 2003. Positioning of the human forearm in tracking movements and their reproduction under conditions of limited visual control. *Neurophysiology* 35: 122-132.
- Mel'nichouk AP, Bulgakova NV, Tal'nov AN, Hellstrom F, Windhorst U, Kostyuk ov AI, 2007. Movement-dependent positioning errors in human elbow joint movements. *Exp Brain Res* 176: 237-247.
- Pilyavsky AI, 1975. Characteristics of fast and slow cortico-bulbar fibre projection to the reticulo-spinal neurtons. *Brain Res* 85: 49-52.
- Pilyavskii AI, Bulgakova NV, Yakhnitsa IA, Pavlasek J, 1982. Changes in postsynaptic responses in spinal motoneurons during repetitive stimulation of the locus coeruleus. *Neurophysiology/Neirofiziologiya* 14: 51-59.

- Pilyavskii AI, Bulgakova NV, Melnichuk AP, Pilyavskii OA, 1998. Postsynaptic activity of spinal motoneurons on early postnatal rats *in vitro*: effects of calcium channel blockers. *Neurophysiology/Neirofisiologia* 30: 362-367.
- Pilyavskii AI, Maznychenko AV, Maisky VA, Kostyukov AI, Hellstrom F, Windhorst U, 2005. Capsaicin-induced effects on *c-fos* expression and NADPH-diaphorase activity in the feline spinal cord. *Eur J Pharmacol* 521: 70-8.
- Vasilenko DA, Kostyuk PG, 1968. Transformation of cortical motor signals in spinal cord. *Proc of the IEEE* 56: 1049-1058.
- Vasilenko DA, Kostyuk PG, 1979. Spinal interneurons. *Ann Rev Physiol* 41: 115-126.
- Vasilenko DA, Kostyuk ov AI, Pilyavsky AI, 1972. Cortico- and rubrofugal activation of the interneurons — sources of propriospinal pathways of the dorsolateral funiculus of the spinal cord. *Neurophysiology/Neirofiziologiya* 4: 489-499.
- Vasilenko DA, Kostyuk PG, 1983. Intersegmental neuronal systems of the spinal cord, Naukova Dumka, Kyiv, 208 p.
- Yakhnitsa VA, Pilyavskii AI, Limansky YP, Bulgakova NV, 1996. Modulation of the activity of midbrain central gray substance neurons by calcium channel agonists and antagonists *in vitro*. *Neuroscience* 70: 159-67.