

This Week's Citation Classic®

Kostyuk P G, Krishtal O A & Shakhovalev Y A. Separation of sodium and calcium currents in somatic membrane of mollusk neurons. *J Physiol.—London* 270: 545-68, 1977. [Bogomolez Institute of Physiology, Ukrainian Academy of Sciences, Kiev, USSR]

This paper describes the method of intracellular perfusion of isolated nerve cells that allowed, for the first time, a complete separation of calcium inward currents generated in the somatic membrane. Extensive description of activation kinetics, selectivity, inactivation, and modulation of the putative calcium ion channels is given. (The SC/© indicates that this paper has been cited in more than 330 publications.)

Separation of Calcium Currents in Nerve Cells

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I was a graduate student in physiology at Kiev University when I met D. Voronov who later became my supervisor. He was always deeply interested in cellular mechanisms of excitation and inhibition and had, in 1925, described the intimate relations between the action of polarizing current and different cations on impulse conduction in nerve fibers.¹

The beauty of the analysis impressed me very much, and, although for many years after graduation I was engaged in spinal cord physiology, I always felt a motivation to switch to more simple systems that could be put under complete experimental control. Then, in 1964,¹ was joined by two young biologists—V. Gerasimov and V. Maisky—who also were looking for more simple structures to study nerve cell functions. We turned to snails (*Helix pomatia*), which are very numerous around Kiev. They have beautiful neurons in their transparent ganglia, easily identifiable under a microscope and just ready for the insertion of microelectrodes. To our surprise, we discovered that sodium ions are not obligatory in the bath solution for the generation of a full-sized action potential in these neurons,

and the latter is obviously produced by the influx not of sodium but of calcium ions into the cell. Then we learned from the literature that the same was already observed on another mollusk (*Onchidium*) in Japan by Y. Oomura and coworkers.²

The highlights of everybody trying to analyze the intimate nature of nervous activity were, of course, the experiments on perfused giant axons that separated so convincingly the nerve impulse into separate specific ionic currents and gave them a quantitative description.³ Why not try to use this approach, not only with axons, but also with cell bodies—at least the big ones?

I was now collaborating with scientists who graduated from the faculty of physics—O. Krishtal and V. Pidoplichko—and we started trials. Finally a method was found to make a permanent pore in the membrane of an isolated nerve cell and to connect it with a perfusing system. We discovered that, in such a way, the soluble ingredients of cell interior can be washed out quite rapidly and replaced by extrinsic ones.

First, we replaced potassium ions inside the cell wall with impermeable ions, switching off outward transmembrane currents; the remaining inward sodium and calcium currents were then separated by changing the composition of extracellular solution. The first results of this separation were published in *Nature* in 1975.⁴

The perfusing system turned out to be very effective, and our subsequent joint work with Krishtal generated a wealth of data about activation kinetics, selectivity, inactivation, and modulation of voltage-operated calcium channels in nerve cell membrane.⁵ Quantitative analysis was carried out by Yu. Shakhovalev who composed the corresponding computer programs. This work started a whole series of investigations in our and other laboratories that continues to expand.

1. **Woronov D S.** Wie schnell stellt der konstante Strom die Leitungsfähigkeit des mit einigen Salzen behandelten Nerven wieder? *Pflügers Arch.* 207:279-86, 1925.
2. **Oomura Y, Ozaki S & Maeno T.** Electrical activity of a giant cell under abnormal conditions. *Nature* 191:1265-7, 1961.
3. **Gerasimov V D, Kostyuk P G & Maisky V A.** Excitability of giant nerve cells of various representatives of pulmonary mollusks in the sodium-free solutions. *Bull. Exper. Biol. Med.—Engl. Tr.* 58:3-7, 1964.
4. **Kostyuk P G, Krishtal O A & Pidoplichko A I.** Effect of internal fluoride and phosphate on membrane currents during intracellular dialysis of nerve cells. *Nature* 257:691-3, 1975. (Cited 150 times.)
5. **Kostyuk P G & Krishtal O A,** eds. *Intracellular perfusion of excitable cells.* Chichester, England: Wiley, 1984. Received March 12, 1991