ХРОНІКА

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My first 20 years in Neuroscience

У 60-ті роки XX століття акад. Платоном Григоровичем Костюком та його співробітниками отримані інтригуючі нейрофізіологічні дані, що потенціали дії (ПД), які реєструються при внутрішньоклітинному відведенні (в експериментах in vitro) за допомогою мікроелектродів при прямій деполяризації гігантських нейронів у різних видів молюсків, виявляють різну чутливість до зміни іонного складу розчинів. Було встановлено, що у гігантських нейронах виноградного слимака (Helix) генерація ПД добре підтримується прямим електричним подразненням мембрани у розчинах без іонів натрію і з високою концентрацією іонів кальцію або іонів барію. Амплітуда ПД і питомий опір мембрани зростають у лінійній залежності від логарифму концентрації Ca^{2+} або Ba^{2+}. Збільшення вмісту Ba^{2+} у розчині призводить до появи подовжених ПД на пряме внутрішньоклітинне подразнення гігантських нейронів Helix. Також було встановлено (1962–1963), що на відміну від молюска Helix, гігантські нейрони легеневих молюсків Planorbis і Limnea втрачають можливість генерувати ПД у розчині, в якому відсутні іони натрію.

На початку 70-х років XX століття учнем П.Г. Костюка (В.О. Майським) у відділі анатомії Роттердамського університету (Голландія) вивчався ретроградний аксонний транспорт пероксидази із хрону (ПХ) від спинного мозку до нейронів різних структур стовбура мозку у кішки. Цими нейроанатомічними дослідженнями керував відомий вчений, проф. Генрікус Куйперс. Як і передбачалося раніше, мічені нейрони виявлялися у ретикулярній формації, вестибулярних ядрах, а також у червоному ядрі. Вперше було показано, що введена у різні сегменти спинного мозку ПХ ретроградно транспортується по ушкоджених аксонах до нейронів блакитної плями (Locus coeruleus, subcoeruleus) і до пришлункової ділянки гіпоталамуса – паравентрикулярного ядра. Таким чином, у 1975 р. вперше було відкрито у мозку кішок гіпоталамо-спінальну нейронну систему (Kuypers a. Maisky, 1975). Тільки через рік у США ці дані були підтверджені на щурах (Saper et al.,1976). На другому етапі нейроанатомічних експериментів з численними пошкодженнями спинного мозку у кішок були встановлені канатикові траєкторії низхідних супраспінальних шляхів. Показано, що прямі шляхи від паравентрикулярного ядра гіпоталамуса спускаються у спинному мозку у складі латеральних канатиків, іпсилатерально (Kuypers a. Maisky, 1977).

About author. DSc. Vladimir A. Maisky is well-known scientist in field of neurophysiology and neuroanatomy. Together with his teachers and colleagues he has obtained for the first time the important data about ultrastructural organization of the propriospinal projections in spinal cord (in co-authorship with Acad. Platon Grygorovych Kostyuk, *Brain Res.*, 1972), axonal transport of horse radish peroxidase and fluorescent substances in the brain (Acad. F.N. Serkov, *Dokl. AN USSR,* 1984), organization of hypothalamic and spinal serotonergic projections (Acad. V.N. Kazakov, *Neuroscience,* 1993), *c-fos* expression and NOS activity in the brain in animal model of Parkinson's disease (Corr. Member V.F. Sagach, *Parkinson. Relat. Disord.,* 2002), distribution of NO-generating

neurons in the cardiovascular centers of the brain (Acad. A.A. Moibenko, *Comp. Biochem. and Physiol.,* 2003), *c-fos* expression in the brain induced by muscle fatigue (Acad. V.M. Moroz, *Fiziol. Zh.,* 2006). In 1975–1976 DSc. V.A. Maisky worked (using IBRO/UNESCO fellowship) in the Department of Anatomy, Erasmus University (Rotterdam, the Netherlands) headed by the famous neuroanatomist Professor H. Kuypers. Prof. H. Kuypers together with DSc. V.A. Maisky found for the first time the existence of the straight pathways from hypothalamus to spinal cord. The obtained data were published in leading scientific journals (*Neurosci. Lett.*, 1975; *Brain Res.,* 1977).

In this issue of our journal, we publish an assai by DSc. V.A. Maisky dedicated to the memory of Acad. Platon G. Kostyuk (August 20, 1924 – May 10, 2010), the first teacher of the author. This literary composition also deals with scientific interests his two other great teachers and his colleagues during the period of neuroscience revolution of the 1960s and the 1970s (Ed.).

1. The search for truth through the microelectrode tip (1960–1970)

Within the first 10 years, we focused on the search for novel biophysical approaches in order to gain a clear insight into the functioning of individual nerve cells. We all, young associates from the Lab. of General Physiology of the Nervous System (GPNS) headed by P.G. Kostyuk, were filled with awe when we read scientific papers by A. Hodgkin, B. Katz, and J. Eccles. I was the second postgraduate student of young DSc. P.G. Kostyuk, and naturally I worked under his guidance. We still value in our hearts the recollection of this beautiful time, so nicely expressed by the words of this song:

"How we once young were,
"Как молоды мы были,
How we once young were,
Как молоды мы были,
How loved sincerely,
Как искренне любили,
How had faith in oneself!"
Как верили в себя!"
(Original text in Russian)

(Music by A.N. Pakhmutova, poetry by N.N. Dobronravov. Ed)

I kept firmly in my mind the gospel truth: "If I have been able to see further, it was only because I stood on the shoulders of giants" (Sir Isaac Newton, *Letter to Robert Hook*. Ed.). I am paraphrasing it as follows: "To be able to see further, it is rather necessary for us, postgraduate students, to stand on the shoulders of our great teacher!" In 1961, my chief and mentor has come back from Australia (he had a long duty journey to the University of Canberra, the Dept. of Physiology) where Prof. J. Eccles opened him the secret doors to the mysteries of the nerve cell. It is obvious that 1962 was the period of triumph of my chief. In this year, he published scientific articles in co-authorship with J. Eccles and T. Araki, as well as the first paper in *Nature*. This was Kostyuk's glorious victory, and also a victory for our Lab. Then, the Lab. was reorganized in the Dept. of GPNS. At that time, we all made only the first steps towards the disclosure of the truth of functioning of the nerve cell and its membrane. My University education is in radiophysics. I became a biophysics under the influence of my first chief. When I began postgraduate course, I already possessed professional skills necessary for operating and maintenance of radar stations. I also was strong in higher mathematics, since several years earlier I graduated from the Kyiv Polytechnic Institute. My skills were very useful for my studies carried out in the Dept. of GPNS. At that time, in the early 1960s, we were up against a difficult problem. Our boss set a task before us: as soon as possible (during one year), we must create a complex of electronic equipment for biophysical studies of the ionic processes occurring at the surface membranes of the nerve cells using glass microelectrodes. We

also must develop reliable method for intracellular stimulation of the studied neurons, acoustic control, and recording electrical responses of the neurons to applied stimulation. We had no the slightest idea where we can purchase all necessary equipment for such a complex task and where we can find animals having giant nerve cells. The Directorate of the Bogomoletz Institute of Physiology, and Military District in Kyiv gave us a helping hand. For our Dept., I managed to get four radar stations which were unregistered in military units. At that time, such help was a major contribution to the realization of the projects of our chief. In 1961, B.Ya. Pyatigorskii, V.D. Gerasimov, and I invested the part of our modest salaries in the purchase of additional electronic components to build necessary special transformers. Using the following Ukrainian idiom (a novel Soviet watchword) popular at that time, I can say that indeed we all worked together under this comic, but not horrifying, slogan:

"We don't want the sun if the Party provides light!

We don't want the bread! Give us hard work and full-time job!"

"Нам сонця не треба – нам партія світить!

Нам хліба не треба – роботу давай!"

(Original text in Ukrainian)

A set of devices for electrophysiological investigations ("3V trend brand") was developed and made already in 1962 due to efforts of V. Pyatigorskii (Vilya, alias Benya), and two Vladimirs, V. Gerasimov and V. Maisky. In this year, we successfully used the 3V set in biophysical studies. Later on (in 1965), we described the 3V equipment in our paper published in Fiziol. Zh. (Kyiv). It seems impossible that the 3V set, after quite a number of modifications is still used by PhD V.D. Gerasimov in his biophysical studies carried out in the Dept. of Physic-Chemical Biology of Cellular Membranes (PCBCM). Unfortunately, DSc. B.Ya. Pyatigorskii, one of developers of this 3V set, passed away long ago.

At the same time, our chief initiated in our Dept. electrophysiological studies on the cat spinal cord; these investigations were under his patronage. He always corrected out work schedule in accordance with the data reported in current scientific literature (published in German, French, or English) and their further careful analysis.

I believe that our boss as one may say was inductive thinker. During the next years his image in the world science was grooving together with extensive career grows that has great impact on his first postgraduate students. He gave us scant personal attention. However, at that time, in spring and summer of 1962 and 1963, V.D. Gerasimov, B.Ya. Pyatigorskii, and I, as well as other associates of our Dept. were joyfully running bare-foot rather frequently (while the morning dew was on the ground) around modest cottage ("dacha") of our teacher (33 km from Kyiv) gathering in gardens, ponds, and small lakes pulmonary mollusks *Helix pomatia, Limnea stagnalis, and Planorbis corneus*. We already knew that "giant neurons" (200 μm in diameter) are localized in subpharyngeal ganglia in these "the creations of God." We sacredly worshipped these animals. One day our convivial intercourse in the Dept. was unexpectedly interrupted by graduate student D.A. Vasilenko, who swallowed a whole big live *Helix pomatia* with no shell, followed by the small glass of vodka, and read us a short lecture about the utility of mucus and the protein hemocyanine of the *Helix* "blue blood" for the human stomach. It should be noted that, due to superficial localization of giant neurons in the ganglia of these mollusks, it was possible to rapidly change the ion composition of the medium around the studied neuron. In addition, since these neurons are huge, we could impale them with two microelectrodes, without any damage the cell's membrane. We also could polarize the membrane and record the electrical activity from the neuron during many hours.

Intriguing facts were obtained in the first electrophysiological investigations [1, 2] that the action potentials (APs) produced by direct depolarization of the cell membrane in different species of mollusks showed specific dependence on the external ionic composition. In *Helix* neurons, the generation of AP was well maintained in sodium-free solutions with high calcium or barium content (fig. 1, a–h). In such case the amplitude of the spike overshoot was linearly related to the logarithm of calcium concentration. It is interesting that the increase in the external calcium or barium ions decreased the conductance of the resting membrane (R_o) also in linear relation to the logarithm of Ca^{2+} or Ba^{2+} concentration. It was found for the first time that addition of Ba^{2+} to the external solution produced in the neurons well-developed prolonged (protracted) APs in all cases. On the contrary, the excitability of *Planorbis* and *Limnea* neurons was rapidly (during 3 min) and reversibly depressed in sodium-free solutions (fig. 1, i–p).

Later on, my chief has proposed me more complex problem, namely, to use mathematical apparatus from the "cable theory" (mathematical modeling of spreading electrotonic potentials (EPs) along giant axons of squid, Hodgkin – Rushton, 1946) in order to predict the biophysical characteristics of individual muscle fibers (*m. sartorius*) of frog *in vitro* experiments on the effects of the changes in composition of the external solution. Within the framework of this theory, the following partial differential equation was proposed:

$$-\lambda^2 \frac{\partial^2 V}{\partial x^2} + \tau \frac{\partial V}{\partial t} + V = 0,$$

where λ is the first coefficient of attenuation of the amplitude of EP (V) along the cable expressed in terms of specific resistance of the axoplasm (sarcoplasm) (Ri) and resistance

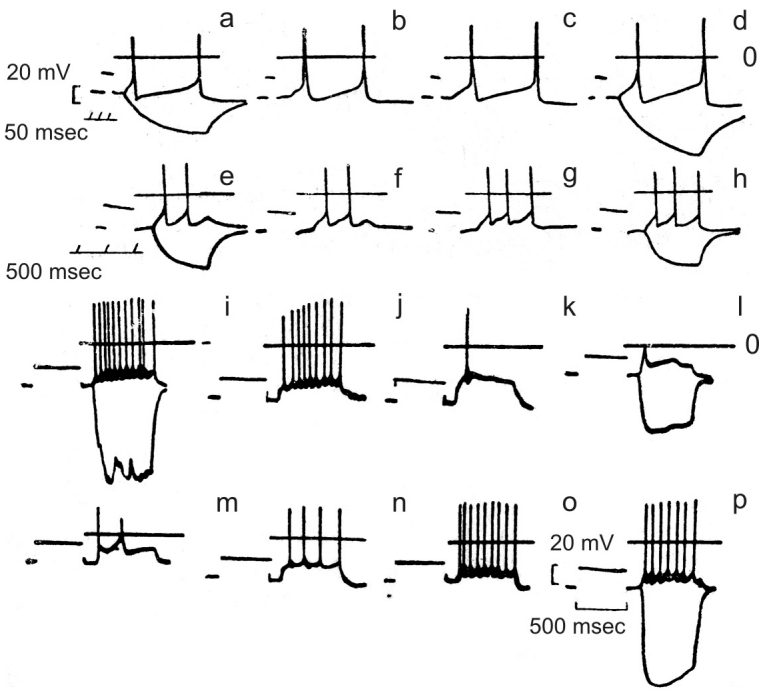

Fig. 1. Changes of electrotonic potentials and APs of the same neuron of *Helix* (a–h) and *Planorbis* (i–p) in isotonic solution $CaCl_2$. Note APs and electrotonic potentials in Ringer's solution without Na^+ (a) and after 1, 2, 3 min registration of the same characteristics (b-d), in isotonic solution of $CaCl_2$, correspondently; again in normal solution without Na^+ after 1, 2, 3, and 4 min registration of the same characteristics (e-h), correspondently; the same changes in these characteristics in normal (i) and isotonic solution of $CaCl_2$ (j-l); again in normal solution (m-p) after 1, 2, 3, and 4 min, correspondently. Hyperpolarized and depolarized currents are $1 \cdot 10^{-8}$ A and $0.3 \cdot 10^{-8}$ A, correspondently (modified from [1, 2])

of the membrane per unit surface (Rm). The second attenuation coefficient τ, or the time constant, is related to the membrane capacity (Cm) per unit surface. At earliest possible date, I developed the special electronic device and original protocol for recording of EPs along the membrane of excitable fiber. The device included a complex radio-frequency set for uncoupling of the biological object from the stimulator. The membranes of individual muscle fibers were polarized by weak hyperpolarizing current pulses ($2 \cdot 10^{-8}$ A) passed through one microelectrode, while another electrode was used for recording of EPs along this fiber at different distances (100 – 2000 μm) from the polarizing electrode. Throughout 1962, I carried out these complex experiments. For the majority of the studied muscle fibers of frog, the following mean biophysical parameters were obtained in the normal Ringer solution: Rm = 3960 ± 480 Ω·cm^2, Ri = 87 ± 10.3 &!Чcm, and Cm = 8 ± 3.2 μF/cm^2. These parameters decreased with increase in the concentration of K^+ in the external solution. The data that I obtained in these experiments were described in the paper published in the Moscow scientific journal [8]. It should be noted that the data attracted lively interest from biophysics.

In those memorable times (namely of the 1960s), our research activities sometimes alternated with informal gatherings with music. We knew that piano concerts in philharmonics Kyiv and Moscow are another strong passion or the second dominant after science for our teacher. We knew that he wonderfully plays the piano himself and also loves to hear great pianists like S. Richter or A. Rubinstein who could make the piano sing. Each of us was just "a copy cat" and we also loved to hear great pianists and singers at the opera or on vinyl. I remember how our trade union organization bought a piano "Ukraine" for the Institute, and we put this musical instrument in the director's office. The next day we all, connoisseurs of art, were invited to listen to the music. Platon Grygorovych emotionally played for us a part of Chopin's Piano Concerto No.1 in E minor. We listened to the music with bated breath. It was so beautiful! We have remarked that our maestro put his own feeling into sounds and told us the pleasant and lively story. Indeed, a Kostyuk's master class was example of the brilliant emotionally-semantic inversion.

Many years have passed invisibly. *Tempus fugit* (time flies. Ed.). These decades seem to have flown by as inexorable fate has willed; time changes us all, and all around us. However, our glorious past, the 1960s, will always remain in our memory. This period of time our cohort: Platon Kostyuk, Lida Savoskina, Zoya Sorokina-Marina, Galina Skibo, Lysia Vihreva, Nellya Pogorelaya, B. Pyatigorskii, V. Gerasimov, N. Preobrajenskii, A. Pilyavskii, nonpareil D. Vasilenko, and I enthusiastically worked together in the Dept. of GPNS.

However, I also have had some conflicts with my boss. I have a recollection of the end of the cold winter in 1965. Before, my five original manuscripts (in co-authorship with my teacher P.G. Kostyuk) were submitted to the Moscow scientific journals. It was snowing. I saw snow-covered streets and snow-covered Bogomoletz Park from the first floor of my room. I was sitting at my desk making some notes. Just as my boss came in, he started out by attacking me and out of a clear sky. He told me about a transfer of my assistance N. Babetz to another research group in order to perform technical service of the new French-made setup "Racia" enabling scientists successfully to run electrophysiological studies on the cat spinal cord. I had an objection. I was not a diplomat yet. I was only 32 years old and my supervisor was older, 41 years old. "It is my order!" my supervisor said. And he spoke to me as if we established new contact, namely manager-subordinate relationship but not teacher – student one. He told me that he is the chief, and I am just only collaborator of the Dept. It was rather painful for me to hear

such words. I was silent. I crumpled a sheet of paper covered with my writing and began to cry… "I'm only graduate student" was in my mind and in all my body… The next day I have written the letter of voluntary resignation addressed to the Director of the Institute Acad. A.F. Makarchenko. I was the first and last "victim" of such circumstances. Many years have elapsed. My boss became an excellent diplomat; his students had never heard rude remarks from him. He could only say: "I am surprised." My vivid recollections are the homage which I pay to my first great teacher Platon Kostyuk.

2. Ukrainian endocrinology of socialist realism

At the beginning of 1965, by the decree of the Ministry of Public Health of the Ukr. USSR, Akad. V.P. Komissarenko founded a new research institution in Kyiv, the Institute of Endocrinology and Metabolism, and occupied the position of its first director. From February 10, I occupied the position of his scientist (Dr. Sci. K.P. Zak, a student of Akad. V.P. Komissarenko, was also the scientist). During the early years, the Institute was located in the beautiful two-storied mansion just at the corner of Shevchenko's Boulevard 8 and Pushkinskaya Street. Here, there was rather big patio. I took on a job (my first job): it was necessary to re-equip field service, organize the work shop, and storehouses. The scientific staff of the Institute, administrative staff, and support personnel has mushroomed up. My second chief was 58 years old. Over a long period of time, he worked under the guidance of Akad. A.A. Bogomoletz. Until the end of 1964, Akad. V.P. Komissarenko was the head of the Department at the Bogomoletz Institute of Physiology of the Akad. Sci. of the Ukr. SSR. He also was well-trained in the Laboratory headed by Prof. H. Selye (the founder of the theory of stress and general adaptation syndrome, GAS) at the Institute of Experimental Medicine, Montreal (Canada). Thus, Prof. A.A. Bogomoletz and Prof. H. Selye were indeed his great teachers. There was an ambitious task in front of our energetic, full of bush fire, and talented leader and associates of his Institute: to lay the first stone of fundamental endocrinology as a science in Ukraine. It was impossible for us to stand against his energy and charm, and all of us worked wholeheartedly. In the first year, Acad. Komissarenko offered a job at his Institute for many young and talented scientists from Kharkov and Odessa (PhD Reznikov A.G. and others). In our environment, in this difficult period of formation of the Institute, a unique aura of high respect and mutual support was formed. I was Senior Res. Ass. in the Laboratory of Physiology. I again began my career from scratch. As before, I began to actively seek for biochemical equipment, amplifiers, oscillographs, and stimulators. I remember that we received a great number of items of equipment retired from the Institute of Cybernetics nearly in the mid-1965. We also bought biochemical reagents. Our experimental infrastructure and machine shops took on life. I again began to create a set of electrophysiological equipment for recording of neuronal activity in mollusks. I should emphasize that our director was a very respected within the circle of communist party functionary, government officials, and even writers in Kyiv and Moscow. One day (it was in the autumn of 1965), my second great boss, the master of science and life, said me that Japanese exhibition of high-resolution electronic microscopes (JEOL) should be opened in Moscow and that he wants to go to Moscow with me. I agreed with my boss with ineffable joy. I should note that I already published my first paper entitled "Submicroscopic Structure Giant Neurons of the Mollusk *Planorbis corneus*." I remember the interesting events in Moscow. We are in Japanese exhibition; we are burning with curiosity. Here, booklets, badges, and even 3-D postcards with Japanese women that winked are distributed… but

there are only four high-resolution microscopes for all 16 republics of the former USSR. Guests and visitors of this exhibition were talking about partitioning the spoils system by the rules of the well-known character Popandopulo: "…this is my… (Moscow), and this is… also my!" Of course, I lost all hope that Ukraine should receive an electronic microscope, but Vasiliy Pavlovich smiles mysteriously and repeats: "Choose the best!" (i.e., JEM–100). Later on, it came to my knowledge that Vasiliy Pavlovich had personal encounter with N.B. Podgornyi (the first Secretary of the Central Committee of the Communistic Party of Ukraine up to 1963 and the Chairman of the Presidium of the Supreme Soviet of the USSR from 1965) who already gave the special order specifically to send one electronic microscope JEM-100 to Ukraine. This JEM-100 was the first high-class electronic microscope in Ukraine that was actively used in the research at Komissarenko's Institute (in winter of 1966), and I together with DSc. K.P. Zak began electron microscopic examination of blood cells in dogs. The first experimental paper was published already in 1967 [12].

In that time, I also actively studied electrical activity of the giant neurons, located in the buccal ganglia of mollusks. These giant neurons of *Planorbis corneus* exhibited intense electrical activity periodically (after 3 sec) substituted by deep (up to 30 mV) and prolonged (nearly 2 sec) inhibitory hyperpolarization. These parameters sharply changed with the addition of different neurotransmitters (or other biologically active substances used in very high dilutions) to superfusing solutions. I remember that, one day, Vasiliy Pavlovich invited high-profile officials of the Ministry of Public Health, writers, and journalists in order to demonstrate at the Institute the electrical activity of these giant nerve cells of *Planorbis* using microelectrode technique for recording of action potentials, combined it with video and sound control. When guests saw alternate impulse discharges of a separate neuron on the screen of oscillograph and heard how this neuron "speaks," they were truly amazed. A well-known Ukrainian writer Natan Rybak even exclaimed: "This is real Ukrainian endocrinological science of socialistic realism!" After inspection of other Labs and JEM-100, all guests (as well as associates of the Institute) went to the monument of the leader of world proletariat (V.I. Lenin) to give him "thanks for the miracles of socialistic science" in Ukraine. The monument was constantly visible from the windows of the first floor of our Institute. Our valuable time passed in such a way, in painstaking labour and vain efforts. My second boss (Acad. V.P. Komissarenko) decided for the expansion of investigations. Then, an excellent German EM (Carl Zeiss Jena) and some optical microscopes were additionally purchased for Labs. Somewhat later my first chief P.G. Kostyuk (from 1966, Corr. Member AN USSR and director of the Bogomoletz Institute of Physiology) was invited to the Komissarenko's Institute. Of course, he met me and asked me about my results obtained using EM and my electrophysiological investigations in the Institute. Then he simply said that a long time elapsed since our last "fight". The summer, but not winter, is now onside and that I can come back in the Dept. of GPNS. I decided to return "home" to the Bogomoletz Institute of Physiology. More than 40 years have elapsed, but I remember now only my deep emotions, tears in my eyes and the last words of my dear second teacher: "Volodya, I do not object you returning "home", to your *Alma Mater*.

3. Revolution in neuroanatomy of the 1970s and my third great mentor

At the beginning of 1970s, methods based on cell biology and chemistry, aimed as "pathway tracing" via anterograde and retrograde axonal transport of horse radish peroxidase

(HRP) and techniques for immunohistochemical localization of neurotransmitters in the brain have been introduced. Acad. P.G. Kostyuk remained abreast of scientific developments. His very interesting papers have been published in International Journals; a paper describing ultrastructural organization of propriospinal projections in cat was published in co-authorship with me in *Brain Res.* (1972). At that time, my mentor already became internationally renowned and occupied the position of Vice-President of IBRO/UNESCO (1974–1979). With the help of my supervisor, I have got Invitation to work for the period of 10 months (using IBRO/UNESCO fellowship, 1975–1976) in the Dept. of Anatomy of the Erasmus University in Rotterdam (the Netherlands) headed by the famous European scientist Prof. H. Kuypers. Thus, Prof. H. Kuypers (1925–1989) became my third great teacher. Indeed, all of us who were privilege to contact with him or work with him are fully aware of the really important scientific contributions that he made during his lifetime. At the beginning of our experiments on cats and rats, we found that microinjections of HRP into the spinal gray matter resulted in retrograde labeling of only a very limited number of brain stem neurons. About a year before, Prof. K. Kristensson from Sweden has found that effective retrograde transport of HRP in transected axons really exists. Thus, as the first step I studied temporal relationships between transport of HRP (Sigma, USA; Boehringer, Germany) and induction of chromatolysis. Prof. H. Kuypers, DSc. J. Siegel (from USA), me, and two young scientists from the Dept. of Anatomy (C. Catsman-Berrevoets and I. Molenaar) worked together. In this Dept., there was also a "secret" scientific Lab. with which Prof. H. Kuypers during 1975–1976 had begun developing a new retrograde labeling technique employing fluorescent substances (Primuline, Fast Blue, Evans Blue, and others) for double labeling of neurons in rat through divergent axon collaterals. Only in 1977, the new technique was published by Prof. H. Kuypers and young scientist Marina Bentivoglio (from Italy, using IBRO/UNESCO fellowship). However, at the beginning of 1975 our group included Prof. H. Kuypers, two technicians, and me. We all made subsequent spinal injections of HRP into the gray and white matter of the rat's or cat's spinal cord and in such way damaged as many axons as possible.

The method, as we found, was very effective even in the large animals (in cats). Too many, about twenty cats (two animals per months) were used for investigation of retrograde axonal transport of HRP from spinal cord (C2, C8/TH1, TH5, L2, and L4 segments, respectively). The most important part of experimental work (contralateral or bilateral spinal lesions at C2, C8/TH1, which in the respective cases spared small portions of the different funiculi, unilaterally), was done only by Prof. H. Kuypers himself and his two very skill assistants using special binocular microscope and vacuum pump. I analyzed experimental material (sections of the cat's brain and spinal cord) and prepared illustrations. I must note that cats were extraordinary spinal animals. After operation, all these cats needed special conditions for rehabilitation. Only one week later, spinal HRP injections were made below the lesions in both animal groups (30 μl of 30 % HRP was deposited in the case). We found that, after injections of HRP, the enzyme was transported retrogradely to brain stem neurons in the bulbar medial reticular formation, the vestibular complex, and the red nucleus. We obtained intriguing facts in our investigations: we recorded the labeled neurons in the *locus coeruleus* and *subcoeruleus*, as well as in the paraventricular hypothalamic nucleus [6]. The existence of straight pathways from hypothalamus to the spinal cord (as can be seen in fig. 2, A) has not been demonstrated earlier. The next step of our study was to determine the corresponding spinal funiculi

where descending fibers (from various brain stem cell groups) are located. Indeed, in our pioneer studies, we found that the fibers from the hypothalamus, which descend throughout the spinal cord, are located mainly in the lateral funiculus, ipsilaterally (fig. 2, B).

My stay in this beautiful country was going to the end. As often as I came in the evening to the downtown at the bus station Rotterdam – Istanbul and sadly looked after the bus with laborers from Turkey: there, to the east, is also my country, my Ukraine. I kept firmly in my mind (as the prayers) Kostyuk's hard directions he gave me in the bar of Moscow hotel of the Acad. Sci. of USSR. "You should obtain good results and publish a paper in the International Journal as soon as possible!" he said. He went to Switzerland, the some time as I went to the Netherlands. I remembered that he was on probation in Australia during

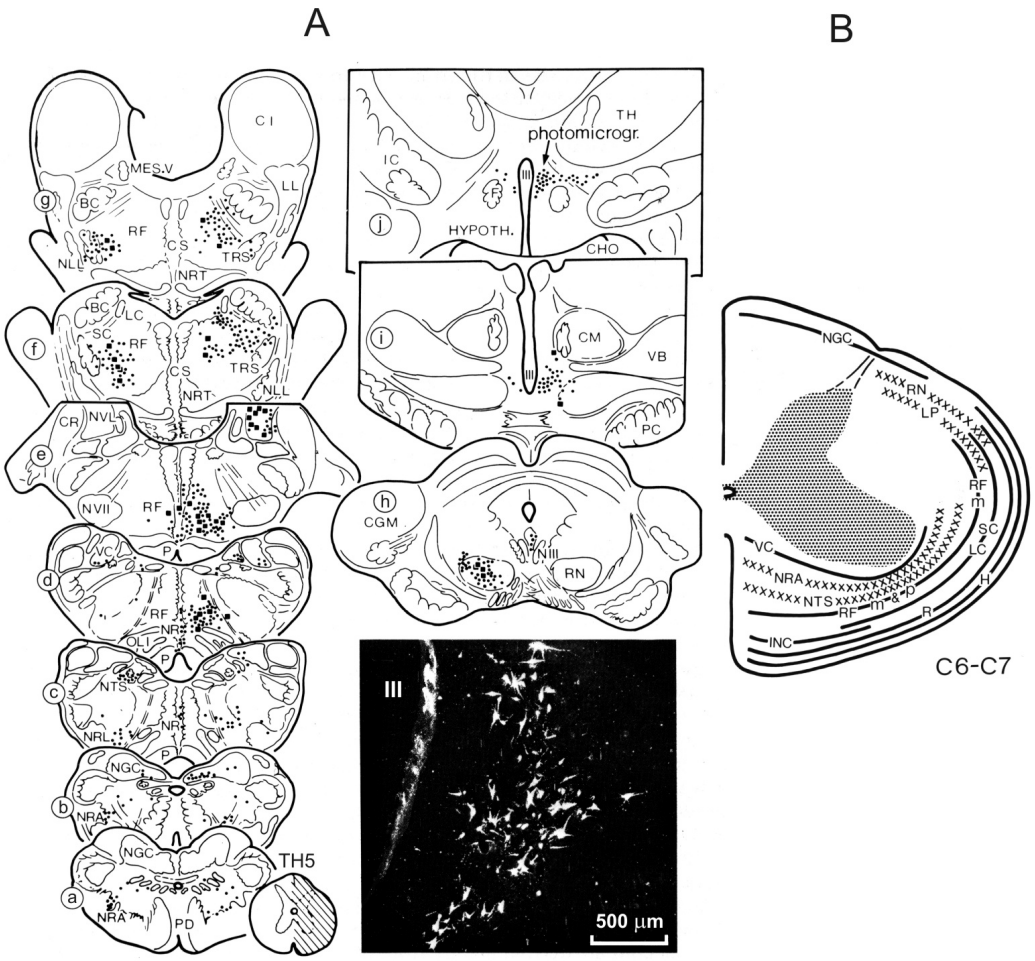

Fig. 2. Cells of origin of descending brain stem pathways to the spinal cord in cat. (A) distribution of retrogradely labeled neurons (dots and squares) at various brain stem levels (a–j) after unilateral (right) injection of HRP at TH5. Note labeled neurons in the *locus coeruleus* (LC) and the nucleus *subcoeruleus* (SC) at levels f and g, and in the paraventricular nucleus of hypothalamus (H) at levels i, j ipsilaterally, as well as in the lateral pontine tegmentum (LT), adjoining the rubrospinal tract (TRS) contralaterally. Dark-field photomicrograph shows the labeled neurons in periventrscular (III) area (level j) in the hypothalamus; (B) funicular trajectories of descending brain stem pathways in C6–C7 segments. Abbreviations: CM, center medium; F, fornix; INC, interstitial nucleus of Cajal; NGC, nuclei gracilis and cuneatus; NRA, nucleus retroambiguus; NTS, nucleus of the tractus solitarius; P, pyramidal tract; R, raphe nuclei; RFm, medullary medial reticular formation; RFp, pontine medial reticular formation; RN, red nucleus; TH, thalamus (modified from [6, 7])

five months and, shortly after this probation, he published (in co-authorship) five scientific papers in 1962. But I had here (in the Netherlands) one problem. Prof. H. Kuypers even not guessed that he is a person of considerable talent. Each scientific problem he touched always turned into pioneering discoveries in Neuroscience. But he was also the father of six children. He lived in the beautiful realm together with his horses, car, certainly, with his beloved wife and dear science. Surely he did not know our "specific" idiom (in Russian) extensively used in our real life in the ex-USSR, such as "*рвать подметки на ходу!*" ("be a fast climber," or "not to waste any time!"). Really, at the end of the first part of my academic mission (in late spring of 1975), I prepared all primary materials for our scientific paper to be published. It was necessary to write at least a short communication and submit it to the International Journal. However, Professor "dragged" preparation of our manuscript. His teaching load and extensive international cooperation strongly distracted his efforts from the main subject – the manuscript. In this situation, I was running out of time absolutely. Academic circles "boiled" like the samovar; this was like a three-ring circus: "The winner is the scientist who first publishes the novel data!" Nearly two weeks before my return to Kyiv. I again insisted on the necessity to get through with our manuscript. I realized that my dear maestro did not comprehend my uneasiness. "You discovered for the first time the direct pathway from the hypothalamus to the spinal cord along its whole length!" I exclaimed... and then my voice suddenly trembled, and my tears dropped on Israel strawberries covered with sour cream and sugar. It has worked. Teacher put his hand on my shoulder, and then he had merely said: "I understand!" Later on, it came to my knowledge that, after our talk, Prof. H. Kuypers intensely worked during four days in his room with our manuscript. On the 5th day, in the morning Professor left by Euro-Express, together with our manuscript, for Germany to

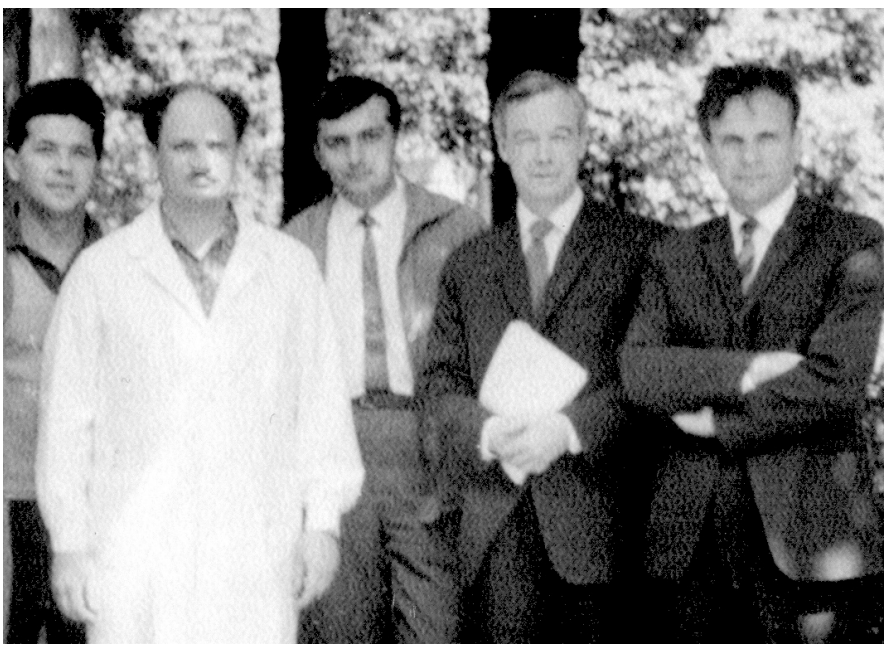

Fig. 3 (Foto). Our glorious past (the 1970s), Akad. Kostyuk P.G., Nobel prize winner Hodgkin A.L. and three yang scientists (Vasilenko D., Pyatigorskii B. and Preobrajenskii N.) in Bogomoletz Park.

Prof. M. Zimmermann (II Physiological Institute in Heidelberg). He was the Editor of a new International Journal *Neuroscience Letters*, which first issue was supposed to be published in a month. Platon Kostyuk and I were pleased, since this paper was published in the first number of the Journal and on its first pages [6]. Yes, indeed, it was my victory achieved through enormous efforts. Later on, a known research group of morphologists (Cowan's laboratory, USA) published their own data about straight pathways from the hypothalamus to autonomic centers of the rat spinal cord [9]. It was also shown that direct projections from hypothalamus to spinal cord arises in paraventricular nucleus and ends in the intermediolateral column and marginal zone of the dorsal horn [10]. To emphasize the contribution of Prof. Kuypers to Neuroscience, I must note that only Pavlov's term *conditioned reflex* (1903), Bykov's postulates about transfer between hemispheres in Pavlov's Lab., and our intriguing facts about straight pathways from the hypothalamus to the spinal cord in cat [6] were discussed and cited in the last issue (50, № 5/6, 1999) of the millennium Brain Res. Bull, which was devoted to the achievements in Neuroscience of 20th Century (see [10]).

P.S. We have Shakespeare at our tongue's end:

"I hold the world but as the world, Gratiano,
A stage where every man must play a part."

(William Shakespeare. The Merchant of Venice)

The reality in Neuroscience (here severe competition reigns) means to sing certain parts of "science services" (by analogy "church services"), since we are devoted ourselves wholly to the service of Science. My first 20 years in Neuroscience will be finished. I had three great "mentors" and three times I cried bitterly. I am going home in order "to sing my own song" tomorrow! However, on the next day… the new *stage* and new singers will appear (see [3–5, 11]).

V.A. Maisky

MY FIRST 20 YEARS IN NEUROSCIENCE

Intriguing facts were obtained in the first electrophysiological investigations (1964) that the action potentials (AP) produced by direct depolarization of the cell membrane in different species of mollusks showed specific relations to changes in external ionic composition. In *Helix* neurons, the generation of AP was well maintained in sodium-free solutions with high calcium or barium content. The amplitude of the spike overshoot in the case was linearly related to the logarithm of calcium concentration. It is interesting that increase in external calcium ions decreased the ionic conductance of the resting membrane (R_o) also in linear relation to the logarithm of Ca^{2+} or Ba^{2+} concentration. It was found for the first time (1965) that addition of Ba^{2+} to the external solution produced in the neurons well-developed prolonged (protracted) APs in all cases. However, the excitability of *Planorbis* and *Limnea* neurons was rapidly (during 3 min) reversibly depressed in sodium-free solutions. We found that, after injections of horse radish peroxidase (HRP) in the spinal cord of cats, the enzyme was transported retrogradely to brain stem neurons in the bulbar medial reticular formation, the vestibular complex, and the red nucleus. We obtained (1975) intriguing facts in our investigations: we recorded the labeled neurons in the *locus coeruleus* and *subcoeruleus*, as well as in the paraventricular hypothalamic nucleus. The existence of straight pathways from hypothalamus to the spinal cord has not been demonstrated earlier. The next step of our study was to determine the corresponding spinal funiculi where descending fibers (from various brain stem cell groups) are located. Indeed, in our pioneer studies (1977), we found that the fibers from the hypothalamus, which descend throughout the spinal cord, are located mainly in the lateral funiculus, ipsilaterally.

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