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The historical retrospective of neurophysiological laboratory at the Faculty of Medicine in Novi Sad

Историјска ретроспектива неурофизиолошке лабораторије на Медицинском факултету у Новом Саду

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SUMMARY

In the course of several decades at the Faculty of Medicine in Novi Sad, fundamental studies in the field of neurosciences were of great importance and continually up-to-date with global scientific achievements. These studies were applied by using the stereotactic method, single unit recording and electroencephalography. Laboratory of Neurophysiology was established in 1965 and since 1978 microelectrode and microiontophoretic techniques important for the registration and analysis of the activity of individual neurons were fully developed. Under the great influence of Russian neurophysiological school (P.K. Anokhin, K.V. Sudakov) the emphasis was on the study of the theory of Anokhin's functional systems. Recently, epilepsy, brain ischemia and the influence of different medications, auxiliary medicinal products and physical agents (electromagnetic radiation) on central nervous system, behaviour, learning and investigated pathological conditions have been studied. Scientific collaboration with renowned institutions in the country and abroad was established, numerous scientific projects were relized, expert international meetings were organized and numerous significant studies were published. These results have often been the basis for further clinical investigations and the improvement of preventive or curative treatment of patients. Researchers from the Laboratory participated in the education of new generations of neurophysiologists encouraging their scientific curiosity and love for fascinating mechanisms of nervous system functioning.

Keywords: history, 20th century; physiology, history; Schools, Medical/history; Serbia

САЖЕТАК

Током више деценија на Медицинском факултету у Новом Саду су велик значај имала фундаментална истраживања у области неуронаука, која су константно ишла у корак са глобалним научним достигнућима, а спровођена су применом метода стереотаксије, микроелектродне регистрације активности појединачних неурона и електроенцефалографије. Лабораторија за неурофизиолошка истраживања је основана 1965. године, а од 1978. у потпуности је разрађена микроелектродна и микројонтофоретска техника, значајне за регистрацију и анализу активности појединачних неурона. Под великим утицајем руске неурофизиолошке школе (П.К. Анохин, К.В. Судаков) акценат је био на изучавању теорије функционалних система Анохина. У новије време су истраживане епилепсија, исхемија мозга и утицај различитих медикамената, помоћних лековитих средстава и физичких агенаса (електромагнетних зрачења) на централни нервни систем, понашање, учење и истраживана патолошка стања. Установљена је научна сарадња са истакнутим институцијама у земљи и иностранству, успешно су реализовани научни пројекти, организовани стручни међународни скупови и објављиване бројне значајне научне публикације. Ови резултати су често били фундамент за даља клиничка истраживања и унапређење клиничког и превентивног рада. Истраживачи ове лабораторије су учествовали у едукацији нових нараштаја неурофизиолога, подстицали њихову истраживачку радозналост и љубав за фасцинантне механизме функционисања нервног система.

Кључне речи: историја, 20. век; физиологија, историја; медицински факултет, историја; Србија

INTRODUCTION

The purpose of this study was to present the evolution of neurophysiology at the end of the 20th and beginning of the 21st century at the Faculty of Medicine in Novi Sad. There was a tendency for neurophysiological research to keep pace with contemporary attitudes within the Russian school of neurophysiology, but at the same time respecting the achievements and trends of Western physiological thought.

ESTABLISHING OF NEUROPHYSIOLOGY

The beginning of educational and scientific work in the Kingdom of Serbs, Croats and Slovenes in the field of physiology is related to the famous Austrian physician and professor from the Faculty

of Medicine in Leipzig, Dr. Richard Burian (1871-1954). He was invited to organize the educational process. Since 1920, at the Medical Faculty in Belgrade Dr. Burian became a professor and gave enormous contribution in establishing the Institute of Physiology, which even today bears his name [1]. The University of Novi Sad and the Faculty of Medicine were established on the same day, on May the 18th of 1960. In order to organize lectures many prominent domestic and foreign professors were invited. In year 1961 the Department of Physiology has been founded. The first director of the Department and first professor of Physiology was Prof. Aleksandar Sabovljev (1907-1963) from the Faculty of Medicine in Sarajevo, a former student of Prof. Richard Burian. He founded the basic concept of work and development of physiology in Novi Sad as an interdisciplinary branch of medicine [2].

In October 1963, director of Department of Physiology became Prof. Radmilo Anastasijević (1915-1975), also student of Prof. Richard Burian. With the support of state institutions, the initial problems were solved by the construction of new buildings and by providing financial resources for purchasing of specific equipment for scientific-educational process.

The founder of neurophysiology at the Faculty of Medicine and the Laboratory of Neurophysiology (established in 1965) is Prof. Mihailo Bajić. From 1961 to 1967, Prof. Bajić, as a scholar of the USSR's, stayed at the Department of Normal physiology at the Sechenov First Medical Institute in Moscow. He worked under the guidance of brilliant follower of I.P.Pavlov, academician Pyotr Kuzmich Anokhin (1898-1974) itself, author of the theory of functional systems (1968) [3]. Functional systems are extremely dynamic, autonomous central-peripheral organizations, united in cyclic organization in order to provide the ultimate, for an organism useful, result. According to Anokhin, operations and architectonics of functional systems include: afferent synthesis, decision making, forming the action result acceptor, efferent synthesis, the action itself and recurrent afferent information about the result. Prof. Bajić conducted examinations of bioelectrical activity of brain of experimental animals (electroencephalography) in the process of formation of conditioned reflexes after application of strychnine, which facilitates synaptic transmission and effect of acetylcholine. He followed the dynamics of formation of conditioned reflex and registered the reduction in number of involved brain structures through the time of its consolidation. The results are integrated in the candidate dissertation, which was successfully defended in 1966 in front of the Council of The Russian Academy of Sciences, acquiring the scientific degree Candidate of Medical Sciences of USSR. At that time, in Former Yugoslavia there were only 21 doctoral thesis defended in the field of medical sciences [4]. Prof. Bajić also defended his doctoral dissertation in Novi Sad in June, 1969 [5]. In the dissertation he highlighted the significance of reticular formation in the processes of conditioning, learning and excitation of higher parts of nervous system (Figure 1).

In neurophysiological laboratory in Novi Sad, Prof. Bajić introduced stereotactic method for implanting microelectrodes and this enabled the registration of single unit activity in various brain structures in different conditions and during application of biologically active substances. Prof. Bajić

Figure 1. EEG analysis in neurophysiological laboratory on Gallileo brand EEG in June 1974; left to right: Professors: Bajić, Sudakov and Lažetić.

was a visionary, a wide-range scientist, but also an excellent manager. At the time he was a dean of the Faculty (1973–1977) and the director of the Institute of Preclinical Disciplines (1975-1990), Prof. Bajić significantly improved the activities, especially of neurophysiological laboratory, thanks to acquisition of modern equipment, developing cooperation with other institutes in the country and abroad and by generating quality staff and team dealing with demanding and futuristic research in the field of neurosciences. For merits in the development of neurophysiology and international cooperation, Prof. Bajić was awarded the Gold medals in the name of I.M. Sechenov in 1978 and 2005 in Moscow.

THE BEGINNINGS OF NEUROPHYSIOLOGICAL RESEARCH

Since the Department of Physiology did not yet possess EEG machine, research in the field of neurophysiology has actually begun at the Provincial Children's Hospital. The first EEG machine at the Department of Physiology (a fourteen-channel EEG of Gallileo brand - model E 14b), was purchased in 1971 through the funds of the Community of Medical Scientific Institutes of the Republic of Serbia (**figure 1**). In the same year for EEG studies Pavlovian chamber was constructed. It was consisted of three parts: for the registration of EEG in humans; in animals and for sleeping studies. It was a rarity at the time and unique in former Yugoslavia. One of the first works in the field of neurophysiology that emerged from this laboratory was the master thesis "Neurophysiological aspects of the effects of neuroleptics and anti-Parkinsonian drugs" of Prof. Zvonimir Lević (1936-2009), the famous neuropsychiatrist, later also the director of Belgrade Clinic for Neurology [6].

Research of the nervous system were acquisited more slowly because the tracking of activity of central nervous system is more complexed than tracking any other system in organism. Thus, plenty of researchers of neurophysiological laboratory and other segments of the Faculty used EEG, stereotactic method and technique of registrating single unit activities in examinations of various brain structures. Special focus was on the changes of spontaneous bioelectric activity of different brain structures in terms of the formation of functional systems of behaviour.

Prof. Bogosav Lažetić made a series of studies on registrating EEG activity of rabbits during repeated light stimulation and during feeding behaviour (figure 2), starting his experiments in

Figure 2. Representation of experimental environments during making of one of the doctoral dissertations in the Laboratory [7].

Moscow and finishing them in Novi Sad. He examined the structure of functional systems that are the basis for orienting response [7]. He defended his doctoral dissertation in 1979.

Prof. Danka Filipović studied alimentary and motivational excitation of *nucleus caudatus* and sensorimotor cortex and their changes under electro-cutaneous and light stimulation. She registered EEG and neuronal – single unit activity during electro-cutaneous and light stimulation [8]. Registered changes in neuronal activity of *nc. caudatus* and sensorimotor cortex in act of alimentary behaviour have indicated that examined structures participate in the realization of coordinated motor activity, learning and emotional states related to alimentation as dominating motivation. It was also found that specific components of studied structures were involved in certain stages of registered forms of behaviour (Figure 3) [9,10].

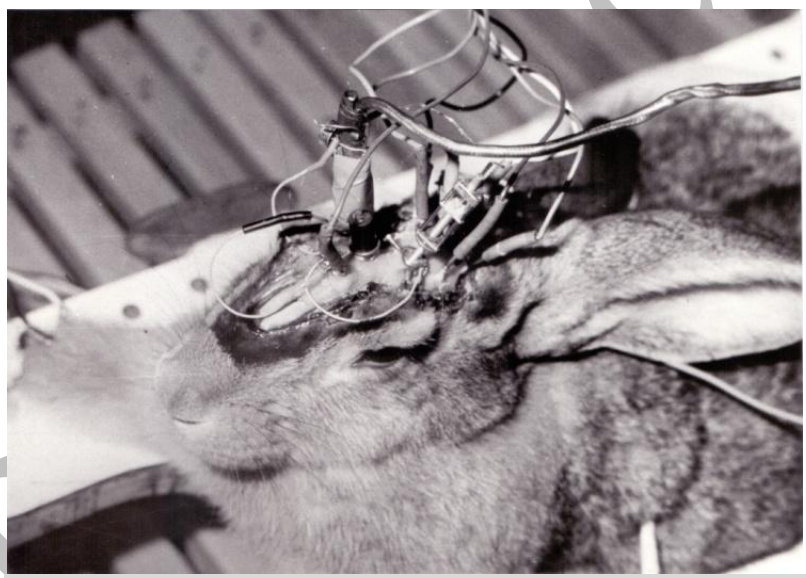

Figure 3. The figure shows simultaneous registration of EEG activity and activity of single neurons using microelectrodes implanted in deep subcortical brain structures on experimental animal (rabbit) [8].

Prof. Vesna Ivetić also registered EEG and single unit activity. Her field of research was low- and high-frequency electrostimulation of hypothalamus, hippocampus and reticular formation and evoked effects on bioelectric activity of cortex, in hungry and satiated animals. Simultaneously, influence of these stimulations was also registered on breathing cycle and behaviour of experimental animals. It was already discovered that hippocampus has been associated with motivation, emotions, learning, memory and goal-directed activity, but Prof. Ivetić studied the way of the activation of hippocampus in the realization of some individual forms of behaviour. She elaborated and explained the activity of neurons of hippocampus (in its areas CA-1 and CA-3) in alimentary behaviour [9,11]. Her dissertation confirmed O'Keefs findings of "place cells" in hippocampus and showed that in the

hippocampus exist neuronal ensembles (so called "goal cells") whose neuronal activity correlates with the development of the goal-directed alimentary behaviour [12]. Professors Ivetić and Filipović were among the first in neurophysiological examinations in the world who monitored neurophysiological functions on laboratory animals during free and feeding behaviour recording it on video tape (Figure 4).

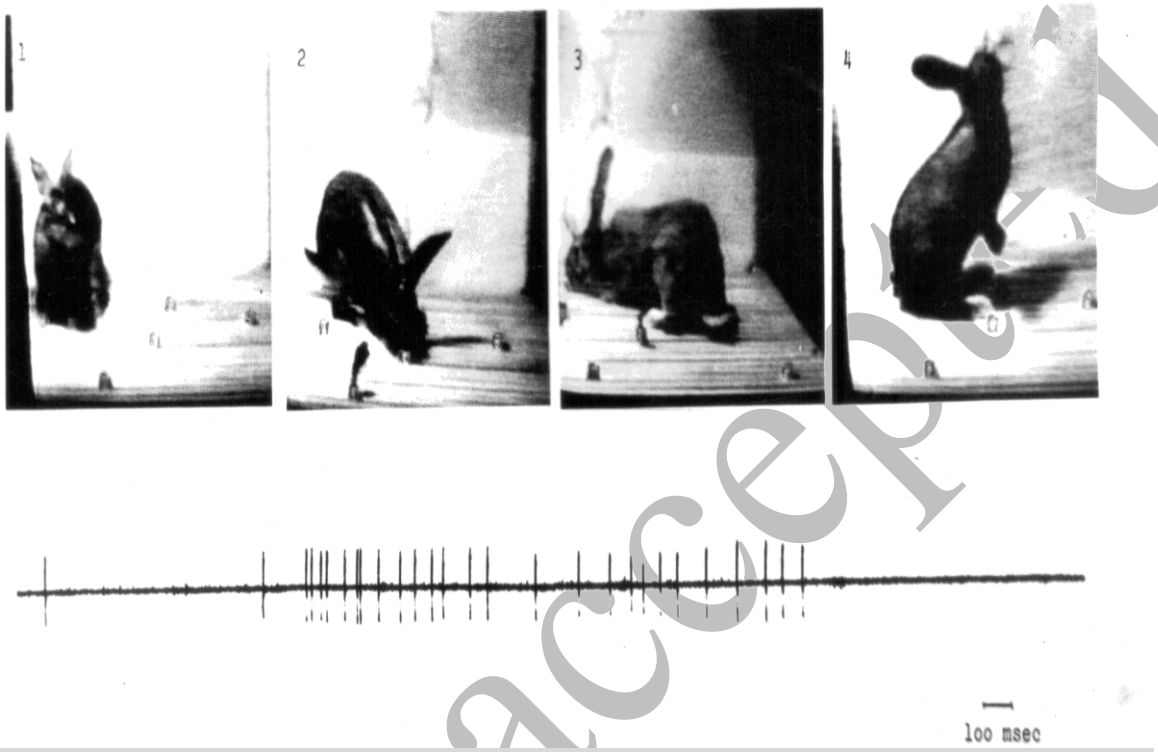

Figure 4. Orienting and searching reaction is fundamental biological reaction. The picture shows rabbit with implanted microelectrodes in *nucleus caudatus*. The neuron was active during searching the food and orienting in newly introduced space (the frames are cut out from a video made in neurophysiological laboratory) [8].

Later on, a microiontophoretic method was also applied in investigations in the Laboratory. Prof. Đorđe Sterio simultaneously examined EEG and single unit activity during microiontophoretic application of primary neurotransmiters – acetylcholine and noradrenalin. In the spotlight of his doctoral dissertation was the influence of cycloheximide, an inhibitor of protein biosynthesis, for which he discovered it slowed down the process of learning. He also concluded that arousal in motivational hypothalamic structures highly specific for orienting response leads to the synthesis of proteins in sensorimotor cortex [13]. Electrophysiological and neurochemical mechanisms of learning that were later in the focus of examinations conducted in the Laboratory aimed to unravel the complexity of this mechanism [14,15]. It became obvious that not only the whole brain, but the single neuron itself also, has an integrative function.

Guided by experience and knowledge of older colleagues from neurophysiological laboratory, Prof. Nada Naumović's first field of research was microiontophoretic application of cycloheximide, actinomycin D and acetylcholine and their effect on learning process and activity of neurons and on cortico-subcortical relations. The objectives of these experiments aimed to reveal how neurochemical

processes regulate the electrogenesis in neuron and which new properties occur in the electrogenesis in the course of learning [16]. As young scientist, eager for new knowledge, she participated in European training program in neural correlates of cognitive processes with presenting as well her own scientific research. The essence of interest of Prof. Naumović during investigation on doctoral thesis was examination of experimental atherosclerosis, cerebral ischemia and influence of specific calcium channel blocker (nimodipine) on their development and it's neuroprotective or neuromodulatory effect on brain activity [17,18]. Based on the findings obtained on experimental animals (rabbits), clinical studies were performed in patients following an ischemic type of stroke. The results showed that nimodipine, in addition to known effects, exhibits an anti-aterogenic and neuroprotective effects [19].

INTERCONNECTION OF BASIC RESEARCH WITH CLINIC

Since 1993, Prof. Sterio continued his successful career at the Department of Neurosurgery at NYU School of Medicine where he uses microelectrode technique for the treatment of Parkinson's disease in humans [20].

Prof. Filipović and Prof. Ivetić completed a specialization in neuropsychiatry and subspecialization in neurophysiological diagnostics and Prof. Naumović physical medicine with medical rehabilitation. The studies conducted on experimental animals have gained a new value because they became the basis for close cooperation with clinics and for the improvement of their work. The outcome of this developmental course was targeted research which was carefully planned in close collaboration with clinics and results that improved the concepts of prevention and provided a better outcome of therapeutic treatment of patients with neurological disorders [21]. Certainly, from this kind of cooperation, a series of multidisciplinary scientific publications emerged.

SCIENTIFIC COLLABORATION

In the middle of 20th century cooperation between Yugoslav scientists with Russian physiological school began. In 1956, Prof. Ljubisav Rakić from Belgrade (1931–) started the international cooperation with institutions in Moscow, USSR. In early 60's, Prof. Bajić maintained and strengthened the cooporation during his stay in Moscow. Two years after he became a dean of the Faculty, he established the Institute of Preclinical Disciplines in 1975 which created a new level of cooperation with Moscow's Institute.

In 1971, a protocol for Scientific-Technical Cooperation between Yugoslavia and USSR was signed in Belgrade. Visit of Russian academician Konstantin Viktorovich Sudakov (1932–2013) to Novi Sad in 1974 (Figure 1) institutionalized scientific cooperation. It was actively maintained for decades, up to the year of 2005. The main directions of research were general mechanisms of behaviour and motivations and integrative activity of neurons. On the basis of this cooperation, several interstate scientific projects were conveyed and even a few symposiums organized with the

issue of monographs. After the last one, organized in 1998 in Novi Sad, on centenary of Anokhin's birth, an extensive monography "Basic and clinical aspects of the theory of functional systems" was publicated [22].

Many associates of the Institute of Normal Physiology from Moscow worked in Novi Sad as well in the international station at the Institute of Marine Biology in Kotor. This institute was opened in 1961 (at that time it was known as Bureau for Marine Biology). Because of its foreign policy interests, American Government partially financed these investigations through the Public Law-480. The examinations in the Institute were also observed by the Central Intelligence Agency and its report from 1973 (declassified in 2009) emphasized significant progress in the field of neurophysiology in Yugoslavia among all other medical fields [4]. In the Institute, in the Laboratory for Brain Research were investigated the characteristics of single unit activities of abdominal ganglia of sea slug *Aplysia depilans* which is analogous to biological object of *Aplysia californica* (used in his decades-long research by Eric Kandel, Nobel Prize winner in 2000). By the application of well established microelectrode and microiontophoretic techniques on this model, the researchers from the Laboratory of Neurophysiology from Novi Sad gave markable contribution to these studies [23]. The characteristics of some neurons of ganglia were examined and influence of different substances on their activity and mechanisms of learning.

Successful cooperation was also maintained with the laboratories of medical faculties in Belgrade, Niš, Kosovska Mitrovica, Banjaluka and Sarajevo as well as with the Faculty of Sciences in Novi Sad. Together with the Military Technical Institute in Belgrade, the influence of various nerve agents on the activity of neurons and the possibility of epileptiform discharges due to the applications of diagnostic ultrasound were studied. By the last decade of previous century, a joint research was realized with collaborators at the University of Rostov-on-Don, especially with the A.B. Kogan Research Institute for Neurocybernetics. Many publications came out from this collaboration [24].

Researchers from the Institute of Preclinical Disciplines actively participated in international meetings of the International Brain Research Organization (IBRO), European Brain and Behaviour Society (EBBS) and European Neuroscience Association (ENA). A fruitful cooperation and validity of scientific results of the Institute has been recognized in scientific community. In August 1987, Institute was honored to organize 19thAnnual Meeting of the European Brain and Behaviour Society in city of Novi Sad. On the conference, prominent and distinguished physiologists from Japan have participated, with whome Prof. M. Bajić and associates have earlier established correspondence and exchange of scientific thoughts. The scientific articles from this congress were printed in entirety by an Oxford-based publishing house – Pergamon Press [9].

MODERN TENDENCIES

By virtue of Prof. Bajić, purchasing of the first computer in 1981 and opening a new workplace for master in electrical engineering were organized. As a result, all subsequent research at the Institute

of Preclinical Disciplines had computerized data processing that was made out targeted and specially designed for each type of research being carried out. Purchase of DELTA 340/10 computer system (Iskra-Delta, Kranj) with 128 KB of internal memory was a huge financial investment but it enabled the analysis of acquisited bioelectric potentials in real time, which only a few centers in the world had opportunity at the time.

During the 20th century, very important issue of influence of artificial electromagnetic fields on human organism appeared. Thorough examinations of this influence have been conducted at the Department of Physiology. Laboratory for magnetobiological research that was formed in 1980's used different devices to expose experimental animals to magnetic radiation that were the basis of numerous studies [25]. Specially designed appliances were used for exposing animals to constant and variable electromagnetic fields and morphofunctional changes in central nervous system were documented. It was shown that the main link to the damages in CNS is in fact the vascular system, which consequently leads to secondary changes in nervous tissue. Many of these studies found their place in monographies dedicated to the topic of magnetobiology [26].

Since the early 1980s, a significant part of the research in neurophysiological laboratory has been dedicated to the study of hyperexcitability of the nervous system and epilepsies [27]. Electroclinical characteristics of wide spectrum of substances on experimentally induced epilepsies in laboratory animals have been studied [28]. These studies found their clinical application in 1995 when the Cabinet for Clinical Neurophysiology has been established. It was oriented not only for routine clinical work, but also for research activities. EEG characteristics after frequent use of cellular phones was investigated [29]. In the end, by the acquisition of modern appliances, the registration and analysis of cognitive evoked potentials (P300) are performed [30]. Beneficial effects of intermediate physical activities on cognitive functions have also been documented [31].

CONCLUSION

The Laboratory of Neurophysiology at the Faculty of Medicine was developed in very specific time. Although, it always has been a part of the University of Novi Sad, at the same time it changed four states and at least two forms of economic and social relations. Researchers from the Laboratory succeded to preserve scientific autonomy and with great scientific zeal and devotion managed to develope it. Professors who led the Laboratory at the same time tried to keep up with modern standpoints as well as to be competitive with the achievements on all levels of international scientific thought. This became possible by the openness for cooperation and appreciation of all the achievements and trends of scientific research conducted in the USSR, as well as throughout Western Europe, USA, Japan and other parts of the world.

In the Laboratory of Neurophysiology stereotactic method was applied in electroencephalographic, microelectrode and microiontophoretic examinations to monitor brain functions during different activities of organisms. At that point in time, Novi Sad was one of a few

centers in the world in which neuronal activity was registered during free behaviour of animals and during their goal-directed behaviour. Monitoring of bioelectrical manifestations in central nervous system in different functional states of experimental animals and in different forms of their behaviour gave a significant contribution to the understanding of neurophysiological mechanisms as well as defining of certain structures involved in particular functional system and form of behaviour of an individuals.

The results obtained in the Laboratory were published in numerous scientific publications in prestigious international journals. International multidisciplinary scientific meetings in Novi Sad were organized and researchers were presenting their works and holding lectures by invitation on an international expert meetings.

The outcomes of basic studies have found their meaning in clinical application. In this sense, the Laboratory was directly involved in improvement of prevention strategy for epilepsy and stroke, as well as in improvement of therapeutic outcome of patients with neurological disorders.

Professors and researchers of neurophysiological laboratory were unselfishly and devotedly transferring their knowledge and experience to new generations of researchers. Based on this work and research, hundreds of student, graduate, master, doctoral and scientific expert papers were written. This extensive educational work made new staff of the Faculty of Medicine in Novi Sad who distinguished themselves on many departments and significantly contributed the continuity of the existence, development and credibility of the Faculty of Medicine, University of Novi Sad.

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