# On The Work Of Spf Animal Facility

*Yu.O. Kalashova [1*], E.V. Kozhenevskaya [1], T.A. Larina [1], E.V. Eremina [1], D.V. Pakina [1], A.D. Gorb [1], V.G. Krut [1], E.D. Ivlev [1], S.A. Melnik [1], A.A. Mironov [1]*
[1] Lobachevsky State University of Nizhny Novgorod, 23 Gagarina ave., Nizhny Novgorod, Russia, 603950
[*] Corresponding e-mail: kalashova@neuro.nnov.ru

**Key words.** SPF animal facility, animal breeding

The animal facility is a complex system of special quarters and equipment for keeping and breeding of laboratory animals. The SPF (specific pathogen free) status of an animal facility means that the laboratory animals are free of particular pathogens causing various infectious diseases in mice and rats. Working with SPF animals under such conditions allows us to answer the question posed in the study. What is more important, the SPF status is required for maintenance of transgenic animals, mostly mice, because they haven`t a pathogenic microflora and possess minimum immunity, therefore, it is necessary to keep them in special conditions. There are only two similar complexes in Russia (at Pushchino and Novosibirsk University), thus the SPF housing room at Lobachevsky State University of Nizhny Novgorod is the third. The animal facility has been built according to strict demands of sterility, corresponding to the international standards of GLP and GMP. It has the seventh cleanliness class according to ISO. The multi-stage protection system prevents the permeation of infections. The clean zone of keeping animals is separated from the external environment by the autoclave and a special gateway.

The SPF animal facility of the UNN Institute of Biology and Biomedicine has been working since June 2015, the first mice have been accepted from the Nursery for Laboratory Animals “Pushchino”. Today, there are more than 1000 animals of 7 different lines in the SPF animal facility of Lobachevsky State University. Today, the planned capacity is 3000 animals, with potential increase up to 8000.

However, the work of the vivarium is not limited by keeping and breeding animals. The technique of embryo transfer is typically used for full breeding of SPF animals. Due to the fact that, there are the several behavioral plants in the SPF housing room such as Coulborn, the Laboras system, Panlab, it is enforceable to conduct a full cycle of all standard behavioral tests, to explore the processes of memory and learning, creation and reproduction of conditioned responses, to conduct a neurobiological, pharmacological and genetic research. Moreover, the operating room allows all necessary surgical manipulations for making different experimental models. Also the educational-practical center has been created on the base of the vivarium, conducting training, operating techniques, etc. in the barrier space.

We have already conducted a joint research work with international and Russian research centers. There is an agreement with the Nizhny Novgorod Medical Academy, Nizhny Novgorod Agricultural Academy, Minin University. What is more, the project for the study of mutagenesis has been carried out with the Institute of immunology of the clinic in Berlin, the German center for neurodegenerative disorders. Scientific researches of schizophrenia, epilepsy, Alzheimer's disease, memory at all levels (molecular, subcellular, cellular, tissue, up to behavioral characteristics) is going to be conducted on the basis of the SPF housing room at Lobachevsky State University of Nizhny Novgorod.

# A Single Episode Of Seizures Induced By Pentylenetetrazole Is Followed By A Cognitive Decline

*V.A. Aniol [1*], A.Yu. Ivanova-Dyatlova [1], A.O. Tishkina [1], V.V. Fominykh [1], A. Kvichanskii [1], N.V. Gulyaeva [1]*
[1] Institute of Higher Nervous Activity and Neurophysiology, RAS, Moscow, Russia
[*] Corresponding e-mail: aniviktor@narod.ru

**Summary.** Single episode of generalized tonic-clonic seizures induced by pentylenetetrazole led to slowly developing memory impairments in rats, accompanied by elimination of excessive newly generated young cells which were born in the hippocampus soon after the seizures and transient activation of microglial cells in this neurogenic niche.

**Key words.** Pentylenetetrazole, seizures, neurogenesis, neuroinflammation

## INTRODUCTION

According to different studies, between 5% and 10% of people suffer a single isolated seizure at some time in their life. However, little is known about effects of a single seizure on the cognitive function, and clinical investigations of this issue are not easy to perform. The aim of our study was to follow the time course of delayed effects of generalized clonic-tonic convulsions on learning and memory functions in rats.

## MEMORY TESTS

We have injected rats with a submaximal dose of a hemoconvulsant pentylenetetrazole (PTZ, 70 mg/kg) and measured their memory performance during three subsequent months using novel object recognition test and social recognition test (Fig.1). In both tests, PTZ-induced generalized tonic-clonic seizures were accompanied by a slowly developing decline in short-term non-spatial memory.

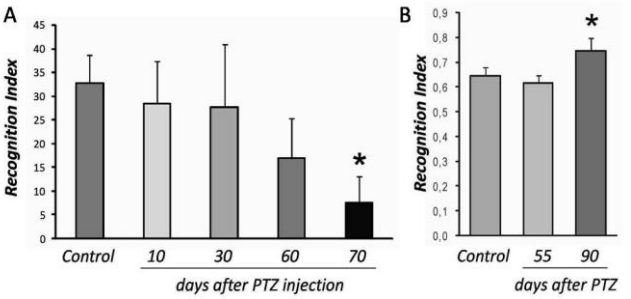


*Fig.1. Performance of short-term memory in novel object recognition test (A) and social recognition test (B) after PTZ-induced seizures in rats.*

## IMMUNOHISTOCHEMICAL STUDY

The temporal profile of progression of memory impairments along with the absence of profound neuronal damage (as assessed by vanadium acidic fuchsine stain) after PTZ-induced convulsions allowed us to suggest the involvement of aberrant seizure-induced adult neurogenesis in the pathogenesis of observed memory dysfunction. To verify this hypothesis, we injected the rats with S-phase marker 5-bromo-2-deoxyuridine

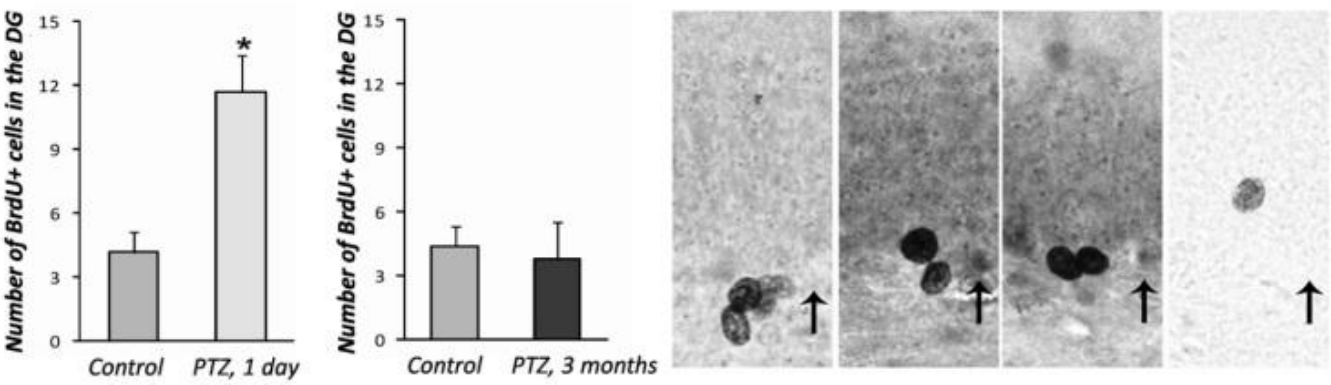


*Fig. 2. The number of BrdU+ cells after PTZ-induced seizures and their integration into DG.*

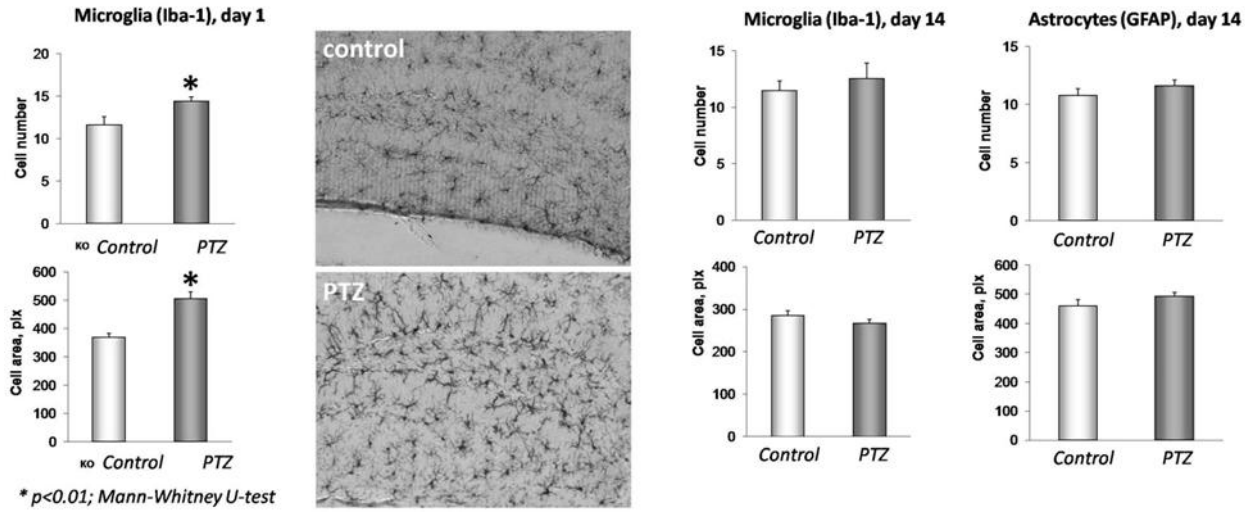


*Fig.3. Expression at different time points after the seizures*

(BrdU) and counted the number of labeled cells in the dentate gyrus (DG) at different time points after the seizures (Fig. 2). We have found that simultaneously with development of memory impairments, an elimination of the neuroinflammatory alteration of the local microenvironment within the neurogenic niche. To check this supposition we stained rat brain slices for glial markers Iba-1 (microglial cells) and GFAP (astrocytes) and analyzed their expression at different time points after the seizures (Fig. 3). On the next day after the seizures an activation of microglial cells occurred in the DG.Two weeks later, no signs of microglial activation were present; moreover, astrocytic glia was also not increased in number and size as assessed by GFAP immunostaining suggesting no chronic neuroinflammation after single PTZ-induced convulsion. excessive young cells occurs in the germinative area of the hippocampus. The possible mechanism of aberrant maturation of the newly generated cells in the absence of their visible structural abnormality can be launched by

**CONCLUSIONS**

Single episode of generalized tonic-clonic seizures induced by PTZ led to slowly developing memory impairments in rats, accompanied by elimination of excessive newly generated young cells and transient activation of microglial cells in this neurogenic niche. The study was partially supported by RFH grant # 13-36-01277 and RFBR grant # 14-04-3152.

## The Influence Of Brain-Derived Neurotrophic Factor (Bdnf) On Functional Activity Of The Culture Hippocampus During Hypoxia (In Vitro Modelling)

*T.A. Astrahanova [1], M.V. Vedunova [1,2], E.V. Mitroshina [1,2], I.V. Mukhina [1,2]*

[1] Lobachevsky State University of Nizhny Novgorod, 23 Gagarina ave., Nizhny Novgorod, Russia, 603950

[2] Medical University Nizhny Novgorod Medical Academy, Ministry of Health of the Russian Federation, 70 Gagarin ave., Nizhny Novgorod, Russia, 603950

Oxygen deficiency is the major cause of cell death at a large range of pathologies. The neurons are among body cells, which are the most sensitive to lack of oxygen, concerning the problem of brain hypoxia retains emergency medical and biological significance. The purpose of research is studying the impact of brain-derived neurotrophic factor (BDNF) on the functional activity of dissociated cultures of hippocampus in modeling normobaric hypoxia. In the in-vitro study we used dissociated hippocampal cell cultures derived from CBA mice 18 day embryos. On the 14th day of the cultivation, the cells exposed to hypoxia. 1ng / ml BDNF was preemptively added in the examined cultures. To measure the functional activity of the hippocampal cultures RNA detection probe SmartFlare was used. To assess the changes in the functional activity of the 1st day after the simulation hypoxia detection of mRNA BDNF was carried out.For detection we used RNA probe SmartFlare, whose fluorescence was determined as helium-neon laser with $\lambda$=543. During examination of the percentage BDNF mRNA-positive cells in primary cultures of dissociated hippocampal cultures between 7 and 14 days of development (Fig. 1) we found a significant increase of BDNF mRNA-positive cell group which preventively got BDNF, relatively to the control group. There also a slight increase in mRNA BDNF-positive cells relatively to controls at 21 days of development, but no significant differences were found. During analyzing the changes in mRNA BDNF-positive cells in the temporal dynamics, we found that the proportion of BDNF mRNA was significantly increased at the 14th day of development in comparison with 7 days. Next 21 hours of significant drop of the mRNA BDNF. Statistical differences between 7 and 21 days was found. These data suggests that the preventive addition of BDNF percentage to dissociated primary hippocampal cultures on the 14th day of the development affects the synthesis of endogenous BDNF in the most positive way.

## RECEIVING OF ADENOVIRAL VECTOR FOR THE STUDY FUNCTIONS OF SYNAPTOPODIN, THE PROTEIN OF SPINE APPARATUS

*E.V. Borisova, E.A. Epifanova, V.A. Salina., A.A. Babaev*

Lobachevsky State University of Nizhny Novgorod, 23 Gagarina ave., Nizhny Novgorod, Russia, 603950

Synaptopodin is the founding member of a novel class of proline-rich actin-associated proteins highly expressed in telencephalic dendrites and renal podocytes. That protein expresses in dendrites of mature neurons in telencephalon. Synaptopodin exists in 3 isoforms: neuronal Synpo-short (685 AA), renal Synpo-long (903 AA), and Synpo-T (181 AA). All 3 isoforms specifically interact with alpha-actinin and elongate alpha-actinin-induced actin filaments. According data from recent studies, we can suggest that dendritic spines containing sinaptopodin greatly differ in structural and functional properties from the neighboring spines that do not contain sinaptopodin. Clusters of synaptopodin in spines colocalize with internal functional flow of calcium. Thus,