

*Igor O. Gojković¹, Smiljana D. Paraš²,
Radoslav B. Gajanin³, Milica D. Matavulj⁴,
Vesna K. Rajković⁴, Goran D. Spasojević⁵*

¹ Clinic of Orthopedic Surgery, Clinical Center Banja Luka, RS, BiH

² Department of Biology, Faculty of Science, University of Banja Luka, RS, BiH

³ Department of Pathology, Clinical Center Banja Luka, RS, BiH

⁴ Department of Biology and Ecology, Faculty of Science, University of Novi Sad, Serbia

⁵ Department of Anatomy, Faculty of Medicine, University of Banja Luka, RS, BiH

THE INFLUENCE OF EXTREMELY LOW-FREQUENCY ELECTROMAGNETIC FIELD ON THE BASAL GANGLIA STRUCTURES OF THE RAT BRAIN

ABSTRACT: We studied the influence of extremely low-frequency electromagnetic field (ELF EMF) to subcortical structures of a brain, i.e. basal ganglia, of sexually mature rats of Wistar strain. The animals were exposed to nonhomogenous ELF EMF, intensity of 50-500 μ T, 50 Hz frequency, 7 hours a day, and 5 days a week during three months. Histological and stereological analysis established a reduction in volume density of ganglia cells in the area of basal ganglia, an increase of their nucleocytoplasmic volume ratio, and presence of an intensive edema of pericellular (perineural) type.

KEY WORDS: extremely low-frequency electromagnetic field (ELF EMF), basal ganglia, pericellular edema

INTRODUCTION

Basal ganglia, located in subcortex, are consisted of three large masses of nucleuses: nucleus caudatus, putamen, and globus pallidus, as well as of their functionally connected structures such as nucleus subthalamicus, substantia nigra, and nucleus ruber that are also located immediately below cortex. There are numerous projections of motoric, premotoric cortex, and thalamus to striatum. The striatum projects to the substantia nigra and globus pallidus both directly and indirectly via the subthalamic nucleus, which also receives cortical input. The globus pallidus, from its own side through ansa lenticularis (the main efferent route from basal ganglia) projects itself to ventrolateral, to the front of the ventral nucleus of thalamus, as well as to subthalamic nucleus, nucleus ruber, and brain stem. Since the thalamus is projected to the cortex,

the functional reverberation circle with the elements of back circuit is closed in this way.

Basal ganglia are also distinctive by presence of nigrostrial dopaminergic system (the bodies of the neurons of this system are located in a dark substance while their axons are located in caudate nucleus), by high consumption of oxygen and by significant concentration of copper in their cytoplasm.

In spite of many earlier discoveries precise function of basal ganglia are still insufficient (K o r n h u b e r , 1971). It is believed that they are involved in planning and programming of movements i.e., in the processes that abstract thought, the plan of action, convert into an actual movement.

In the process of studying neurological effects of electromagnetic field (EMF) exposure different parameters of these fields as intensity, frequency, duration of EMF exposure, the co-occurrence of the static magnetic field (both the natural earth's magnetic field and anthropogenic fields), the presence of the electrical field, the magnetic field, or their combination have been taken into account and, also, whether electromagnetic field is sinusoidal, pulsed or in more complex wave forms. In our daily lives we are all exposed to different sources of EMFs of extremely low frequency (ELF) (below 300 Hz) and low intensity (below 2 mT), such as residential power installations, domestic electrical appliances, etc. (G a n d h i et al., 2001, G a u g e r , 1985). Question of influence of such ELF-MEMFs on the central and peripheral nervous system remains open and there are many controversial data about this issue (C a r r u b b a et M a r i n o , 2008). The main reason for this is the obvious problem of detecting ELF-EMF effects in classical neurophysiological signals such as electroencephalograms (EEG) or evoked potentials (C o o k et al., 2004). There is evidence that neurological response to ELF EMFs influence include changes of human and monkey response time (H o m e r , 1968, G a v a l a s et al., 1970, G a v a l a s - M e d i c i et D a y - M a g d a l e n o , 1976), changes of EEG, GABA level and changes in calcium ion binding in cerebral tissue of cat and chick (B a w i n and A d e y , 1976, B a w i n et al., 1978, B a w i n et al., 1975) and also changes in expression of brain protein c-Jun in mice (S t r a š á k et al., 2009).

However, until now there are only few data on morphological changes of subcortical basal ganglia influenced by ELF EMF. In this study, we examined the effect of ELF EMF for the characteristics that are the most commonly occurring in human living and working environment, and regarding the structural characteristics of rat's basal ganglia, having in mind that many neurodegenerative diseases, primarily Parkinson disease, are related to the changes of these ganglions.

MATERIALS AND METHODS

The experiment was performed on 26 male Wistar rats. Animals were housed in laboratory conditions with $22\pm 2^{\circ}\text{C}$ temperature and subjected to a natural photoperiod. Access to tap water and palate food was unlimited. A

total of 13 animals were exposed to EMF from 24 h after birth, 7 hours a day (from 07:00 A.M. to 14:00 P.M.), 5 days a week for a period of three months. Thirteen animals served as controls and they were maintained in a separate room free of any appliances involved in generation of EMFs. The investigation was made with permission of the Ethical Committee on Animal Experiments of the University of Novi Sad.

The exposure system was made of a single coil of 2.5 mm thick copper wire placed on a wooden frame in 1320 turns. The coil was energized from a standard power supply of 220 V, 50 Hz, and 16 A via an autotransformer. The autotransformer provided a 60 V output and was used in order to reduce the electric field. The value of the electric field at any point in the room was less than 10 V/m. Cages with animals were placed symmetrically on both sides of the coil. The ELF EMF produced by the coil was inhomogeneous and of decaying intensity along the animal cages with a 500 μ T value on the side of the cage near the coil to 50 μ T on the opposite side.

After the decapitation, removed brain tissue was fixed in Bouin's solution and processed using a standard procedure for paraffin embedding. Samples were cut in a frontal plane on a rotary microtome (LEICA RM 2125, Leica Microsystems, Wetzlar, Germany) in 4-6 μ m thick serial sections. For the histological analysis, paraffin slices were stained with hematoxylin-eosin (HE) (both stains by Merck, Darmstadt, Germany). Histological and stereological analysis was performed on every 5th, 10th, 15th, 20th, 25th and 30th HE stained section per animal using a multipurpose stereological grid M42 (Weibel et al., 1966) placed in the ocular of a light microscope under a total magnification of x400. The volume density of nucleus and cytoplasm of ganglion cells in subcortical nuclei was determined. The obtained numerical values were used to further calculate the total volume density of ganglion cells as well as the nucleocytoplasmic ratio. The estimations were made by the same observer. The data were statistically analyzed by Student's t-test. *p* values less than 0.05 were considered significant.

RESULTS

Our histological studies indicated that after three months of exposure to ELF EMFs altered the structure of the rat basal ganglia cells. Damaged neurons were seen as condensed dark neurons, intermingled with normal neurons in basal ganglia (Fig. 1a-b). These dark neurons, as have been proposed by Sugimoto et al. (1990), have three main characteristics: (1) irregular cellular outlines, (2) increased density of chromatin and cytoplasm, and (3) intensely and homogeneously stained nucleus. All these properties regarding dark neuron were recorded in basal ganglia in our EMF exposed animals (Fig. 1b and 2b).

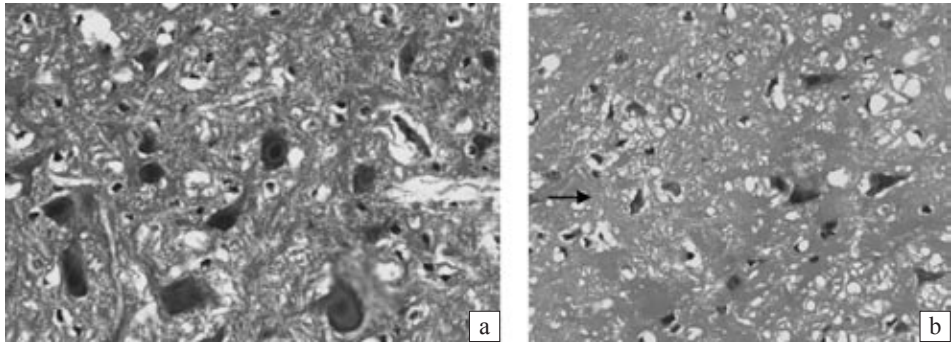


Fig. 1 – Basal ganglia cells; (a) control animal; (b) animal exposed to EMF. Among the normal big and pale blue nerve cells, there are interspersed black and shrunken nerve cells (arrow). Hematoxylin and eosin stain, x400.

Apart from the occurrence of dark neurons, in basal ganglia of the animals exposed to EMF, a great variability was also observed in the size of the cells, as well as in their shape and size of their nuclei (Fig. 2a, b).

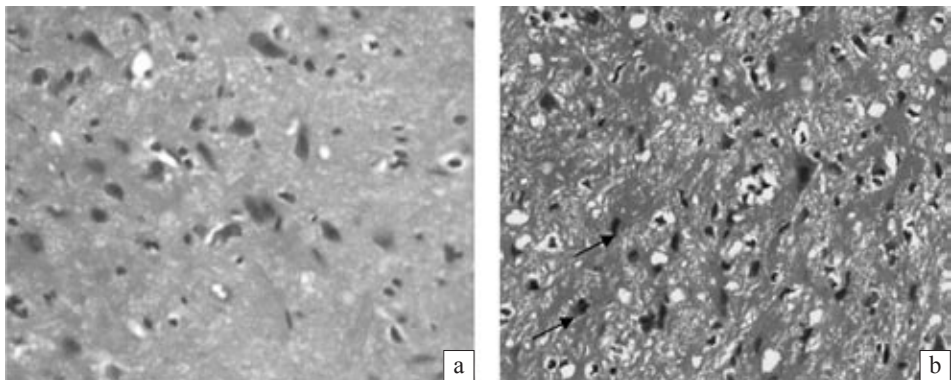


Fig. 2 – Basal ganglia cells; (a) control animal; (b) animal exposed to EMF. Note the shrunken dark blue stained (basophilic) cell bodies (arrow). Adjacent histologically intact neurons are present. Hematoxylin and eosin stain, x400.

Stereological analysis indicated statistically significant decrease of volume density of cytoplasm of EMF exposed animal basal ganglia cells compared to the control animals (Fig. 3).

However, this treatment caused statistically significant increase of volume density of basal cells nuclei compared to control animals (Fig. 4) and significant decrease of the entire volume density of these cells (Fig. 5).

The data from Fig. 3 and Fig. 4 were also used to calculate the changing mean nucleo-cytoplasmic ratio, which was found to significantly increase in animals exposed to ELF EMF (Tab. 1), due mainly to decrease in the volume density of basal cells cytoplasm in this treatment.

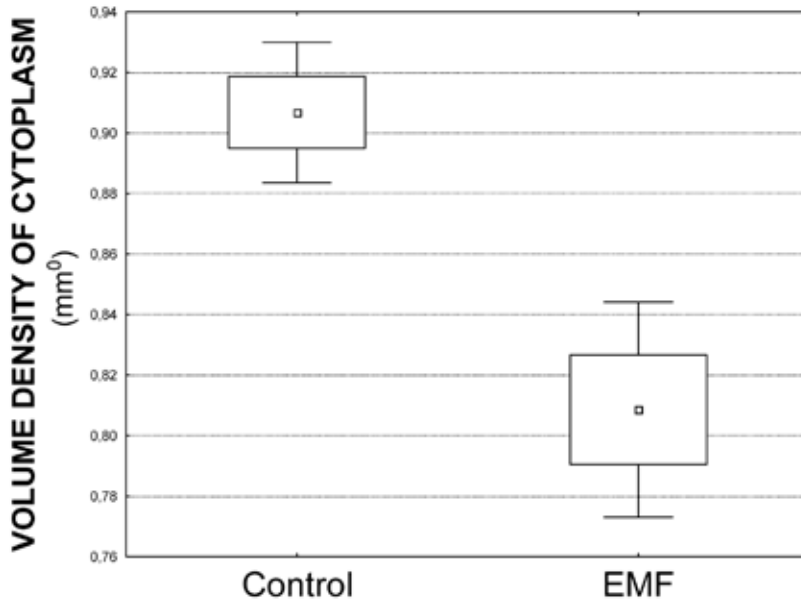


Fig. 3 – Volume density of the basal ganglia cell cytoplasm in control animals and animals exposed to EMF.

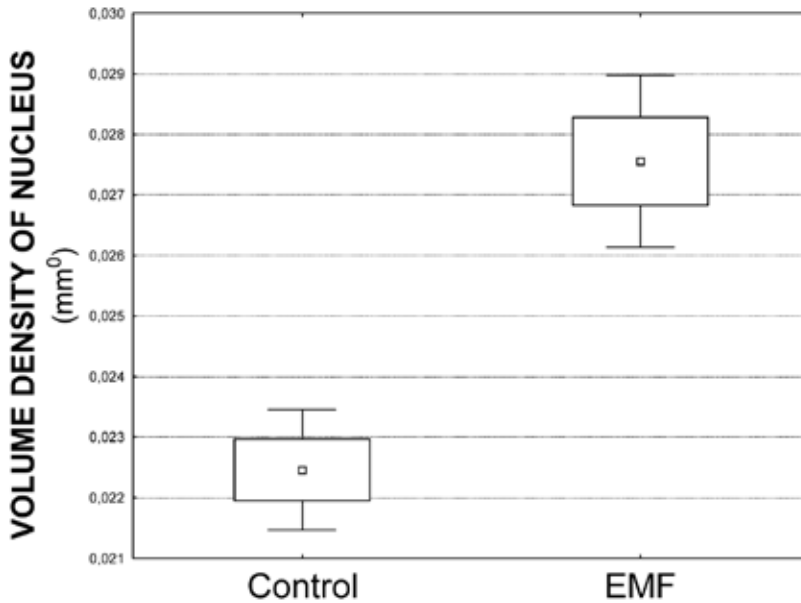


Fig. 4 – Volume density of the basal ganglia cell nuclei of control animals and animals exposed to EMF.

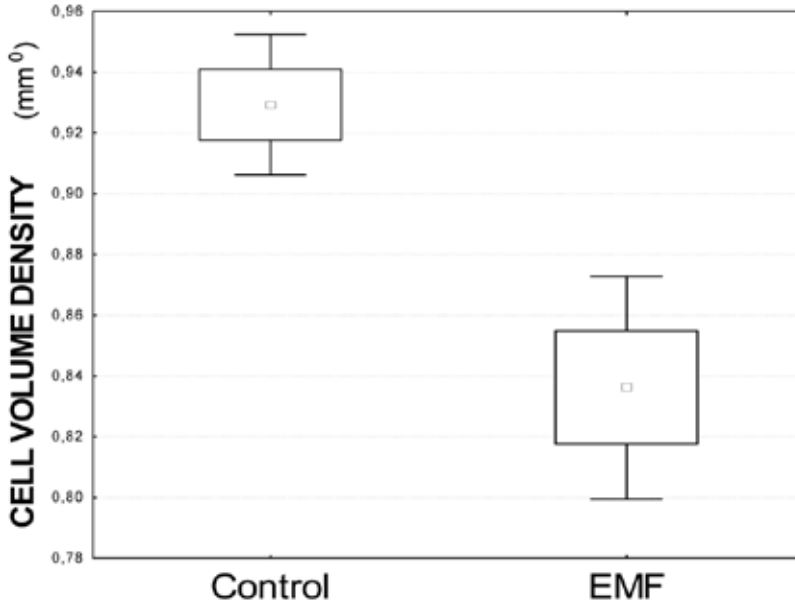


Fig. 5 – Volume density of the basal ganglia cells of control animals and animals exposed to EMF.

Tab. 1 – Mean values with the standard error of the mean (SE) of nucleo-cytoplasmic volume ratio of basal ganglion cells of control and EMF exposed animals are given.

	Control	EMF	p-level
Nucleocytoplasmic ratio	0.0248 ± 0.0007	0.0341 ± 0.0006	0.000000

Besides the change in the shape and volume of basal ganglia neurons of animals exposed to EMF, numerous small vacuole are observed in cytoplasm and, also, bubbly appearance of chromatin in their nuclei (Fig. 6).

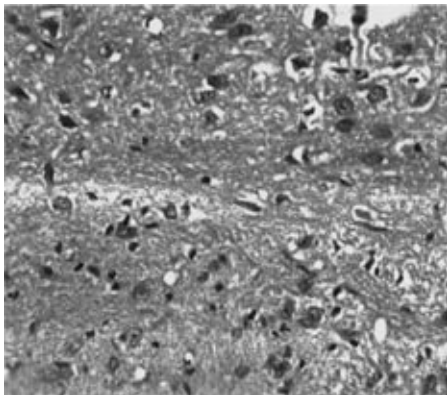


Fig. 6 – Basal ganglia cells. Animal exposed to EMF. The prominent cytoplasmic vacuolization. Hematoxylin and eosin stain, x400.

Well distinguished change in basal ganglia of the animals treated with EMF is an incidence of intensive edema of pericellular (perineural) type with clearly distinguished, blank, white, round or improperly shaped haloes, resistant to color and filled with water (Fig. 7 and 8a, b).

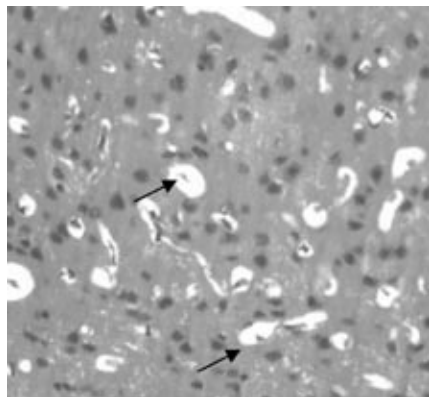


Fig. 7 – Basal ganglia cells. Animal exposed to EMF. Perineural edema (arrow) is observed. Hematoxylin and eosin stain, x400.

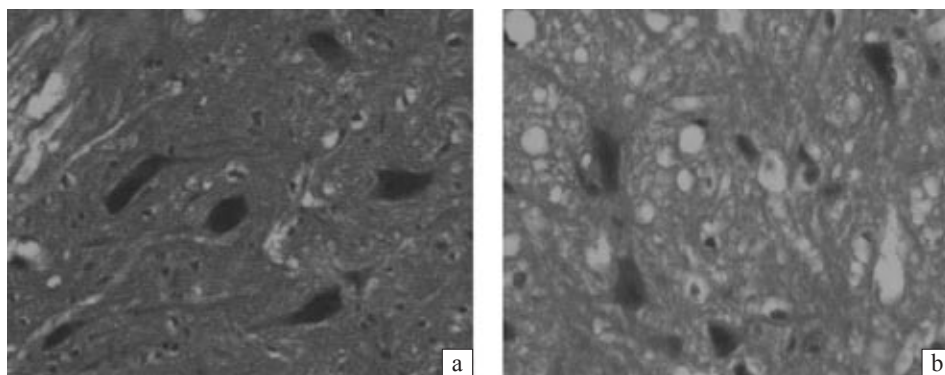


Fig. 8 – Basal ganglia cells; (a) control animal; (b) animal exposed to EMF, area with very prominent perineural edema. Hematoxylin and eosin stain, x400.

DISCUSSION

In our study, we found evidence for neuronal damage caused by ELF EMFs. Damaged neurons and very prominent perineural edema were recorded in the basal ganglia in the brains of EMF exposed rats. Changes described here would seem to indicate a serious neuronal damage, which may be mediated through some organelle damage and blood-brain barrier (BBB) leakage.

Damaged neurons assigned as dark neurons that occurred in basal ganglia in our experimental condition also occurred after exposure to GSM (Global System for Mobile Communications) (Nittby et al., 2009, Eberhardt et al., 2008), in connection to experimental ischemia (Kövesdi et al., 2007),

hypoglycemia (Gallias et al., 2005), and epilepsy (Södenfeldt et al., 1983). Many authors suggest that the BBB leakage is the major reason for nerve cells injury and appearance of dark neurons (Fredriksson et al., 1988 Salahuddin et al., 1998, Sokrab et al., 1988, Hassel et al., 1994). Physiologically, the central nervous system (CNS) microvasculature differs from that of peripheral organs. It is characterized not only by its tight junctions, which seal cell-to-cell contacts between adjacent endothelial cells, but also by the low number of pinocytotic vesicles for nutrient transport through the endothelial cytoplasm and its lack of fenestrations; and the five-fold higher number of mitochondria in BBB endothelial cells compared to muscular endothelia in rat (Oldendorf et al., 1977). All this speaks in favor of an energy-dependent transcapillary transport. Electromagnetic fields increase permeability of BBB (Nittby et al., 2008). When this barrier is damaged in some pathological conditions, the normally excluded molecules can pass into the brain tissue. EMFs have the potency to significantly open the BBB such that the animal's own albumin passes out of the bloodstream into the brain tissue and accumulates in the neurons and in glial cells surrounding the capillaries (Salford et al., 1992).

The results obtained in this study indicate that ELF EMF affects basal ganglia, causing reduction in their volume and increasing their mean nucleocytoplasmic ratio. Observed an intensive edema, which is mainly of pericellular type, can damage the cells that at the end it results in cells death. This is a cytotoxic edema and in CNS it is always a consequence of hypoxia (Aarts and Tymiansky, 2005). As a rule, hypoxia causes cell membrane damage and also causes disturbance of cellular processes as production of high-energy phosphate and an ion balance. Hypoxia is a consequence of mitochondrion and oxidative phosphorylation cycle damage, resulting in reduced ATP production. This primary influences on active transport, which mostly depends on ATP-dependant Na/K pump. The lack of ATP causes malfunction of electrogenic pump, and that is why many Na and Cl ions enter the cell, causing an increase of intracellular osmolarity and as a consequence the water entrance to (maybe as a consequence of water APQ channels activations) cell causes its swelling. Swelling induces an increase of cell volume, damaging of cell membranes and organelles, and also induces losing of membrane phospholipids and staining ability of nucleus. Reduced ATP production is, as a rule, followed by the activation of phosphofructokinase enzyme, increased decomposition of glycogen and production of milk acid, pH reduction, and the increase of intracellular acidosis. The acidosis caused in this way brings up the activation of non-active acid-sensitive ionic channels such as ASIC, SUR-1, NC-ca and TRP channels (Allen and Atwell, 2002). In swelling cell concentration of intracellular Ca^{2+} rises as a consequence of its increased entrance in cell and its release from depot of cell (Xiong et al, 2006). In addition, calcium enters in mitochondria and causes additional inhibition of oxidative phosphorylation (MacDonald et al., 2006).

However, despite the large number of experiments conducted, there is yet no consistent scientific evidence in support of a plausible neurocarcinogenic mechanism for ELF-EMF (50- or 60-Hz) exposure. A possible hypothesis is

that ELF-EMF affects cell membrane structure and its permeability to small molecules (Baureus Koch et al., 2003; Grassi et al., 2004; Marino et al., 2003). Also, some data from literature have described redox-related cellular changes following ELF-EMF exposure (Regoli et al., 2005; Wolf et al., 2005; Zwirska-Korczala et al., 2005). According to this theory, ELF-EMF may interfere with chemical reactions involving free radical production (Simk'o et Mattsson, 2004). These effects could be even more pronounced in neuronal cells, partly due to relatively low levels of antioxidant defenses and, but mainly, because of great amounts of polyunsaturated fatty acids in their membranes what is potential target of oxidative attack (Falone et al., 2007). It is generally recognized that neuronal cells are very susceptible to oxidative injury and, in addition, some studies have evidenced greater incidence of tumors in human nervous system after exposure to ELF-EMF (reviewed by Feychting et al., 2005). Results of Falone et al., (2007) support redox-mediated ELF-EMF biological effects. They observed a positive modulation of antioxidant defenses as well as a shift of cellular environment towards a more reduced state after exposure to these fields.

The results obtained in this study may be significant in the light of evidence that Parkinson's disease causes reduction in total number of cells in basal ganglia. Nowadays, Parkinson's disease almost becomes epidemic and cannot be interpreted only by extended life span of human race, but also by drawing attention to the environmental factors, such as electromagnetic fields present in highly urban society.

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УТИЦАЈ ЕКСТРЕМНО НИСКО-ФРЕКВЕНТНОГ ЕЛЕКТРОМАГНЕТНОГ ПОЉА НА СТРУКТУРЕ БАЗАЛНИХ ГАНГЛИЈА МОЗГА ПАЦОВА

Игор О. Гојковић¹, Смиљана Д. Параш², Радослав Б. Гајанин³, Милица Д. Матавуљ⁴, Весна К. Рајковић⁴, Горан Д. Спасојевић⁵

¹ Клиника за ортопедску хирургију, Клинички центар Бања Лука, 12 беба бб, 78000 Бања Лука, Република Српска, Босна и Херцеговина

² Одељење за биологију факултета наука, Универзитет у Бањалуци, Младена Стојановића 2, 78000 Бања Лука, Република Српска, Босна и Херцеговина

³ Одељење за патологију, Клинички центар Бања Лука, 12 беба бб, 78000 Бања Лука, Република Српска, Босна и Херцеговина

⁴ Одељење за биологију и екологију Факултета наука, Универзитет у Новом Саду, Трг Доситеја Обрадовића 2, 21000 Нови Сад, Србија

⁵ Одељење за анатомију, Медицински факултет, Универзитет у Бањалуци, Саве Мркаља 14, 78000 Бања Лука, Република Српска, Босна и Херцеговина

Резиме

У нашем раду смо проучавали утицај екстремно ниско-фреквентног електромагнетног поља (ENF EMP-а) на субкортикалне структуре мозга, односно базалне ганглије, полно зрелих пацова соја Вистар. Животиње су излагане дејству нехомогеног ENF EMP-а јачине 50-500 μ T, фреквенције 50 Hz, 7 сати дневно, 5 дана седмично током 3 месеца. Хистолошка и стереолошка анализа је показала смањење волуменске густине ганглијских ћелија у подручју базалних ганглија, пораст њиховог нуклео-цитоплазматског односа и присуство интензивног едема перичелуларног (перинеуралног) типа.