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THE IMPACT OF ONE-TIME EXPOSURE TO COMBINED STRESS ON THE NEURONAL PARAMETERS OF NEOCORTEX AND HIPPOCAMPUS OF OLD RATS

ABSTRACT

The purpose of this study is to assess morphological and morphometric parameters of the neocortex and hippocampus neurons of old rats exposed to one-time combined stress. Experimental rats were divided into 2 groups: male old intact and male old rats. The last group exposed to the one-time combined attack of noise, light and immobilization for 1 hour. Experimental materials were taken by decapitation, on the 7th day in the morning hours. We figured out that the influence of combined stress enhances significantly the process of neuronal death in the neocortex of the aging animals' brain. Morphological changes are manifested by increasing number of hyperchromic neurons, vacuolization and increasing cell cytoplasm's area. In the hippocampal neurons of the CA1 area morphological changes were not detected.

Keywords: neurons, morphometry, stress, neocortex, hippocampus, ageing.

INTRODUCTION

For centuries, scientists from various fields pay their attention on the problem of ageing. There are several theories about the development of aging, but now the most widely accepted is the free radical theory. According to this theory, a change in the balance between the intensity of free radical formation and antioxidant protection is the main universal mechanism of aging and damage to living systems [1,5]. It is also known that during the process of age involution resistance to stress factors naturally decreases [1,4]. There are data that stress-induced effects lead to intensification of lipid peroxidation and development of oxidative stress in the brain [2,3]. It leads to damages and death of neurons through apoptosis or necrosis. At the present time, the role of stress factors in the formation of age-related changes in the morphology and metabolism of neurons are not well known, therefore this is of significant interest.

The purpose of this research is to study the morphological and morphometric parameters of neocortex and hippocampus neurons of old male rats exposed to one-time combined stress.

MATERIALS AND METHODS OF THE RESEARCH

In the research we used old (20-24 months) male white rats, weighing 350 – 400 g (n = 10). Animals were divided into 2 groups: 1 group – old intact (n = 5), 2 group – old rats, stressed one-time by combined exposure of noise, light and immobilization for 1 hour (n = 5).

The combined stress was modeled by placing the animals in a narrow plastic box with simultaneous exposure to white noise and 100W light at a distance of 50 cm. The experiment was carried out under the conditions of a vivarium; the animals were kept in ad libitum conditions.

All procedures and manipulations on animals were carried out in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals and the principles of the World Medical Association Declaration of Helsinki. Experimental materials were taken by decapitation on the 7th day in the morning hours.

The materials were fixed in a Carnoy's fluid. Sectioning tissues was made by standard paraffin method. The morphological study was carried out on sections (7 μm) of the parietal lobe, stained by methylene blue and hematoxylin-eosin. The program PhotoM1.21 was used for morphometric research. Statistical analysis of the

research data was realized using the program Statistica 10.

RESULTS AND DISCUSSION

During the morphological study, we found qualitative and quantitative differences from intact control group. In all cortical layers, we observed multiple hyperchromic neurons, whereas the morphological picture of the intact old rats' neocortex was characterized by just few number of hyperchromic neurons. Hyperchromic neurons with karyopyknosis and cytopyknosis are perishing/pathologically altered neurons, reflecting the processes of natural vital activity and cell death. These neurons are deformed cells with irregular contours, often reduced in size, with wrinkled hyperchromic nucleus and cytoplasm, and characterized by a more intense staining than normal. Hyperchromic neurons did not form clusters and located among unchanged cells in all cortical layers. Also, among neurons of II-IV cerebral cortical layers of stressed rats, we observed a large number of cells with vacuolization, probably characterizing the malfunction of synthetic processes.

Only in the group of stressed animals we observed single cases of apoptotic bodies, as well as died cells with fragmented and deformed neurites. The received morphological data showed the increasing number of perishing/pathologically altered neurons under the influence of one-time combined stress, both in the mechanism of necrosis and apoptosis.

The hippocampus of the stressed group had a normal cytoarchitecture, the CA1 area included medium size pyramidal neurons. There were single hyperchromic cells, also areas of low spatial density of cells both in the intact control group and in the stressed group. Morphometric examination does not indicate credible changes in the areas of the cytoplasm and the nucleus of neurons. Degenerating neurons, related to irreversible changes, as well as decreasing their density, we characterized them as a progressive aging process that do not differ from intact control group.

The number of neurons in the external granular layer (II) has not identified changes from the control group, in the internal pyramidal layer (V) has shown an unreliable decreasing of the number of cells (13%) (Table 1).

Gravimetric and morphometric indicators of brain

Indicator	Group	
	1st group Old male rats (intact)	2nd group Old male rats (stressed)
Brain mass, mg	2170±14,4	2034±38,4
Brain hemisphere mass, mg	777±18,1	736±19,1
Number of neurons:		
- external granular layer (II layer)	12,4±0,51	12,0±0,44
- internal pyramidal layer (V layer)	6,0±0,31	5,2±0,37
Cross-sectional area, μm ^{2},		
- nuclei of neurons (II layer)	55,5±2,28	58,4±2,23
- cytoplasm of neurons (II layer)	44,2±1,68	54,4±2,41*
- nuclei of neurons (V layer)	102,3±3,54	108,3±4,47
- cytoplasm of neurons (V layer)	120,6±4,60	134,4±9,34
- nuclei of hippocampus neurons (CA1)	15,8±0,54	15,1±0,47
- cytoplasm of hippocampus neurons (CA1)	10,1±0,69	9,4±0,55

Note: * - differences are statistically confirmed in comparing to group 1.

Morphometric research of neurons in the II and V layers detected a reliable increasing of the cytoplasm area in the II layer for stressed group (up 22%) (Table 1). In addition, the morphometry data demonstrates in other layers a tendency to increase in size of nuclei and cytoplasm of neurons, but there are no statistically confirmed differences. Thus, the mean values of the nuclei of the II layer neurons increased by 5.4%, the nuclei and cytoplasm of V layer increased by 5.8% and 11.6%, respectively. We suggest these changes caused by destructive changes in neuronal organelles, mainly in mitochondrias, in endoplasmic reticulum and in the Golgi complex, due to intensification of lipid peroxidation.

CONCLUSIONS

According to experimental data we detected morphological changes, characterized by increasing number of perishing/pathologically altered cells in the neocortex. The most distinctive morphological changes are: hyperchromic neurons, vacuolization and increasing in the size of neurons cytoplasm in the external granular layer. Also we can mark unconfirmed increase of cells size parameters in other layers. In the hippocampus we observed degenerating neurons, reducing cells density in both groups. Thus, the stress significantly enhances the neurons death in the brain of aging animals and it is an important mechanism of nervous tissue damages.

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