ORIGINAL ARTICLE

PARTICULARITIES ASSOCIATED WITH THE EXPRESSION OF GLIAL ACIDIC FIBRILLARY PROTEIN ON THE STRUCTURAL COMPONENTS OF CEREBELLUM OF THE RATS INFLUENCED BY THE FOOD ADDITIVES COMPLEX

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ABSTRACT

The aim: To define the degree for glial acidic fibrillary protein expression on the structural components of cerebellum of the rats in health and when rats influenced by the food additives complex.

Materials and methods: In order to determine the degree of expression of the immunohistochemical marker GFAP on the structural components of the cerebellum of rats we applied immunohistochemical, morphometric and statistical methods in our study.

Results: In histological specimens at the end of 1st week of observation in the gray matter of the cerebellum there occurred a gradual increase in 1.16 times of the average number of GFAP-positive cells.

At the end of 4th week of the experimental study, the average number of GFAP-positive cells increased accurately (at $p<0.05$ compared to the control group) in 1.27 times, at the end of 8th week it has increased in 1.99 times, at the end of 12th week in 2.25, and at the end of 16th week in 2.39 times.

Conclusions: The outcomes of our study are as follows the increase in the average number of GFAP-positive cells is directly related to the decrease in the average number of major neurons of the gray matter of the brain, while the fluctuations in the average number of astrocytic glia cells represent a compensatory mechanism in the recovery of gray matter neurons of the brain from neural stem cells with the subsequent development of reactive astrogliosis and, thereafter the possible development of neuropathology.

KEY WORDS: glial acidic fibrillary protein, GFAP-positive cells, astrocytes, cerebellum

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INTRODUCTION

Food is and has always been an integral part of our lives. But with the progress of technologies, ecologically pure products were turned into a mixture of a large number of food additives and chemical impurities. Which allowed us to reduce the time for cooking and to decrease its cost thereafter to facilitate people's lives in an economic crisis and high rate of life. But a large number of food additives pose a threat to human health. Some of the already known additives are considered as life-threatening and therefore are prohibited.

Nevertheless, synthetic colorants E102, E110, E122, E124 are authorized to use in Ukraine, although they are included in the so-called "Southampton list" and are banned in the EU [1]. In our study, we focused on dietary supplements such as monosodium glutamate (dietary supplement E621), ponso 4R (dietary supplement E124) and yellow synthetic colorant "sunset" (dietary supplement E110).

The main consequences of taking a large number of these supplements are the following: abdominal and chest pain, headaches, nausea, tachycardia and weakness. But special attention should be paid to the negative effects on the nervous system, especially on the cerebellum, which is easily affected, the development of allergic reactions and a variety of diseases such as Parkinson's, Alzheimer's and Huntington's diseases, amyloid polyneuropathy, type II diabetes etc. [2, 3, 4].

In our turn, we plan to examine the effect of dietary supplements intake on changes in the structures of the cerebellum of rats using the immunohistochemical marker GFAP, which is for indicating changes in astrocytes. Nowadays there occur more and more experiments aimed at the detection of contribution of astrocytes in the structural formation of synapses, in the synthesis, trap and release the neuro- and glio-transmitters as in synapses as in the extrasynaptic areas [5].

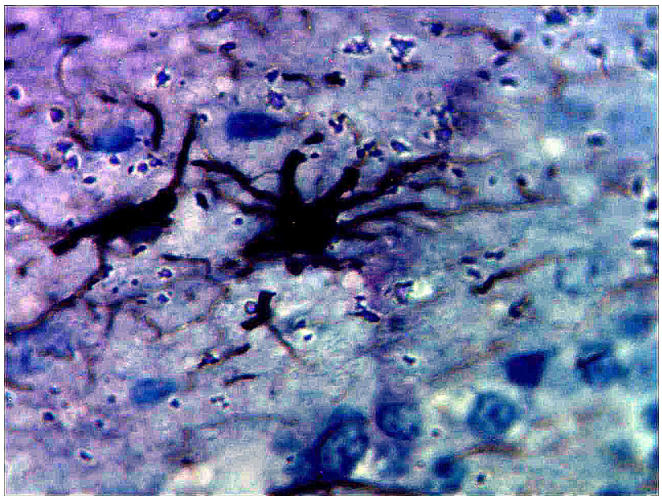

Fig. 1. Morphology and histotopography of GFAP-positive cells of the cerebellum of control rats. Staining: immunohistochemical reaction with glial fibrillar acidic protein with Mayer's hematoxylin staining.

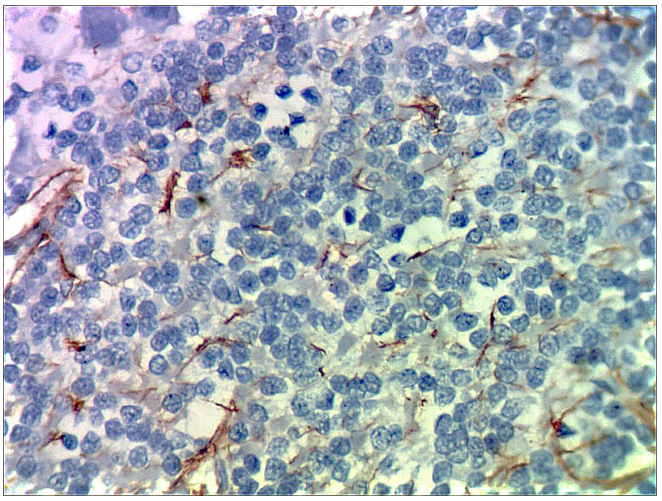

Fig. 2. Morphological changes in the granular layer of the cerebellar cortex at 1 week of experimental study. Staining: immunohistochemical reaction with glial fibrillar acidic protein with Mayer's hematoxylin staining.

THE AIM

To define the degree for glial acidic fibrillary protein expression as an immunohistochemical marker on the structural components of the cerebellum of rats when influenced by the food additives complex.

MATERIALS AND METHODS

100 white rats were used in our study, with average weight of 258.1 ± 0.67 g. The experiment involved rats under normal conditions (C - control) and rats influenced by the complex of chemicals (monosodium glutamate, ponso 4r and yellow synthetic colorant "sunset" (E - experiment and week number).

Experimental animals were treated and all manipulations on them were carried out in accordance with the "Rules for the use of laboratory experimental animals" (2006, Annex 4) and the Helsinki Declaration on the Humane Treatment of Animals, the Law of Ukraine "Protection of Animals from Cruelty" 2006) according to the requirements of the Commission on Bioethics of the Ukrainian Medical Stomatological Academy, concordant with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) [6,7].

We implied immunohistochemical, morphometric and statistic methods to define the expression degree of the immunohistochemical marker GFAP on the structural components of the cerebellum of rats. In order to determine morphological features, cerebellar biopsies were sealed in paraffin according to conventional methods. Slices of 4-5 μm thick were cut from paraffin blocks, then subjected to immunohistochemical reaction according to the manufacturer's protocol with the primary antibody to GFAP (Dako, Denmark).

Visualization of immunohistological reaction products captured by the detection system EnVision ™ FLEX (Dako, Denmark). Each slice was additionally stained with Mayer's hematoxylin. On the next stage, we examined histological sections examined using a light microscope with digital microphotolens adjustment Olympus C 3040-ADU with installed programs adapted for our study (Olympus DP - Soft, license № VJ285302, VT310403, 1AV4U13B26802) and Viorex 3 (serial number 5604).

We have defined the number of astrocytes expressing the GFAP marker morphometrically. Morphometric studies were performed with a visual analysis histological specimens system. Images of histological specimens were displayed on a PC monitor from different sources such as microscope and a Vision CCD Camera. Morphometric studies and histogram both were made using VideoTest-5.0 program, KAAPA ImageBase and Microsoft Excel in a personal computer.

Statistical analysis of the study outcomes was made with the computer, using applications for statistical processing of data from medical, biological and epidemiological studies "InStat". The program allows to obtain research outcomes in the form of the following predicted values: M - average value; 0 - is the standard deviation; m -is the standard error of the mean. Student's T-criterion was used to compare quantitative values in dual series. The difference was considered probable at values of $p < 0.05$.

RESULTS

Thus, as an outcome of our study on histological specimens there were found elongated GFAP- positive cells, axon branched from star-shaped perikaryon and from 9 to 11 dendrites. Their average number in animals of the control group was (3.96 ± 0.35) in 100 fields of view. According to morphological features, (in respect their histotopographic features most of them were located in the gray matter), in our opinion, GFAP-positive cells should be identified as neuroglial macrogliocytes, namely protoplasmic astrocytes, which are directly involved in the formation of the

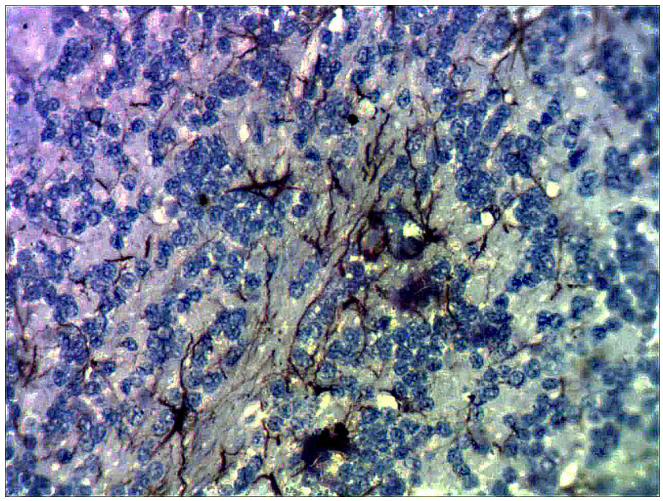

Fig. 3. Morphological changes in the granular layer of the cerebellar cortex at week 4 of the experimental study. Staining: immunohistochemical reaction with glial fibrillar acidic protein with Mayer's hematoxylin staining.

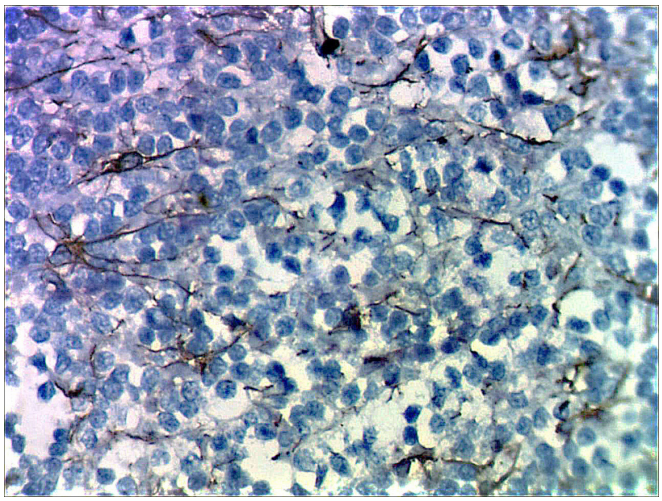

Fig. 4. Morphological changes in the granular layer of the cerebellar cortex at week 4 of the experimental study. Staining: immunohistochemical reaction with glial fibrillar acidic protein with Mayer's hematoxylin staining.

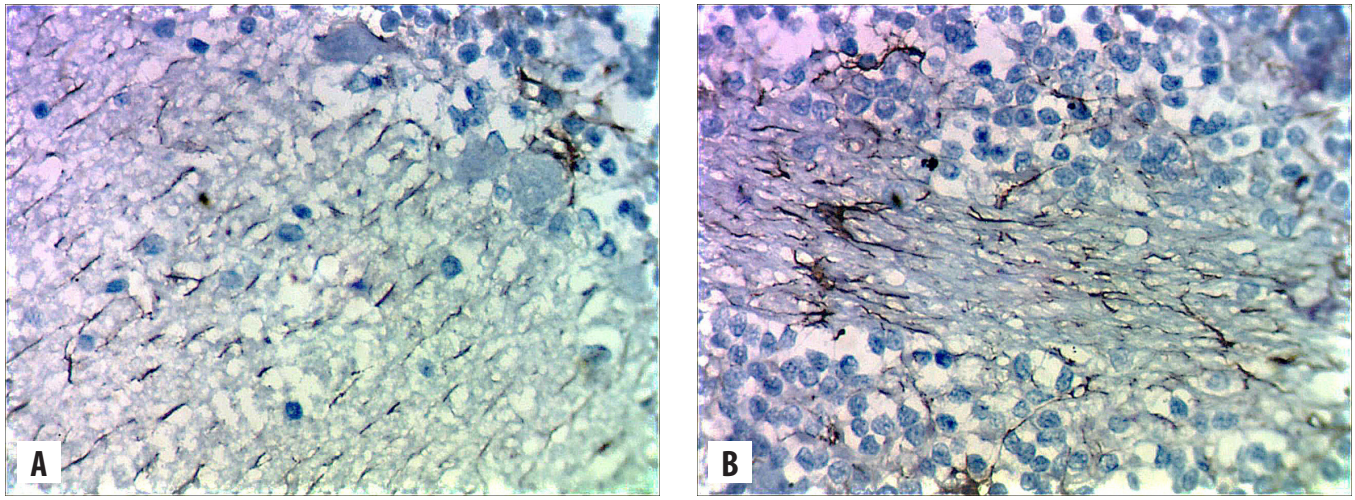

Fig. 5. Morphological changes in the granular layer (A) of the cerebellar cortex and in the white matter (B) of the cerebellar of rats at 12 weeks of experimental study. Staining: immunohistochemical reaction with glial fibrillar acidic protein with Mayer's hematoxylin staining.

cytoskeleton. In the same time, numerous small granular and fibrous GFAP-positive structures were visualized, which in our opinion are identified as areas of astrocyte fibers (Fig. 1).

At the light-optical level in histological specimens at the end of 1st week of observation in the gray matter of the cerebellum there was a gradual increase in 1.16 times of the average number of GFAP-positive cells. Their perecarions were singly located between the neurocytes of the corresponding layers of the cerebellar cortex. A slight enhancement of the pattern formed by the fibers of astrocytic microglia also attracted our attention (Fig. 2).

We determined morphometrically the fact that at the end of 4th week of the experimental study, the average number of GFAP-positive cells significantly (at $p<0.05$ compared to the control group) increased in 1.27 times. Comparing to the previous observation period, their average number also increased in 1.09 times, but the calculation range was within the statistical error. Thus, we are able to state that in the period from 1st to 4th weeks there appears a tendency to increase in the average number of GFAP-positive cells (Fig. 3).

Concurrently with the increase in the average number of astrocytic macroglia cells, an increase in the granular layer of grain cells should be measured. They visualized as cells with basophilic cytoplasm and were not singly located, but in a kind of conglomerate. Taking into account their functional significance, we are able to assume the strengthening of excitatory effects which are transmitted by the fibers of the latter from moss-like fibers to pear-shaped neurocytes (see Fig. 3).

At 8th week of the experimental study, we have found that the average number of GFAP-positive cells significantly increased (at $p<0.05$) in comparison to both the control group of animals and to the previous observation period in 1.99 and 1.57 times, respectively. Neurocytes of

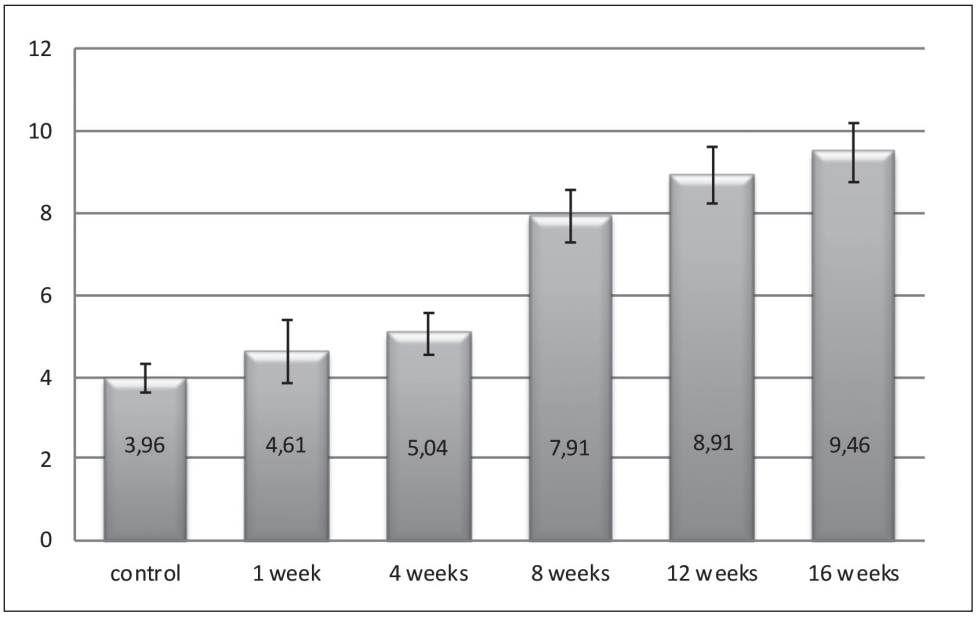

Fig. 6. Changes in the average number of GFAP-positive astrocytes in the dynamics of the experiment.

a granular layer in comparison with the previous period of observation lost cytoplasm basophility and had the signs of destruction (Fig. 4).

After analyzing morphological and morphometric changes in the structural components of the cerebellum influenced by the chemical additives complex at 12th of the experimental study, we noted that the average number of GFAP-positive cells significantly increased (at $p<0.05$) in 2.25 times compared to the control group of animals. In comparison with the previous observation period, the abovementioned indicator has also increased in 1.12 times, but the data is inter-correlated within the statistical error (Fig. 5).

At the light-optical level in the granular layer of the cerebellar cortex for the above-mentioned observation period, areas with completely destructed cell structures were identified, which were actively inoculated with GFAP-positive structures (macrocytic glia astrocytes), and their processes penetrating these areas created a kind of fibrillar frame.

We detected the strengthening of the fibrous pattern in the white matter of the cerebellum. Astrocytic processes passed in almost parallel rows penetrating the nervous tissue of the white matter of the cerebellum. Spherical nuclei, singly placed in the white matter, had basophilic cytoplasm and oxyphilic one. Areas with fine-spongy degeneration and sometimes with spherical dust-like cavities were detected around basophilically stained nuclei (see Fig. 5,6).

At the end of 16th week of the observation and the ending of the experimental study, we have found that the average number of GFAP-positive cells significantly increased (at $p<0.05$) in 2.39 times compared to the control group of animals, and comparing with the previous observation period, the abovementioned value has also slightly increased in 1.06 times, but remained within the statistical error.

On histological specimens, on the 16th day of the experimental study, in the granular layer of the cerebellar cortex there were determined the cases of active colonization by matured grain-cells of areas that were dystrophically altered during previous observation periods. These neurocytes were located between GFAP-positive structures and had a clear tendency to recover their average number compared to the control group of animals.

In the white matter of the cerebellum there remained the foci of fine-spongy degeneration with spherical cavities, which merged with each other at the boundary of the granular layer and the white matter of the cerebellum. Single GFAP-positive processes were sent deep into the white matter, but the fibrous pattern was less intense in comparison with one in the previous observation period. We noted that the spherical nuclei of white matter had no signs of destruction, which in our opinion is caused by the passing of the recovery stage of the pool of neurocytes in response to long-term intake of a food additives complex.

Such processes can be regarded as compensatory-adaptive, and GFAP-positive structures play the role of the fibrillar framework for the restoration of the structural components of the cerebellum.

Summarized data on changes in the average number of GFAP-positive astrocytes are on the Figure 6.

DISCUSSION

Thus, our study with use of glial acidic fibrillar protein as an immunohistochemical marker proved that an increase in the average number of GFAP-positive cells in the dynamics of the experiment was directly related to a decrease in the average number of major neurons of the cerebellum gray matter. Fluctuations in the average number of astrocytic glia cells are the compensatory mechanism in the recovery of neurons of the cerebellum gray matter from neural stem cells with the subsequent development of reactive astrogliosis and thereafter, the possible development of neuropathology.

At the end of 4th week of observation, we have determined an increase of the average number of grain-cells

in the granular layer, which were visualized as cells with basophilic cytoplasm and were not located singly, but gathered in some kind of conglomerate.

At the end of 8th week of the experimental study, we have noted that the perikaryons of the granular layer of neurocytes, in comparison with ones in the previous observation period, lost the basophilicity of the cytoplasm and had signs of destruction.

At the end of the 12th week of the experimental study we have detected areas of nervous tissue affected by the cases of fine-spongy degeneration, which are surrounded by spherical, dust-like cavities. In these areas we identified processes of GFAP-positive cells which penetrated the latter and had a parallel direction.

Thus, astroglial syncytium was detected by immunohistochemical reactions using glial fibrillar acidic protein which indicates on the protoplasmic astrocytes in normal states, while the change in their average number indicates on the compensatory-adaptive responses of the cerebellum after the influence of a food additives complex.

CONCLUSIONS

The outcome of our study is that the increase in the average number of GFAP-positive cells is directly related to the decrease in the average number of major neurons of the cerebellar gray matter, and fluctuations in the average number of astrocytic glia cells is the compensatory mechanism in the recovery of cerebellar gray matter neurons from neural stem cells with the subsequent development of reactive astrogliosis and, thereafter, the possible development of neuropathology.

We have also found that the change in the average number of protoplasmic astrocytes indicates on the compensatory-adaptive reactions of the cerebellum influenced by the food additives complex.

We have noted that in both gray and white matters of the cerebellum the compensatory-adaptive processes are aimed at partial restoration of structural components of the cerebellum, and GFAP-positive structures play a major role in this process as a fibrillar frame.

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Conflict of interest:
The Authors declare no conflict of interest.

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