Morphology and Morphometry of Neurons in Front-Parietal Lobe of the White Rat Cerebral Cortex under the Influence of Lead Acetate

Olga Sergeevna Shubina*, and Olga Ivanovna Komusova.

Federal State Budgetary Educational Institution of Higher Professional Education “M. E. Evseyev Mordovian State Pedagogical Institute”, Mordovia, 430007, Saransk, Studencheskaya Street, 11A.

ABSTRACT

The morphologic and morphometric peculiarities of neurons in the front-parietal or somatosensory lobe of the cerebral cortex of white rats under the influence of lead acetate were studied. To clarify this question the microscopy of the histologic sections of the cerebral cortex by means of the microscope MT 4000 Series Biological Microscope with software for image analysis Bio Vision Version 4.0 was applied. The photographing of specimens was performed with the help of an inboard digital camera using 10×10, 40×10 and 100×10 enlarging.

Keywords: cerebrum, lead acetate, necrotic neurons, perikaryons.

*Corresponding author
INTRODUCTION

The study of anthropogenic influence of heavy metals on human and animal health is one of the basic problems in biology and medicine. It is well-known that lead is a poison of a polytropic action. Many scientists think that the nervous system, the cerebrum in particular, is first to react on lead intoxication [2, 3, 5]. But the clarification of the changes in the microscopic structure of the cerebral cortex under the influence of heavy metals is still relevant and requires further investigation [1, 7].

The aim of the research was to study morphologic and morphometric peculiarities of neurons in the front-parietal lobe of the cerebral cortex of white rats under the influence of lead acetate.

TECHNIQUE

The research was carried out in 50 white outbred sexually mature male Wistar rats weighing 200 – 250 g. 25 animals that had the general feeding regime in the vivarium were taken as a control group. 25 animals that had the general feeding regime in the vivarium and received lead acetate Pb(CH$_3$COOH)$_2$ x 3H$_2$O in a single dose at a rate of 45mg/kg body weight each day orally for 7 days were taken as an experiment group.

The animals were anesthetized with the use of the ether-chloroform (1:1) mixture and slaughtered via decapitation. All the principals of humanity set out in the directives of the European community (86/609/EEC) and in the Declaration of Helsinki were covered and the regulations requirements to the fulfillment of work with the use of experimental animals were kept.

The sections of the rat cerebral cortex in the front-parietal lobe were the data for study.

To get the data the skin and muscles were removed from the skull surface with scissors making the bone tissue lay bare. Then cervical vertebrae and the occipital bone were pitched off with forceps and the last one was removed. Then the branch of forceps, being placed under skullcap firstly from one side then from the other one, pitched parietal and temporal bones off which were also removed. The cerebrum bared in such a way was lifted upwards from frontal lobes, the visual tract was cut off, after that the cerebrum was lifted even higher, the pachymeninx covering the cerebellum was cropped and the cerebrum was extracted after cutting the neuraxis off in the cervical spine. After cutting the membranes the extracted organ was weighed and put into a jar of formalin for fixation [2, 3, 4, 6].

The cerebrum fixation was performed by means of 10 % formalin, prepared on a 0,2 М phosphate buffer, and Carnoy’s fluid. When there were no any macroscopically visual damages of the organ, the lengthwise section at the level of the medulla was made. Making the microscopic sections was carried out by means of cutting blocks filled up with paraffin spheres. The paraffin sections, 4 – 5 mcм in thickness, were stained with hematoxylin and eosin for demonstration purposes and with the methylene blue according to Nissl – for studying cytoarchitectonics. Studies of the histologic specimens were performed with the help of a digital microscope MT 4000 Series Biological Microscope that has software for image analysis Bio Vision Version 4.0. The photographing of specimens was performed with the help of an inboard digital camera using 10×10, 40×10 and 100×10 enlarging.

The static handling of the received digital data was performed in the program Exel. The testing of statistical hypotheses was accomplished according to Student’s t-test. While valuating statistical hypotheses the following significance levels were accepted: p≤0,01, p≤0,05.

MAIN PART

While the cytologic research it was revealed that the neocortex in both control and experiment groups consists of 6 layers of neurons that are placed in the following order: 1. the molecular layer; 2. the external granular layer; 3. the external pyramidal layer; 4. the internal granular layer; 5. the internal pyramidal layer; 6. the multiform layer (Fig. 1).

Fig. 1.
Fig. 1. The cerebral cortex of rats (the control group) containing 6 layers of neurons. Hematoxylin and eosin stain. Enlarging 10×10: 1 – the molecular layer; 2 – the external granular layer; 3 – the external pyramidal layer; 4 – the internal granular layer; 5 – the internal pyramidal layer; 6 – the multiform layer.

The study showed the differences in the morphologic structure of neurons in the cerebral cortex of white rats in the control and experiment groups.

In the control group the molecular layer contains few neurons of a slightly elongated or oval shape, 4.19±0.51 mcm in diameter. The average surface of the cell is 21,07±1,19 mcm². The cytoplasm of the cell has a small grain structure, the surface of the nuclei is 8,24±0,53 mcm², the diameter is 1,36±0,23 mcm. Cell processes, axons and dendrites, form the most part of the molecular layer volume. The thickness of the layer is 88,44±0,94 mcm (Fig. 2, Table. 1).

The perikaryons of the molecular layer settle themselves in short “chains” under the influence of lead acetate. In comparison with the control group the neurons are enlarged and have an oval shape, the average surface of the cell is 39,61±0,77*mcm² (p≤0,01), the diameter is 6,09±0,41*mcm (p≤0,01). The nuclei of the neurons are well-visible, their surface is 13,74±1,12* mcm² (p≤0,01) and the diameter is 3,41±0,43* mcm (p≤0,01). The thickness of the layer under the influence of lead is 282,00±1,05* mcm (p≤0,01) (Fig. 3, Table 1).

The external granular layer of the control group is formed out of large neurons having a round or pyramidal shape, 10,11±0,43 mcm in diameter. The average surface of the cells is 74,58±1,92 mcm². The neurons contain nuclei of a slightly elongated or oval shape, 22,47±0,91 mcm² in surface and 4,69±0,44 mcm in
diameter. The cytoplasm of the cell has a coarse-grain structure. The cells are placed tightly, so they form a strictly separated layer, 62.81±0.46 mcm in thickness (Fig. 4, Table 1).

In the experiment group the external granular layer is formed out of large oval cells, 104,15±0,58* mcm² (p≤0,01) in surface and 1,84±0,59* (p≤0,01) mcm in diameter. The perikaryons are placed tightly to one another and hardly have any intercellular space. The nuclei are small, have a regular oval shape. Their surface is 8,46±0,48* mcm² (p≤0,01) and the diameter is 3,32±0,60* mcm (p≤0,01). It was stated that the necrotic or “melting” neurons are significantly enlarged. The thickness of the layer under the influence of lead is 46,94±0,69* mcm (p≤0,01) (Fig. 5, Table 1).

The external pyramidal layer is formed out of innumerous pyramidal neurons of a conic shape, 27,14±0,67 mcm² in surface and 6,87±0,48 mcm in diameter. The neurons contain small nuclei of a round shape. The average surface of the layer is 8,82±0,47 mcm², the diameter is 4,14±0,39 mcm. The cytoplasm of the cells has a smooth non-granular structure. The thickness of the layer is 119,18±1,74 mcm (Fig. 6, Table 1).

As a result of the experiment with lead acetate the external pyramidal layer comes to be formed out of pyramidal cells placed in short “chains”. The average surface of the cells is 45,79±0,88* mcm² (p≤0,01), the diameter is 7,86±0,54* mcm (p≤0,01). The nuclei are small and more frequently settle themselves close to the cell membrane, their surface is 5,94±0,52*mcm² (p≤0,01), the diameter is 2,95±0,51* mcm (p≤0,01). The structure of the layer is not heterogeneous. The thickness of the layer is 76,45±0,97* mcm (p≤0,01) (Fig. 7, Table 1).
In the control group the internal granular layer contains small stellate cells, \(31.60 \pm 1.14 \text{ mcm}^2\) in surface and \(5.93 \pm 0.43 \text{ mcm}\) in diameter. The nuclei of the perikaryons have a round shape and a clear-cut structure. The average surface of the nuclei is \(4.38 \pm 0.54 \text{ mcm}^2\), the diameter \(2.18 \pm 0.32 \text{ mcm}\). The cytoplasm in the cell is arranged evenly without any large protein concentrations. The thickness of the layer is \(664.38 \pm 0.66 \text{ mcm}\) (Fig. 8, Table 1).

While studying the internal granular layer in the experiment group of animals it was noticed that the number of cells decreased, that the shape of the neurons changed and became oval and that their size also slightly changed. According to changes, the average surface of the cells is \(26.02 \pm 0.87^* \text{ mcm}^2\) (\(p \leq 0.01\)), the diameter \(5.89 \pm 0.59^{**} \text{ mcm}\) (\(p \leq 0.05\)). The nuclei of the perikaryons are small, \(2.09 \pm 0.26^* \text{ mcm}^2\) (\(p \leq 0.01\)) in surface, \(2.04 \pm 0.26^* \text{ mcm}\) (\(p \leq 0.01\)) in diameter. The structure of the layer is heterogeneous with frequent “gaps”, the thickness of the layer greatly decreased and came to \(240.30 \pm 1.20^* \text{ mcm}\) (\(p \leq 0.01\)) (Fig. 9, Table 1).

The internal pyramidal layer is formed out of large neurons (Betz cells, Meynert cells) and innumerous stellate cells.

The study of the control group of animals showed that Betz cells are the largest neurons of the cortex being \(149.31 \pm 1.18 \text{ mcm}^2\) in surface and \(13.66 \pm 0.89 \text{ mcm}\) in diameter and have a long apical dendrite going out of the vertex of the pyramid into overlying layers and other dendrites going from sides and from basement of the perikaryon. The axon goes from the basement of the pyramid to the white substance. The cytoplasm doesn’t contain concentrations, the nuclei are large, the surface is \(28.83 \pm 0.61 \text{ mcm}^2\), the diameter is \(6.34 \pm 0.57 \text{ mcm}\). Meynert cells are quite large neurons localized over the whole fifth layer of the cerebral cortex. The average surface of the cells is \(86.13 \pm 0.96 \text{ mcm}^2\), the diameter is \(9.23 \pm 0.40 \text{ mcm}\). The cells have a pyramidal shape but in comparison with Betz cells they have no any large apical and lateral dendrites, their nuclei are relatively large, round and their surface is \(16.59 \pm 0.67 \text{ mcm}^2\).

The perikaryons of the stellate neurons are of a round, polygonal or triangular shape, their diameter is 9–14 mcm. The average thickness of the internal pyramidal layer is \(285.61 \pm 0.99 \text{ mcm}\) (Fig. 10, Table 1).

The internal pyramidal layer of the front-parietal lobe of the cerebral cortex in the experiment group is formed out of cell distinguishing by a noticeably smaller size from those of the control group.

According to the received data, Betz cells have the surface of \(114.04 \pm 0.53^* \text{ mcm}^2\) (\(p \leq 0.01\)) and the diameter of \(15.24 \pm 0.82^* \text{ mcm}\) (\(p \leq 0.01\)). Meynert cells under the influence of lead have even structure with a small amount of concentrations. The nuclei of cells occupy almost all the cell space and have the surface of \(10.94 \pm 0.39^* \text{ mcm}^2\) (\(p \leq 0.01\)) (Betz cells) and \(3.02 \pm 0.32^* \text{ mcm}^2\) (\(p \leq 0.01\)) (Meynert cells). The structure of the layer is heterogeneous having solitary cystic formations of an oval shape that are spread all over the layer thick. The thickness of the layer is \(240.33 \pm 1.23^* \text{ mcm}\) (\(p \leq 0.01\)) (Fig. 11, Table 1).
### Table 1: The morphometric indices of neurons in the front-parietal lobe of the cerebral cortex of white rats in normal state and under the influence of lead

<table>
<thead>
<tr>
<th>The layers of the cerebral cortex</th>
<th>The surface of a cell, mcm²</th>
<th>The diameter of a cell, mcm</th>
<th>The surface of a nucleus, mcm²</th>
<th>The diameter of a nucleus, mcm</th>
<th>The thickness of a layer, mcm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>experiment</td>
<td>control</td>
<td>experiment</td>
<td>control</td>
</tr>
<tr>
<td>The molecular layer</td>
<td>21,07±1,19</td>
<td>39,61±0,77*</td>
<td>4,19±0,51</td>
<td>6,09±0,41*</td>
<td>8,24±0,53</td>
</tr>
<tr>
<td>The external granular layer</td>
<td>74,58±1,92</td>
<td>104,15±0,58*</td>
<td>10,11±0,43</td>
<td>11,84±0,59*</td>
<td>22,47±0,91</td>
</tr>
<tr>
<td>The external pyramidal layer</td>
<td>27,14±0,67</td>
<td>45,79±0,88*</td>
<td>6,87±0,48</td>
<td>7,86±0,54*</td>
<td>8,82±0,47</td>
</tr>
<tr>
<td>The internal granular layer</td>
<td>31,60±1,14</td>
<td>26,02±0,87*</td>
<td>5,93±0,43</td>
<td>5,89±0,59**</td>
<td>4,38±0,54</td>
</tr>
<tr>
<td>The internal pyramidal layer (Betz cells)</td>
<td>149,31±1,18</td>
<td>114,04±0,53*</td>
<td>13,66±0,89</td>
<td>15,24±0,82*</td>
<td>28,83±0,61</td>
</tr>
<tr>
<td>The internal pyramidal layer (Meynert cells)</td>
<td>86,13±0,96</td>
<td>51,39±0,72*</td>
<td>9,23±0,40</td>
<td>7,18±0,38*</td>
<td>16,59±0,67</td>
</tr>
<tr>
<td>The multiform layer (granule cells)</td>
<td>76,35±0,76</td>
<td>56,23±1,81*</td>
<td>9,58±0,55</td>
<td>9,82±0,43**</td>
<td>16,08±0,92</td>
</tr>
<tr>
<td>The multiform layer (pyramidal cells)</td>
<td>71,97±1,55</td>
<td>98,56±1,53*</td>
<td>7,05±0,57</td>
<td>13,92±1,00*</td>
<td>11,83±0,45</td>
</tr>
<tr>
<td>The multiform layer (oval cells)</td>
<td>29,47±0,80</td>
<td>25,75±0,93*</td>
<td>6,32±0,47</td>
<td>5,86±0,39*</td>
<td>8,22±0,32</td>
</tr>
</tbody>
</table>

Note:  
* - p≤0.01 – in comparison with the animals of the control group  
** - p≤0.05 – in comparison with the animals of the control group;
The multiform layer in the control group is formed out of numerous neurons of different size and shape and also of some amount of pyramidal and granule neurons (Fig. 12). The cells are arranged in chains. The pyramidal neurons have a slightly elongated shape with evident prolongations, their surface is 71,97±1,55 mcm². The nuclei are not large with even structure, their surface is 11,83±0,45 mcm². The granule cells have a subangular shape, their size is 74–77 mcm. The cytoplasm has large protein concentrations that give granularity to the inner content of the cells. The nuclei of the cells are elongated, their surface is 16,08±0,92 mcm², the diameter is 5,15±0,36 mcm.

The neurons of an oval shape with a small apical process, 28 – 31 mcm in size, were also found in the multiform layer. The outlines of the cells are even, the cytoplasm has a weak structure, the nuclei are small, their diameter is 3,27±0,53 mcm. The average surface of the cells is 29,47±0,80 mcm². The thickness of the multiform layer is 580,63±1,66 mcm. (Fig. 12, Table 1).

The structure of the multiform layer in the experimental group contains frequent “gaps”. The neurons are arranged in chains, the most part is formed out of “dark” hyperchromophilic perikaryons. In comparison with the control group the surface of the pyramidal neurons is enlarged and comes to 98,56±1,53 mcm² (p≤0,01).

In its turn, the surface of the granule neurons is decreased and comes to 56,23±1,81 mcm² (p≤0,01). The oval neurons have the surface of 25,75±0,93 mcm² (p≤0,01) and the diameter of 5,86±0,39 mcm (p≤0,01). The surface of the nuclei varies from 2 to 7 mcm². Frequent cystic formations of a round shape are observed in the layer structure, the thickness of the layer is 554,85±1,51 mcm (p≤0,01) (Fig. 13, Table 1).
CONCLUSION

The studies showed the differences in the morphologic structure of neurons in the cerebral cortex of white rats in the control and experiment groups.

1. The molecular layer in the control group contains few neurons of a slightly elongated or oval shape. Axons and dendrites form the most part of the molecular layer volume. Under the influence of lead acetate the perikaryons of the molecular layer settle themselves in short “chains”. The neurons are enlarged in comparison with those of the control group.

2. The external granular layer of the control group is formed out of large neurons having a round or pyramidal shape. The cells are placed tightly, so they form a strictly separated layer. In the experiment group the external granular layer is formed out of large oval cells. It was stated that the necrotic or “melting” neurons are significantly enlarged.

3. The external pyramidal layer is formed out of innumerous pyramidal neurons of a conic shape. The neurons contain small nuclei of a round shape. As a result of the experiment with lead acetate the external pyramidal layer comes to be formed out of pyramidal cells placed in short “chains”. The nuclei are small and more frequently settle themselves close to the cell membrane.

4. In the control group the internal granular layer contains small stellate cells. The nuclei of the perikaryons have a round shape and a clear-cut structure. While studying the internal granular layer in the experiment group of animals it was noticed that the number of cells decreased, that the shape of neurons changed and became oval and that their size also slightly changed. The structure of the layer is heterogeneous with frequent “gaps”.

5. The internal pyramidal layer is formed out of large neurons (Betz cells, Meynert cells) and innumerous stellate cells. The internal pyramidal layer of the front-parietal lobe of the cerebral cortex in the experiment group is formed out of cell distinguishing by a noticeably smaller size from those of the control group. The structure of the layer is heterogeneous having solitary cystic formations of an oval shape that are spread all over the layer thick.

6. The multiform layer in the control group is formed out of numerous neurons of different size and shape and also of some amount of pyramidal and granule neurons (Fig. 12). The cells are arranged in chains. The structure of the multiform layer in the experimental group contains frequent “gaps”. The neurons are arranged in chains, the most part is formed out of “dark” hyperchromophilic perikaryons.

7. The research allowed describing cytologic and morphometric peculiarities of cellular structure of layers in the front-parietal lobe of the cerebral cortex of white rats in normal state and under the influence of
lead acetate. The studies showed that some layers are exposed to changes in a greater degree; they are the internal granular layer, the internal pyramidal layer and the multiform layer. The changes happening under toxic action of lead in these layers are characterized by appearing hyperchromophilic perikaryons and numerous cystic formations. The decrease of the total surface of the cerebral cortex is revealed under the influence of lead acetate. In such a way the total surface of the cortex in the control group was 1801,05 mcm and the total surface of the cortex in the experiment group came to 1440,87* mcm (p≤0,01) (Table 1).

ACKNOWLEDGEMENT

The study was carried out with the financial support of the Ministry of Education and Science of the Russian Federation within the state programme of FSBEI HPE “M. E. Evseyev Mordovian State Pedagogical Institute” (project “The influence of anthropogenic factors on the morphofunctional state of the organism”).

REFERENCES