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# Comparative Characteristics of Changes in Neuron Organelles During Two-Stage Ligation of the Common Carotid Arteries in Phylogenetically Different Sections of the Brain Cortex of Outbreed White Rats

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**Abstract**

Ultrastructural characteristics of changes in neuronal organelles during two-stage ligation of the common carotid arteries in phylogenetically different sections are important indicators of the degree of brain damage during ischemic exposure, which necessitates the study of this aspect. Two-stage subtotal cerebral ischemia was performed on 24 outbred male rats by sequential ligation of both common carotid arteries with an interval of 7 days, 3 days and 1 day. This method of modeling subtotal ischemia makes it possible to study the dynamics of the consequences of severe cerebral ischemia and the pathogenesis of ischemic brain damage. Thus, compensatory changes in neuronal organelles during two-stage ligation of the common carotid arteries included expansion of the cisternae of the Golgi complex and hyperplasia of the endoplasmic reticulum, as a reflection of the activation of compensation mechanisms during hypoxia.

**Keywords:** rat, cerebral ischemia, hippocampus, parietal cortex

## 1. Introduction

In the world literature, there are data according to which, with insufficient blood supply to the brain, a number of ultrastructural disorders of the brain occur, namely: an increase in the roundness of mitochondria, disorganization and destruction of their cristae; in synthetic and transport organelles, such as the Golgi complex and endoplasmic, an increase in the width of the tubules is observed; ribosomes are split off from the rough endoplasmic reticulin and the proportion of their free fraction in the cytoplasm increases significantly (Maksimovich NE, Bon' EI & Zimatkin SM., 2020; Gusev E. I. & Skvortsova V. I., 2003).

Changes in the organelles of nerve cells can act as a putative diagnostic marker of the severity of hypoxic damage to the nervous system. Thus, establishing the nature of organelle disorders in cerebral ischemia is important and relevant.

The literature describes various methodological approaches to modeling cerebral ischemia (incomplete, focal, complete, etc.).

This article describes the original stepped model of cerebral ischemia, developed by the authors of the article. This model makes it possible to assess not only the severity of hypoxic damage to neurons, but also the degree of implementation of compensatory mechanisms, since, in our opinion, it is the compensatory mechanisms that should be given the most attention when detailing the pathogenesis of cerebral ischemia (Maksimovich NE, Bon' EI & Zimatkin SM., 2020; González Fuentes J, Insausti Serrano R, Cebada Sánchez S, Lagartos Donate MJ, Rivas Infante E, Arroyo Jiménez MDM et al., 2021; Maksimovich NYe, Bon EI & Zimatkin SM., 2021).

For a detailed study of pathological processes, mechanisms of damage development and adaptation in the brain, it is necessary to develop an adequate model of cerebral ischemia, which would increase their life expectancy after surgery.

The problem is solved by ligation of the common carotid arteries (CCA) in rats, with the difference being that first one CCA is ligated, and a second one a few days later (Eklöf B. & Siesjö B. K., 1973; Towfighi J. et al., 1997; Eklöf B. & Siesjö B. K., 1972; Kitagawa K. et al., 1998).

This method of modeling subtotal ischemia by staged (non-simultaneous) ligation of both common carotid arteries makes it possible to study the consequences of severe cerebral ischemia and the pathogenesis of ischemic brain damage in dynamics (Eklöf B. & Siesjö B. K., 1972; Kitagawa K. et al., 1998; Ginsberg M. D., Medoff R. & Reivich M., 1976; Vannucci R. C. et al., 1999).

In this article, the goal was to study changes in neuronal organelles during two-stage ligation of the common carotid arteries in phylogenetically different parts of the cerebral cortex of outbred rats.

**2. Materials and Methods of Research**

The experiments were carried out on 24 male outbred white rats weighing 260±20g. Permission for the study was obtained from the ethical commission of the Grodno State Medical University (protocol No. 1, date 01/05/2022).

Sodium thiopental was used for anesthesia, the solution was administered intravenously at a dose of 40 mg/kg.

Stepwise subtotal cerebral ischemia or SSCI was performed by sequential ligation of both common carotid arteries with an interval of 7 days, 3 days and 1 day (Bon L. & Maksimovich N. Y., 2021; Bon L. I. & Maksimovich N. Y., 2019).

Six rats underwent only a sham operation, they made up a sham group (Bon L. I. et al., 2021).

Stepwise subtotal cerebral ischemia or SSCI was performed by successive ligation of both CCAs with an interval of 7 days (subgroup 1), 3 days (subgroup 2), or 1 day (subgroup 3). The material was taken 1 hour after ligation of the second CCA in each of the subgroups (Bon L. I. & Maksimovich N. Y., 2021).

Immediately after decapitation and quick removal of the brain, sections of the parietal cortex and hippocampus were cut out with a blade and placed in 1% osmium fixative in Millonig's buffer (pH=7.4) for 2 hours at 4°C (Jenkins L. W. et al., 1979).

Next, the sections were washed in a mixture of Millonig's buffer (20 ml) and sucrose (900 mg), dehydrated in alcohols of increasing concentration, a mixture of alcohol and acetone, pure acetone; passed through a mixture of resins (araldite M + araldite H + dibutyl phthalate + DMR-30) and acetone and embedded in a mixture of resins (Bon L. I. & Maksimovich N. Y., 2021; Jenkins L. W. et al., 1979).

Ultrathin sections (about 35 nm thick) were made on an MT-7000 (RMC, USA) ultramicrotome, assembled on support grids, and counterstained with uranium acetate and lead citrate. To do this, the meshes with sections were dipped into a drop of uranyl acetate and kept for 20 minutes in the dark at room temperature, then washed in 3 portions of bidistilled water for 5 seconds and contrasted with lead citrate for 8 minutes, washed in 3 portions of bidistilled water for 5 seconds.

The resulting preparations were studied under a JEM-1011 electron microscope (JEOL, Japan) and photographed with an Olympus MegaView III digital camera (Olympus Soft Imaging Solutions, Germany).

Ultrastructure morphometry was performed using the Image Warp image processing program (Bit Flow, USA).

To prevent a systematic measurement error, brain samples from the compared control and experimental groups of animals were studied under the same conditions. As a result of the research, quantitative continuous data were obtained. Since the experiment used small samples that had a non-normal distribution, the analysis was performed by nonparametric statistics using the licensed computer program Statistica 10.0 for Windows (StatSoft, Inc., USA). The data are presented as Me (LQ; UQ), where Me is the median, LQ is the value of the lower quartile; UQ is the value of the upper quartile. Differences between groups were considered significant at $p<0.05$ (Kruskell-Wallis test with Bonferoni correction) (Rebrova O. Y., 2002).

**3. Research Results**

Modeling of stepped subtotal cerebral ischemia (SSCI) was carried out by sequential ligation of both CCAs with an interval of 7 days (subgroup 1), 3 days (subgroup 2), or 1 day (subgroup 3). Unlike SIGM with simultaneous ligation of both CCAs, this model of cerebral ischemia makes it possible to study the development of compensatory mechanisms.

Stepped subtotal cerebral ischemia with an interval of 1 and 3 days between dressings of both CCAs leads to damage to the neurons of the parietal cortex and hippocampus of rats, which manifests itself in a decrease in

their size, deformation of the perikarya, and an increase in the number of shriveled neurons and shadow cells. The most pronounced changes were observed in the SSCI subgroup with an interval between dressings of 1 day. These disorders are in many respects similar to those in subtotal cerebral ischemia, except for the absence of cells with pericellular edema in the hippocampus and a smaller number of them in the parietal cortex. SSCI with an interval between CCA dressings of 7 days, on the contrary, is manifested by lesser severity of histological changes, especially in the hippocampus.

When studying the ultrastructure of neurons in the brain of rats with stepped cerebral ischemia, the following data were obtained.

In the 1st subgroup of SSCI, with an interval between ligation of both CCAs of 7 days, giant mitochondria were found in the cytoplasm of neurons of both the thymen cortex and the hippocampus of rats. In addition, hyperplasia of the endoplasmic reticulum occurred (Figure 1).

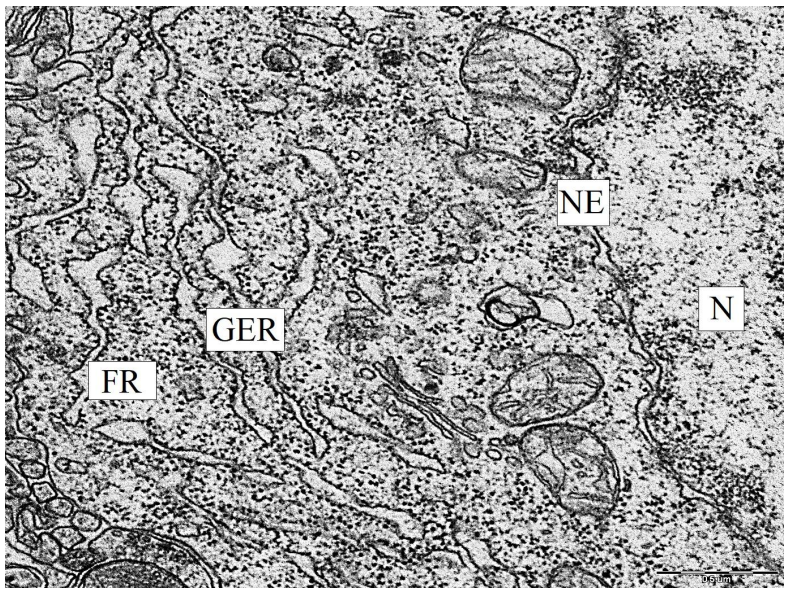


Figure 1. – Endoplasmic reticulum of neurons in the parietal cortex of rats of the 1st subgroup of SSCI. N – nucleus, NE – nuclear envelope, GER – granular endoplasmic reticulum, FR – free ribosomes. Electrongram. Zoom 50000

The number of free ribosomes in the cytoplasm of neurons in the parietal cortex and hippocampus increased by 67(62;72)%, $p<0.05$ and by 59(52;62)%, $p<0.05$ compared with the values in the control group (Figure 2).

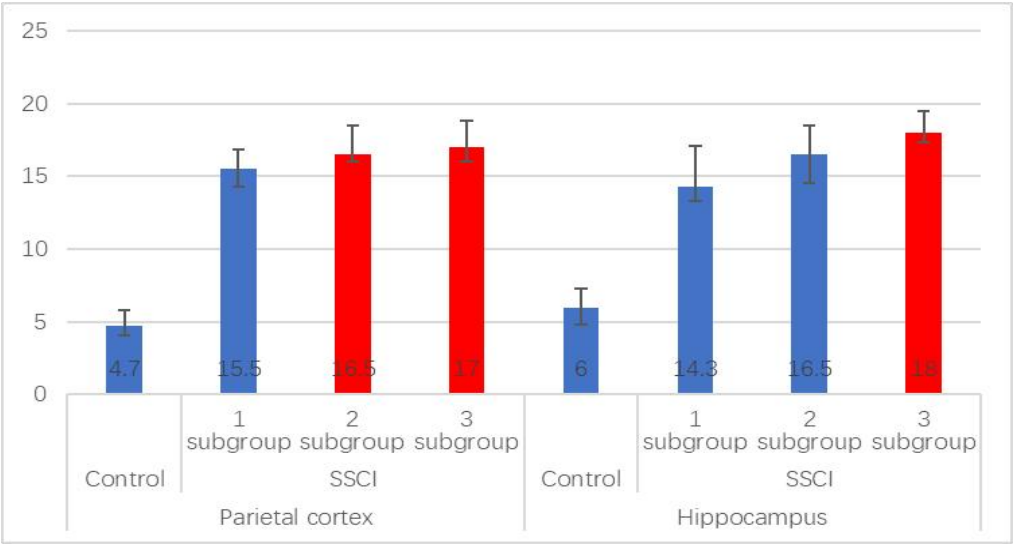


Figure 2. – Number of free ribosomes (μm$^2$) in the cytoplasm of neurons in the parietal cortex and hippocampus. Red color – $p<0.05$ compared to control

Disorganization and expansion of the cisternae of the Golgi complex was noted (Figure 3), the sizes of lysosomes did not differ from the sizes in the control group, however, their density in the cytoplasm of neurons of the parietal cortex and hippocampus of rats of the 1st subgroup of SSCI, compared with the indicators in the "control" group, was more by 50(44;59)%, $p<0.05$ and by 38(32;44)%, $p<0.05$.

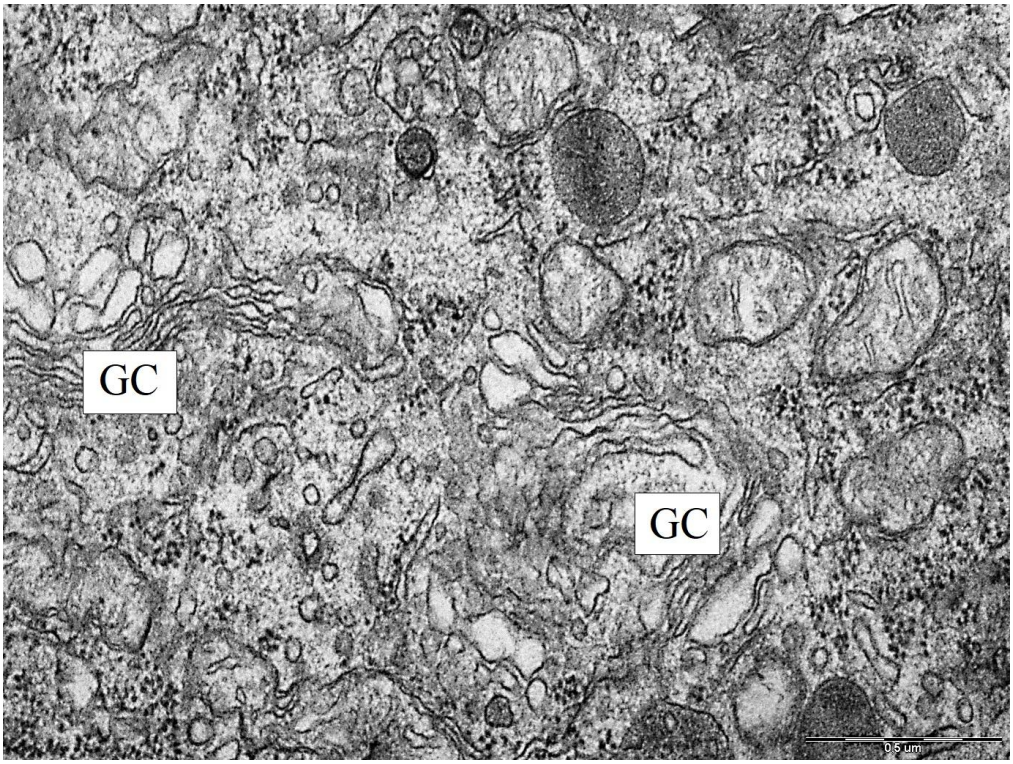


Figure 3. – Golgi complex of neurons in the parietal cortex of the rats of the 1st subgroup of SSCI. GC – Golgi complex. Electronogram. Zoom 50000

Compared with the indicators in the control group, in rats of the 2nd subgroup of SSCI with an interval between CCA dressings of 3 days, the mitochondrial elongation factor in the parietal cortex decreased by 50(47;58)%, $p<0.05$, and factor – increased by 28(17;33)%, $p<0.05$. The density of mitochondrial cristae and their length decreased by 42(35;46)%, $p<0.05$ and by 50(46;55)%, $p<0.05$, respectively. At the same time, compared with the indicators in the 1st SSCI subgroup with an interval between CCA ligations of 7 days, in rats of the 2nd SSCI subgroup with an interval between CCA ligations of 3 days, in the parietal cortex, the mitochondrial elongation factor was less by 39 (32; 43)%, $p<0.05$, and the form factor is higher by 25(22;30)%, $p<0.05$. The density of mitochondrial cristae and their length were less by 41(35;47)%, $p<0.05$ and by 67(63;75)%, $p<0.05$, respectively. In the hippocampus, compared with the indicators in the control group, the mitochondrial elongation factor was less by 24(17;28)%, $p<0.05$, and the form factor was higher by 16(12;18)%, $p< 0.05$, the density of cristae did not differ ($p>0.05$), and their length decreased by 38(34;43)%, $p<0.05$. Compared with the indicators in the 1st SSCI subgroup, the elongation factor was less by 13(11;19)%, $p<0.05$, the form factor was higher by 27(23;32)%, $p<0.05$. The density of cristae did not differ ($p>0.0$), and their length was less by 43(37;48)%, $p<0.05$.

The number of free ribosomes in the cytoplasm of neurons in the parietal cortex and hippocampus of rats of the 2nd SSCI subgroup increased by 72(66;80)%, $p<0.05$ and by 64(56;68) %, $p<0.05$, and compared with the indicators in the 1st subgroup, SSCI did not differ ($p>0.05$).

Vacuolization of cisterns of the endoplasmic reticulum and the Golgi complex was noted, the density of lysosomes, compared with the indicators in the control group, increased by 73(66;78)% in the parietal cortex ($p<0.05$) and by 62(52;64)% – in the hippocampus ($p<0.05$), and their area – by 90(86;95)%, $p<0.05$ and by 75(73;81)%, $p<0.05$, respectively. Compared to the indices in the 1st subgroup of SSCI, the density of lysosomes in the 2nd subgroup of SSCI increased by 47(42;52)% in the parietal cortex ($p<0.05$) and by 38(33;45)% in the hippocampus ( $p<0.05$), and their area – by 90(85;97)%, $p<0.05$ and by 50(48;56)%, $p<0.05$, respectively (Figure 4, 5).

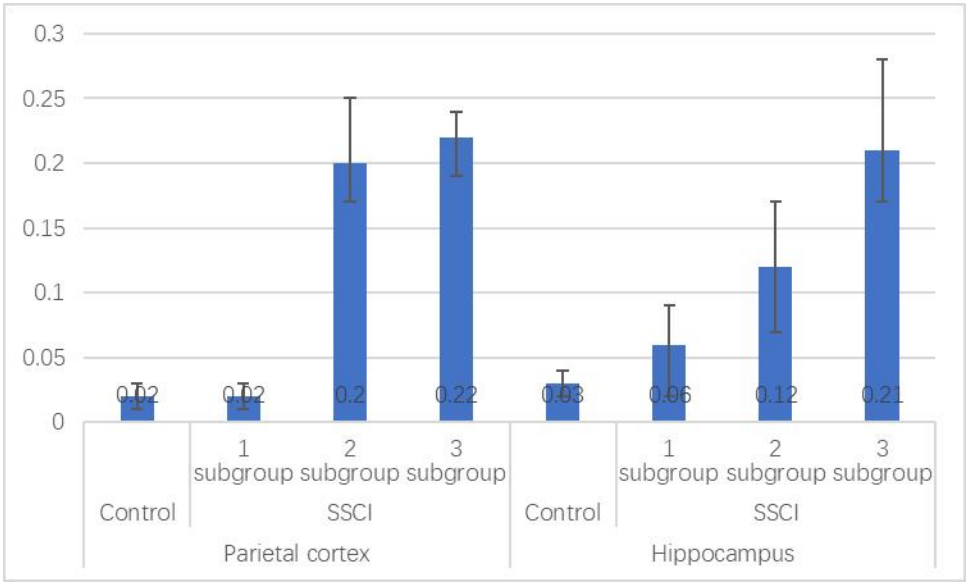


Figure 4. – Area of lysosomes (μm$^2$) in the cytoplasm of neurons in the parietal cortex and hippocampus

Compared with the indicators in the control group, in rats of the 3rd subgroup of SSCI with an interval between CCA dressings of 1 day, in the parietal cortex, the mitochondrial elongation factor decreased by 55(47;59)%, p<0.05, while as form factor increased by 28(23;34)%, p<0.05. The density of mitochondrial cristae and their length were less by 42(35;46)%, p<0.05 and by 50(46;55)%, p<0.05, respectively. Compared with the indices in the 1st SSCI subgroup with an interval between CCA ligations of 7 days, in rats of the 3rd SSCI subgroup with an interval between CCA ligations of 1 day, the mitochondrial elongation factor in the parietal cortex decreased by 45 (35; 47)%, p <0.05, and the form factor increased by 26(21;32)%, p<0.05. The density of mitochondrial cristae and their length decreased by 41(35;47)%, p<0.05 and by 67(63;75)%, p<0.05, respectively.

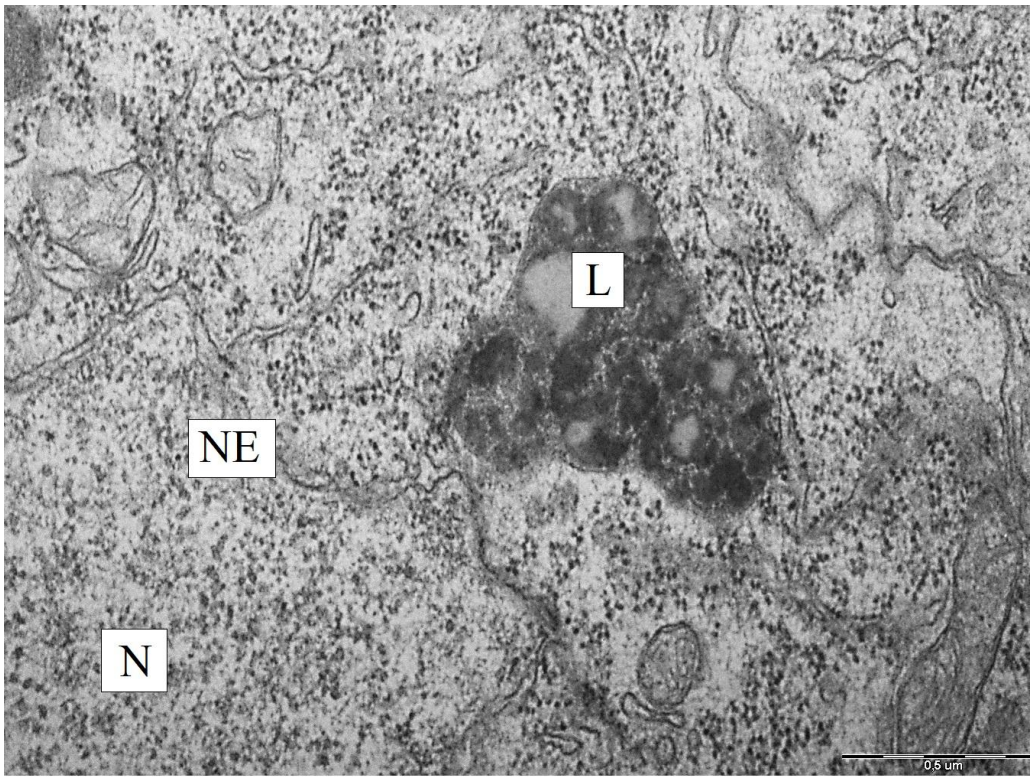


Figure 5. – Lysosomes of neurons in the parietal cortex of rats of the 2nd subgroup of SSCI. N – nucleus, NE – nuclear envelope, L – lysosomes. Electrongram. Zoom 50000

In the hippocampus, compared with the indicators in the control group, in SSCI subgroup 3 rats, the myochondrial elongation factor decreased by 24(17;28)%, p<0.05, and the form factor increased by 17(12; 18)%, p<0.05. The density of cristae did not differ (p>0.0), and their length decreased by 50(47;53)%, p<0.05. In the hippocampus, compared with the indices in the 1st subgroup of SSCI, in rats of the 3rd subgroup of SSCI, the elongation factor was less by 27(18;33)%, p<0.05, and the form factor was higher by 14(10 ;19)%, p<0.05. The density of cristae did not differ (p>0.0), and their length was less by 50(47;58)%, p<0.05 (Figure 6).

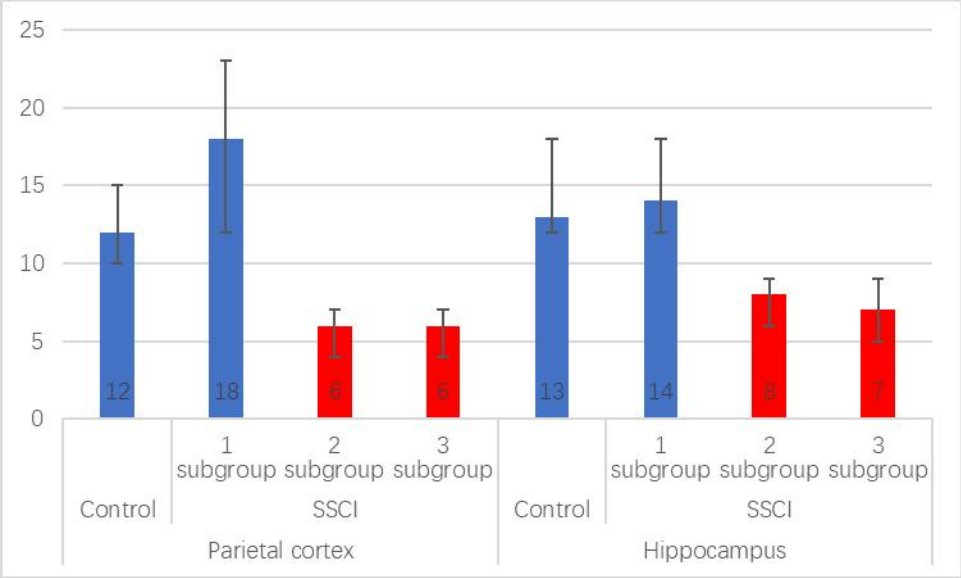


Figure 6. – Length of mitochondrial cristae (μm$^2$) in the cytoplasm of neurons in the parietal cortex and hippocampus. Red color – p<0.05 compared to control

The number of neurons with free ribosomes in the cytoplasm of the parietal cortex and hippocampus of rats of the 3rd subgroup of SSCI, compared with the indicators in the control group, increased by 72(67;79)%, p<0.05 and by 67(52;69 )%, p<0.05. The number of free ribosomes in the cytoplasm of neurons of the parietal cortex and hippocampus in rats of the 1st and 3rd SSCI subgroups did not differ (p> 0.05) "control" by 75(67;79)% – in the parietal cortex (p<0.05) and by 64(53;69)% – in the hippocampus (p<0.05), area – by 91(87;98 )%, p<0.05 and by 86(84;92)%, p<0.05. Compared with the indices in the 1st subgroup of SSCI, the density of lysosomes in the 3rd subgroup of SSCI increased by 50(47;59)% in the parietal cortex (p<0.05) and by 43(25;47)% in the hippocampus (p<0.05), and their area – by 91(85;96)%, p<0.05 and by 71(66;76)%, p<0.05, respectively.

Compared with the parameters in the 2nd SSCI subgroup with an interval between CCA ligations of 3 days, in rats of the 3rd subgroup of SSCI with an interval between CCA ligations of 1 day, the parameters of ultramicroscopic morphometry of the organelles of neurons in the parietal cortex and hippocampus did not differ (p>0.05).

## 4. Conclusion

In subgroup 1 SSCI with an interval between ligation of both common carotid arteries of 7 days, the size and shape of mitochondria and the number and length of mitochondrial cristae did not differ from the values in the control group. There was hyperplasia of the endoplasmic reticulum, which indicates the activation of compensation mechanisms during hypoxia. However, there was an increase in the number of free ribosomes, disorganization and expansion of the cisterns of the Golgi complex. The sizes of lysosomes did not differ from the sizes in the control group, however, their average number in the cytoplasm of neurons increased.

As the time interval between CCA ligations decreased in the 2nd (3 days between CCA ligations) and 3rd subgroups (1 day between CCA ligations) SSCI, the structure of organelles was similar, indicating insufficient inclusion of compensation mechanisms in these types of SSCI modeling.

In the parietal cortex and hippocampus, changes in neuron organelles were similar, except for a decrease in the density of mitochondrial cristae in parietal cortex neurons in SSCI subgroups 2 and 3.

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**Conflict of Interest**

The authors declare no conflict of interest.

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