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Morpho-Functional Changes in Neurons of Spinal Nodes after Experimental Cholecystectomy in Mongrel Dogs

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Abstract In the experiment on mongrel dogs, we studied structural and functional changes of neurons of spinal nodes arising at experimental cholecystectomy. We described the states of neurons in control groups of dogs and in experimental cholecystectomy. Differences in the size indices and the ratio of cells with reactive and destructive changes were found between the two: control and experimental groups of animals.

Keywords Spinal nodes, Neurons, Morphology, Cholecystectomy, Reactive and destructive changes of neurons

1. Introduction

The study of mechanisms of compensatory-adaptive reactions of spinal nodes neurons is one of the urgent problems of modern morphology, since spinal ganglia are primary afferent centers occupying a borderline position between the central and peripheral nervous system. Disruption of the structure and function of receptor neurons forming sensitive ganglia can aggravate the course of the underlying disease and is a condition for inflammatory complications [1,2,3]. In the available literature there are practically no data on the reaction of spinal neurons to cholecystectomy. The aim of the present study was to investigate the morpho functional state of Th_{VII} - Th_X spinal node neurons (SNN) in the dynamics of experimental cholecystectomy [4,5].

2. Materials and Methods of Research

The work was performed on 35 male adult mongrel dogs weighing 3100-3750 g. The animals were kept in individual cages in vivarium conditions with free access to water and food, on a standard diet in accordance with the norms of keeping laboratory animals. Cholecystectomy was performed under sterile conditions. Three groups of animals were formed: a group of control (C) and two experimental groups (early and late terms after surgical intervention). Morpho functional features of neurons in the first experimental group were studied in early terms after surgical interventions, in the second group of animals in late terms. To take experimental material the animals were anaesthetized with xylazine and decapitated. Animals were removed from the experiment on the 1st, 3rd, 5th, 7th, 14th, 28th day in equal groups of 5 animals each, including the control group. Thoracic ganglia Th_{VII} - Th_X were dissected as corresponding to the nerves innervating the gallbladder area. The taken biological material was fixed in Carnoua mixture and poured into paraffin mixture according to the standard technique, then 6 µm thick slices were obtained on a microtome. The obtained slices were stained with haemotoxylin-eosin and according to Nissl's method.

The studies were carried out at the light-optical level, N-300M binocular microscope with a digital camera Quality resolution of 500 megapixel was used, then the obtained images were processed using the programmer ImageJ Ver. 1,38x. To determine the profile field area of neurons, two cell diameters were measured in mutually perpendicular planes and calculated by the formula $S=\pi AB$, where, respectively, A is the larger and B is the smaller diameters. When assessing the state of spinal neurons, we distinguished cells with morphological signs of different functional states: neurons with no pronounced changes at the light-optical level, neurons with reactive or reversible changes, and neurons with destructive changes. The relative number of cells of the described groups was counted and compared. The obtained data were expressed as $P \pm p_i$ (%), where P (%) is the share of cells with the investigated characteristic in the total number of cells in the given group, p_i – is the confidence interval of the sampling fraction. The presence of perinuclear chromatolysis, general hypochromia of cytoplasm, cell shrinkage

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and pericellular oedema were considered to be signs of reactive changes in neurons (Fig. 2). Destructively changed neurons were classified as cells with pronounced pycnosis, shriveling of the nucleus, with possible exit of the nucleus, vacuolisation, extreme hypo- or hyperchromia (Fig. 3). Statistical processing of the obtained results was performed using Stat Soft Statistic 6.0 package. Mann-Whitney test (u) was used to identify differences between groups; the results were considered reliable at $p_u < 0.05$.

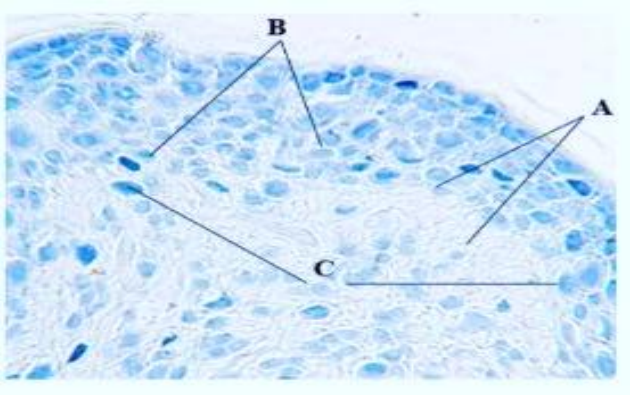

Figure 1. Spinal node neurons in the control group of dogs. A - small cells, B - medium-sized neurons, C - large cells. Magnification x200, line size on the bottom left is 10 µm. Nissl staining

3. Results and Their Discussion

The neurons of the spinal ganglion are represented by pseudounipolar cells, the node itself is surrounded by a connective tissue capsule. In the literature there are many classifications of spinal node neurons based on morphological features, but in most cases 3 groups of spinal node neurons are distinguished: small, medium, large [6,7]. As a result of statistical processing of morphometry data of neurons of the control group of animals, it was found that the size of pseudounipolar cells varied within the limits of 10-18 µm – small, constituted 19±1.8% of the total number of cells, 18-30 µm - medium, 49±5.8% of the total number, 30-61 µm - large neurons, 32±3.7% (Fig. 1). Among the neurons of the animals of the control group, most of them were represented by cells without signs of reactive changes (Fig. 1). In each neuron there is one nucleus of rounded shape with a well-defined nucleus. The Nissl substance was not homogeneous in its content: a part of neurons had large clumps in the perinuclear cytoplasm; in another part of cells tigroid was dispersed throughout the cytoplasm. In the spinal nodes of control animals, the proportion of neurons with signs of reactive changes in the total number was 6.4±3.5%, which, according to literature data [8,9], can be considered a manifestation of normal functional polymorphism of cells.

The changes in the spinal nodes on the 1st day after the operation were characterized by the occurrence of the phenomena of primary irritation of neurons. There was an increase in the number of cells with chromatolysis phenomena - 8.2±0.8% and an increase in the number of cells with enlarged pericellular spaces - 2.8±0.3%.

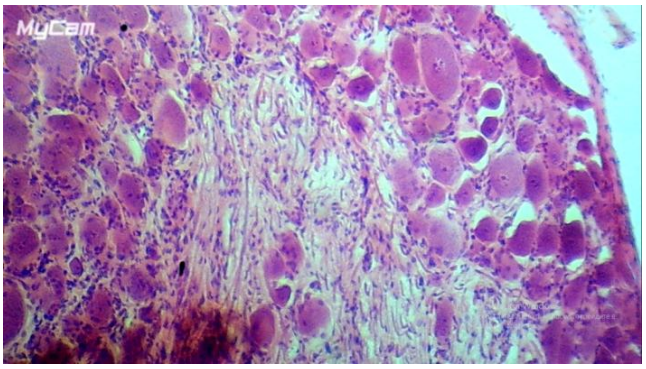

Figure 2. Reactive changes in spinal neurons, 5th day of the experiment. A - cell with chromatolysis phenomena, B - cells with pycnosis phenomena. Magnification x320, line size on the left bottom - 10 µm. Hematoxylin and eosin staining

On the 3rd day of the experiment, chromatolysis phenomena increased in the neurons of the spinal nodes, expressed in the increase of the lumenal zone between the nucleus and the tigroid substance, shifted to the periphery of the cytoplasm. The total number of cells with signs of reactive changes was 28,6±1,5%, and within the histological sections of spinal nodes morphologically changed cells often formed separate groups, outside of which there were unchanged neurocytes. Similar changes in neurons of spinal nodes were detected on the 5th day of the experiment (Fig. 2). One week after experimental cholecystectomy the share of neurocytes with reactive changes increased up to 36,7±3,2%. Among such neurons, cells with condensation of chromatophilic substance under the cell membrane and ectopia of the nucleus were found in large numbers, which can be considered as signs of axonal reaction [10]. Exposure to a complex of posttraumatic factors at this stage leads to partial elimination of nerve cells and initiates destruction processes, as a result of which foci with destructively changed cells appear, the share of which was 15.5±4.2% (Fig. 3).

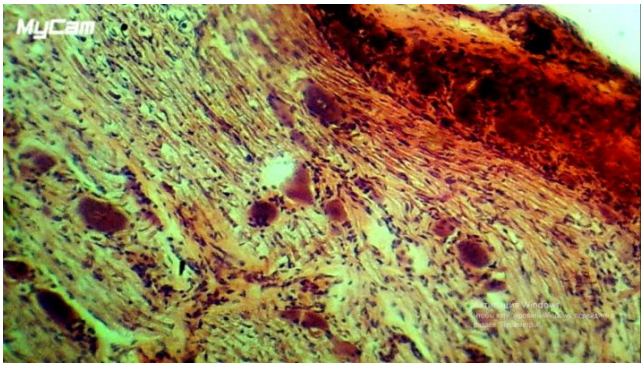

Figure 3. Destructive changes in neurons of spinal nodes on the 7th day after wounding. A - destructive changes in neurons. Magnification x400, line size on the left bottom - 10 µm. Hematoxylin and eosin staining

On the 14th day after the injury the neuronal reaction was most pronounced, a significant part of cells showed sharp irregularity and blurring of contours, shriveling with formation of wide perineuronal cavities. During this period, cells with total chromatolysis and poorly visible borders of nuclei were often met. Due to irreversible changes leading to

neuronal death, single glial nodules were formed as a result of neuronophagy and subsequent migration of satellite glia. The number of cells with reactive changes reached 45.7±4.2%, with destructive changes - 35.6±2.4%.

By 28 days the state of preserved spinal neurons begins to return to normal, there is a restoration of the globular-grained form of chromatophilic substance in cytoplasm. The number of cells with reactive changes decreased and made 24,4±2,4%, but the number of cells with destructive changes remained at high level - 38,4±3,2%. The ratio of cell groups with reactive and destructive changes for animals in the postoperative period is presented in the graph (Fig. 4).

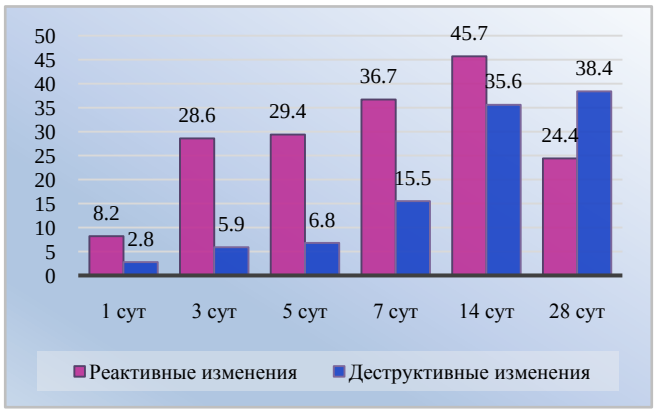

Figure 4. Dynamics of the number of neurons with reactive and destructive changes during cholecystectomy (in % of the total number of cells). Note: reliable difference from the control group, at p<0.5

According to the results of morphometric studies, the average area of neurons of spinal nodes in laboratory dogs of the control group was 2458.3±198.9 μm^2. The dynamics of the mean values of neuron areas for the control and experimental groups is reflected in the table (Table 1). The results of measurements are presented as M±tm, where M is the mean value of the planimetric index, tm is the confidence interval.

Table 1. Profile field areas of spinal cord neurons at cholecystectomy (A) and in control animals (B)

Timing	Neuron profile field area, S μm2 (M±tm)	
	B	A
1 day	2458,3±198,9 μm^2	2557,9±204,9 μm^2
3 days	2449,6±203,6 μm^2	2673,5±214,6 μm^2
5 days	2471,3±203,8 μm^2	2752,9±211,7 μm^2
7 days	2536,3±204,9 μm^2	2926,3±236,1 μm^2
14 days	2478,5±201,8 μm^2	3432,1±275,7 μm^2
28 days	25,36±197,6 μm^2	3215,3±231,5 μm^2

Note: reliable in comparison with control, at p<0.5

Considering in dynamics the average values of neuron areas, it is possible to note that in two experimental groups there was an increase of these indices by 14 days of experiment and a tendency to their decrease in the following terms. The confidence interval for all groups and terms was also quite high.

To form a more accurate picture of changes in the planimetric indices of spinal neurons, at the second stage of the study we analyzed the correlations between groups of neurons stratified by the profile field area into three dimensional groups. It was found that, starting from the 1st day of the study, there was a rearrangement of the ratios of the size groups of cells. In the postoperative period, the number of neurons of the medium-sized group increased throughout the studied period of time, the proportion of small neurons after a constant decrease up to 14 days began to increase smoothly by 28 days of the experiment. The number of large cells decreased throughout the whole period of the study. Changes in the size groups of neurons during experimental cholecystectomy are presented in percentage in Fig. 5.

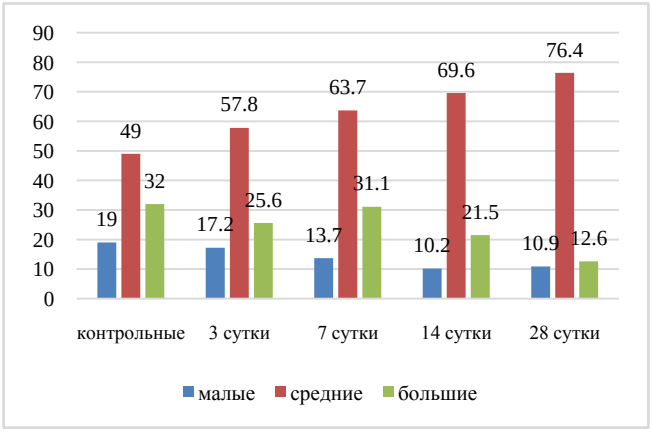

Figure 5. Dynamics of changes in the ratios of neuron size groups in selected terms of the study after cholecystectomy. Note: reliable difference from the control group, at p<0.5

Considering the structural and functional rearrangement of neurons of spinal nodes in aggregate, it can be noted that the change in the ratio of different size groups of cells is a consequence of several processes: increase or decrease in the area of the studied cells and the occurrence of irreversible destructive changes at different terms of the experiment, leading to the elimination of cells of different sizes. Changes in the areas of neuron profile fields, leading to changes in the ratio of cell groups, can be caused by different processes in the cells. According to literature data, a decrease or increase in neuron size is a reflection of the process of functional rearrangement of a nerve cell and can occur both in neurons without visible changes and in reactively changed cells [11,12]. Neuron wrinkling, reflecting the process of cell fatigue [9] and accompanied by a decrease in the profile field area, may also be the cause of cell transition from one size group to another.

4. Conclusions

The study of the dynamics of changes in neurons during the postoperative period reveals successive stages: 1) progressive change of the studied characteristics by 14 days of observation, reflecting the reaction of spinal neurons to

cholecystectomy and the following activation of reparative processes; 2) gradual decrease of the formed disorders by 28 days, corresponding to a favorable outcome of the postoperative process. The complex of changes in spinal neurons accompanying the experimental operation includes an increase in the number of medium-sized neurons. Apparently, this is associated with multidirectional changes in the sizes of large and small neurons. The accelerated dynamics of the increase in the proportion of reactively changed neurons with the subsequent decrease in destructive changes in spinal nodes in dogs can be considered as a consequence of the activation of recovery processes. This corresponds to a more uniform and closer to the control distribution of the size groups of neurons in the experimental group by the end of the observation period.

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