THE POSSIBILITY OF DIVISION OF THE CORTICAL NEURONS

T. V. Ivannikova

Laboratory of Electrophysiology of Nervous Activity (Head, Professor A. B. Kogan),
Rostov-on-Don State University
(Presented by Active Member AMN SSSR N. A. Kraevskii)
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In previous studies of the compensation of analyzer functions in young animals [2, 5] we touched on the problem of the possibility of regeneration of cortical neurons after extirpation of the occipital cortex. The results indicated that a complete functional restoration took place, with anatomical regeneration of the cortex. It remained uncertain whether in these cases there was histological regeneration of the neurons.

Reports have recently been published that division of neurons may occur in the autonomic and spinal ganglia [6, 11], and that regeneration of neurons is possible in the central nervous system [7-10, 12]. It has been demonstrated that the process of regeneration in the central nervous system is stimulated by administration of substances of the pyromen type, which inhibit scar formation [12].

However, the regeneration of neurons is not a generally accepted fact. Some writers, who have failed to observe the division of nerve cells, are doubtful about the ability of neurons to give a regenerative reaction [1]. During recent years, the problem of the possibility of division of neurons has been discussed in the pages of the journal "Arkhiv Anatomii, Gistologii i Émbriologii" [1, 3, 4].

We set out to discover whether, in our previous experiments in which functional restoration took place, this was accompanied by histological regeneration of the cortical neurons, or whether anatomical regeneration resulted simply from the movement of neighboring cortical elements into the region of the defect.

EXPERIMENTAL METHOD

Experiments were conducted on 33 young animals, in 27 of which (20 kittens and 7 puppies) the occipital lobes were extirpated bilaterally, while 6 animals (3 kittens and 3 puppies) were used as controls. The animals were sacrificed at various intervals after operation (from 1 day to 2 years) and the brain was fixed in 10% neutral isotonic formalin solution. The material was embedded in celloidin. Frontal and sagittal sections of the hemispheres were cut and stained with hematoxylin-eosin, picrofuchsin by Van Gieson's method, thionine by Nissl's method, iron hematoxylin by Heidenhain's or Mellendorf's method, for myelinated fibers by Spielmeyer's or Zolotova's method, and by silver impregnation by Kampos's method.

Conditioned optic reflexes, differentiation, and transformation were produced experimentally by a technique described earlier [2, 5]. As in the previous experiments, the forms of optic analysis which we tested in the operated animals after an interval of time were in no case inferior to those in the controls.

The animals were divided as follows in accordance with the times of the morphological investigation: during the first 3 days after operation – 8 kittens and 2 puppies; after 5-7 days – 4 kittens and 1 puppy; after 10 days – 2 kittens; after 2 weeks – 1 kitten and 1 puppy; after 3 weeks – 2 kittens; after 1 1/2 months – 2 kittens; after 3-6 months – 1 kitten and 1 puppy; after 2 years – 2 puppies.

EXPERIMENTAL RESULTS

When the brain was investigated during the first 3 days after the operation, a hematoma was observed in the region of the defect, and at the borders of the defect the normal layering of the cortex was disturbed; further away from the edge of the defect the layers were preserved, but were inclined (radially) to the edge. In the region adjacent to the defect, a considerable proliferative reaction of the neuroglial elements was observed, together with neuronophagy, the formation of granular corpuscles, and the accumulation of disintegration products of myelin; sometimes the pericellular and perivascular spaces were enlarged on account of cerebral edema. In the nerve cells in the zone of the

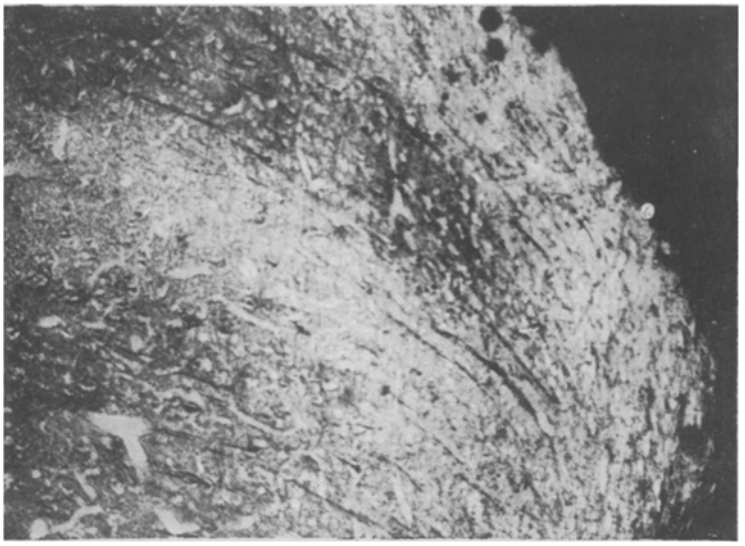

Fig. 1. Displacement of layers of cortex near the defect 2 days after operation. Photomicrograph. Silver impregnation. Objective 6.3, eyepiece 6.6.

defect itself, we observed chromatolysis, vacuole-formation in both cytoplasm and nucleus, and enlargement of the nucleolus; in the same region numerous pycnotic and hypertrophied cells were seen. Near the defect the myelin sheaths were broken up to form large and small droplets of myelin. The nerve fibers frequently pursued an irregular course and showed varices. Besides the phenomena demonstrating degeneration of the nerve fibers, we observed regenerative changes, such as the appearance of bulbs of growth on the ends of the fibers facing the defect. By the third day after the operation, pictures were observed indicating amitosis of the neuroglial and nerve cells (constriction rings in the nuclei, cells with double nuclei superimposed on one another, and cells placed close together, as yet incompletely separated).

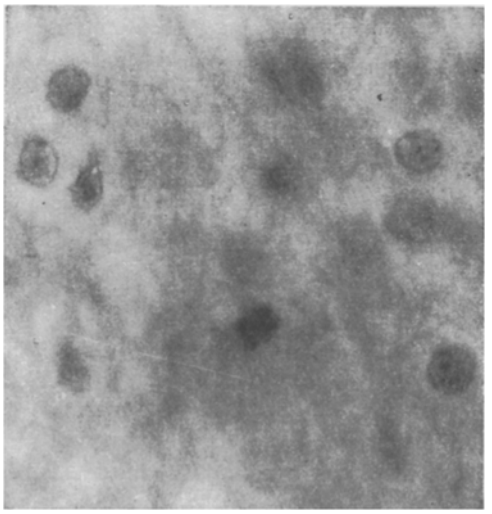

Fig. 2. Mitosis in a neuroglial cell 10 days after operation. Photomicrograph. Stained with iron-hematoxylin. Objective 100, eyepiece 6.6.

The general pattern of arrangement of the cortical neurons in the animals sacrificed 5-7 days after the operation was little different from the previous findings. The regenerative reaction, however, was more marked on account of the developing bulbs of growth and the amitosis of the neurons and, in particular, the neuroglial cells. The amount of scar formation was insignificant.

After 10 days the general pattern of arrangement of the layers and the changes in the zones adjacent to the defect were similar to those after 7 days. The regenerative changes, however, were more intensive – the numerous bulbs of growth, demonstrating regeneration of nerve fibers en masse, the large number of amitoses and the presence of karyokineses in the glial (Fig. 1) and nerve cells (Fig. 2), which were much larger than the surrounding neuroglial cells and sometimes preserved the origin of the processes emerging from them. Although mitoses were frequently found in the nerve cells (prophase, metaphase), we never observed pictures indicating their completion (anaphase, telophase). Figures of karyokinesis were demonstrated by staining with iron-hematoxylin and thionine. The formation of a neuroglial scar began at this time.

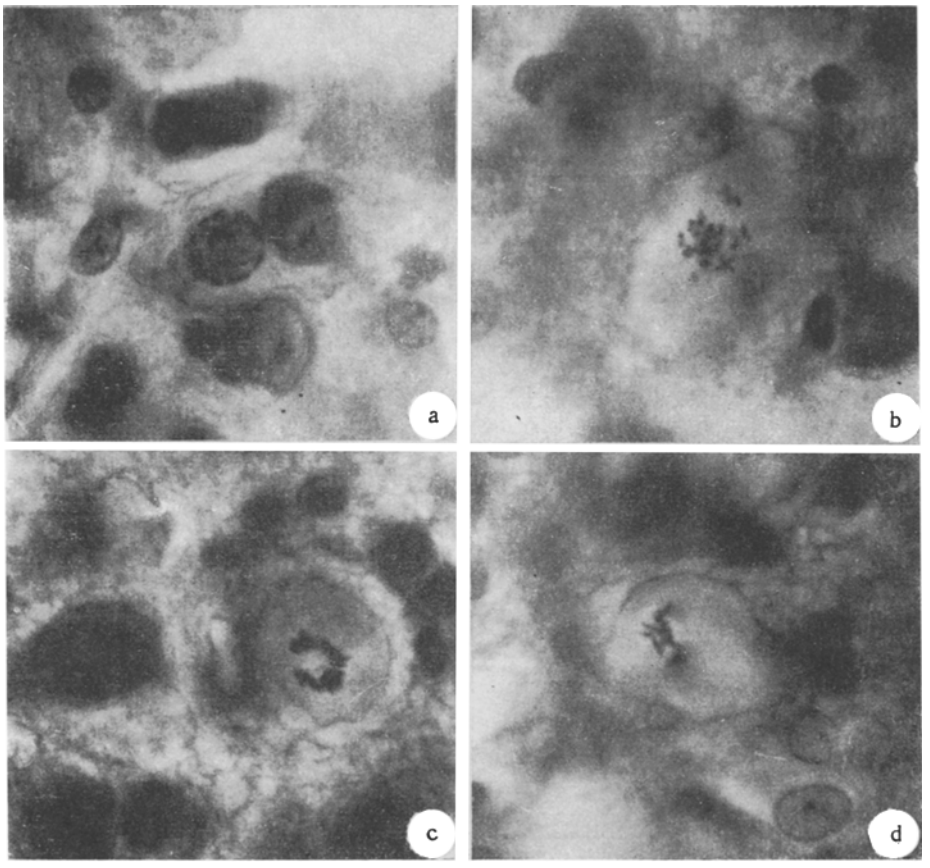

Fig. 3. Mitoses in nerve cells 10 days after operation. a) Beginning of karyokinesis; b) intermediate stage between prophase and metaphase; c) stage of the mother star; d) stage of the equatorial plate. Photomicrograph. Stained with iron-hematoxylin. Objective 100, eye-piece 6.6.

After two weeks, connective tissue elements appeared in the neuroglial scar, although as yet only in small numbers. The general pattern was similar to that after 10 days, although the regenerative reaction was less intensive: amitoses were seen fairly frequently in the glial cells and, from time to time, in the neurons, but there were far fewer cells undergoing mitotic division and fewer bulbs of growth than in the picture observed 10 days after operation.

After three weeks the site of the former defect was largely filled up, partly by nerve tissue displaced there, with sparsely arranged neurons, and partly by a scar formed of neuroglia and connective tissue. The neuroglial scar was permeated with disintegration products of myelin, and many granular corpuscles were observed in it. The scar contained both neuroglial tissue with blood vessels, and nerve elements. At the edge of the scar the normal arrangement of the cortical layers was disturbed, and pycnotic and hypertrophied cells were observed. In the neurons of the zone adjacent to the scar, the chromatophilic substance was in a finely dispersed state and no mitoses were present, while amitoses were frequently seen.

After 1 1/2 months, the defect was filled mainly by neuroglia. Connective tissue with densely packed fibers infiltrated into the neuroglial scar. The neurons were much sparser in the zone adjoining the scar, and the arrangement of their layers was disturbed. Around the scar, neuroglial elements were predominant in the cortex. Here, too, there were large amounts of myelin disintegration products, especially in the deep layers of the defect. Mitoses were absent. Amitoses were seen, more frequently in the glial, and less frequently in the nerve cells. In the neurons dividing by amitosis, hypertrophy of the nuclei was sometimes seen.

From 3 to 6 months after the operation the site of the defect was filled at its center mainly by neuroglial tissue, and at its periphery by nerve cells, arranged haphazardly and showing considerable thinning of the layers as if they were stretched to the scar. Whereas in the cortex of the control animals of this age, if examined with a 7x eye-piece

and a 90X objective, from 18 to 20 nerve cells were found in a field of vision (varying the depth of the fine-adjustment screw), in the same field of vision of the cortex of the operated animals only 4 or 5 nerve cells were found in the region immediately adjoining the scar (the width of this zone in frontal sections was 1500-1700 μ), 9 or 10 neurons were present in the next zone (width about 3000 μ), 13-15 neurons in a zone about 5000 μ wide and, finally, at a distance of 9500-10,000 μ from the scar, there was a normal arrangement of the cell layers and no significant decrease in their density. At the edge of the scar pycnotic and hypertrophied neurons with finely dispersed chromatophilic substance were observed. If the ventricle was involved in the defect during the operation, a cyst was formed, the walls of which contained nerve cells. The cyst was almost closed above by a strip of cortex, and its internal surface was lined by neuroglial tissue, with the formation of granular corpuscles. In the region of the neuroglial scar large amounts of myelin disintegration products were observed. No cell division was seen.

Two years after operation, the picture resembled very closely that observed after 6 months.

Hence, in the early stages after operation an increase in the intensity of the regenerative reaction was observed to a maximum on the 10th day, followed by a decrease on account of the formation of a scar, initially of neuroglia and later of connective tissue. It may be concluded from this investigation that the factor of decisive importance in the restoration of function was the cortex (and, possible, the subcortex), left intact after the operation, and making good the anatomical defect. Nevertheless, this by no means excludes the possibility of regeneration in the central nervous system. On the other hand, the presence of mitoses and amitoses in the nerve cells during the early postoperative period, and their absence in the period of scar formation, demonstrate that the regeneration of neurons is possible in principle, but that special conditions are necessary for it to take place; these conditions are primarily those blocking the formation of scar tissue.

SUMMARY

In young animals (kittens and puppies) there was revealed a practically complete restoration of the visual analysor function and an anatomical restoration of the occipital lobes of the large hemispheres after their excision. Histological investigations demonstrated the presence of mitoses and amitoses in the glial and nerve cells of the cortex at the early postoperative periods and the extinction of a regenerative reaction coinciding in time with the formation of the glial and connective tissue scar. The author came to the conclusion that the regeneration of the cortical neurones is possible theoretically but requires special conditions for its manifestation.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.