Structural and Functional Changes in the Brain of Rats with Different Behavioral Types at Later Stages of Circulatory Hypoxia

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Structural changes in the brain were studied at later (one and three months) stages of cerebral hypoxia caused by occlusion of the common carotid arteries, which were indicative of the development of recovery and destructive processes. Brain ultrastructure was found to be better preserved in rats with an active type of behavior one month after the onset of ischemia than in rats of the other groups; motor activity was restored to the baseline level, thus demonstrating a higher rate of recovery processes. Significant ultrastructural damage to the brain at the same stage of ischemia was observed in rats with a passive type of behavior (especially in rats of the middle group), while motor activity was increased.

Key Words: *circulatory cerebral hypoxia; behavioral type; brain; neurons; neuroglia; synapses; blood vessels*

It was previously established by us [6,7] that the resistance of rats to circulatory cerebral hypoxia, determined by the "48-hr survival rate" parameter, correlates with behavioral type and with the manifestation of compensatory structural and metabolic changes (hypertrophy and mitochondrial division, elevated activity of succinate dehydrogenase) developing in the brain at an early (one hour) stage of ischemia. Structural and metabolic adaptation was higher in rats with the active type of behavior (high resistance to brain ischemia) than in rats with the passive type of behavior (average resistance) and rats of the middle group (low resistance). In other words, we detected individual and typological differences in emergency adaptative responses of the brain tissue to hypoxic stress. In addition, we observed a link between resistance to circulatory hypoxia at the acute stage of brain ischemia and preservation, i.e., mitochondria resistance to the injurious effect which accompanies oxygen deficiency.

The purpose of the present study was to find out whether individual and typological differences developed in the brain structures in the process of long-term adaptation to circulatory cerebral hypoxia.

MATERIALS AND METHODS

Experiments were carried out on 72 male albino rats. The animals were divided into groups of 24 rats each with active, passive and average types of behavior, 15 of which were controls. We studied structural changes in the brain of 42 rats one month after the onset of brain ischemia and in 15 rats after three months. Brain ischemia was induced by bilateral ligation of the common carotid arteries. The behavioral type of animals was determined by tests: "open field" and "enforced swimming"

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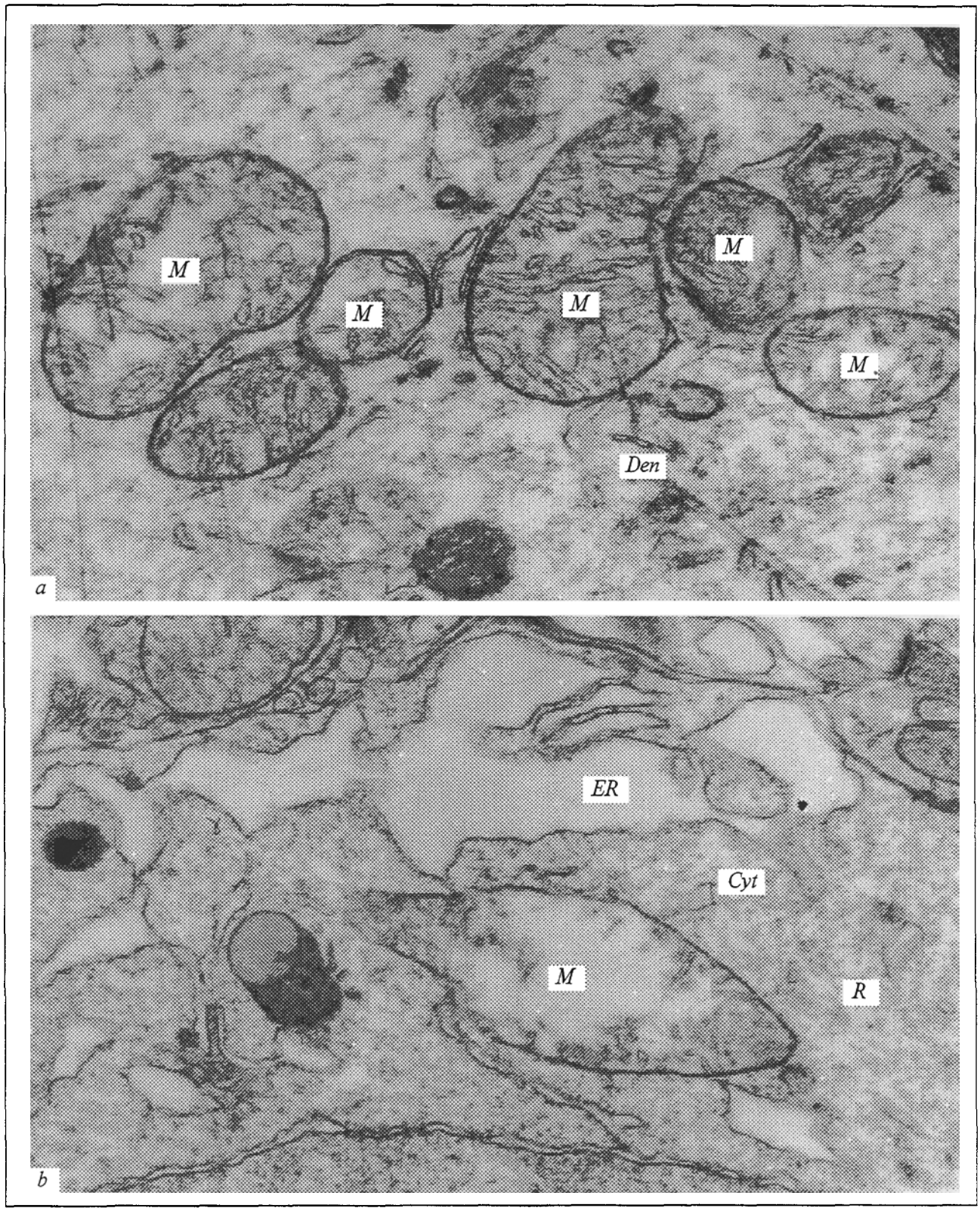

Fig. 1. SAC ultrastructure one month after the ligation of the carotid arteries. *a*) mitochondrial clusters (*M*) in a dendrite (*Den*) of a rat with the active type of behavior. Light areas can be seen inside the mitochondria. ×40,000. *b*) dilation of channels of endoplasmatic reticulum (*ER*) in the cytoplasm (*Cyt*) of a neuron. Destruction of the inner mitochondrial structure (*M*) and a decrease in ribosome number (*R*). ×40,000.

according to the method described earlier [6]. Methods of light microscopy (Nissl, Kajal, Miagawa-Aleksandrovskaya) and electron microscopy were used in the study. Different areas of the cortex and subcortex structures were studied by means of light microscopy; the sensorimotor area of the cortex (SAC) was studied by electron microscopy. The rats were promptly killed by decapitation under light Nembutal anesthesia. The brain was removed and fixed in a 5% solution of glutaraldehyde and then in a 2% solution of OsO_4, dehydrated in alcohols, and embedded in araldite. The sections were prepared on an LKB-III ultratome, contrasted with lead citrate after Reynolds [10] and studied under a JEM-100B electron microscope. For quantitative determination of synapse density in the 2nd-3rd layers of the SAC the brain pieces were fixed in 5% glutaraldehyde and dehydrated in alcohols, and then treated with a 1% solution of phosphotungstic acid in 100° alcohol and embedded in araldite. Synapses were counted on the sections (no additional contrast applied) on the screen of the electron microscope at magnification 20,000×, which corresponded to an actual area of 13.5 μ^2. Statistical analysis of the data was performed according to Student's test.

RESULTS

We detected two major types of changes in the vessels at later (1-3 months) and earlier [6] stages of brain ischemia: 1) greatly dilated vessels resembling paresis [4] and not always filled with blood cells; 2) emptied and often twisted vessels with constricted lumens. Microhemorrhagic foci with microgliacyte aggregations were observed around some vessels. We detected structural changes in endothelial cells of the vessels, especially in capillaries, as has been noted by other authors [2,3]. The departure of astrocyte processes from the walls of emptied capillaries was typical of later stages of brain ischemia. These vascular changes demonstrated that insufficient blood supply of the brain persisted even three months after ligation of the common carotid arteries. A considerable number of brain structural elements were found to be recovered one month and especially three months after ligation of the carotid arteries compared with the acute (48 h) period of ischemia [6]. Intact neurons were more often found in the cerebral cortex; they contained a complete and (mainly) well-preserved set of organelles. Among ordinary type mitochondria many small (newly formed) mitochondria were observed; some of them were moderately hypertrophied, which testified to the recovery process of energy metabolism in the brain. Focal aggregations of mitochondria were seen both in the cytoplasm of neuron bodies and in processes (Fig. 1, *a*). The granular reticulum was better preserved, and in some cells ribosomes and polysomes were increased in number, which indicated resumption of the protein synthesis required for the repair processes. Multiple micronuclei were formed in the nuclei of some cells. Our attention was drawn to the appearance of direct contacts between capillaries and neuron bodies without the formation of an intermediate astrocyte chain (Fig. 2, *a*), which is of great compensatory importance under conditions of insufficient blood supply to the brain. On the whole, the response of the astrocyte glia in the brain was not well marked, whereas the number of satellite glia cells was considerably increased (Fig. 2, *b*). Similar results were obtained by other scientists [11]. Ultrastructures were better preserved in the rats with the active type of behavior than in the rats of the other two groups one month after the onset of ischemia. This testified to a higher rate of repair processes correlating with the recovery of motor activity (number of squares crossed) to the baseline level (77.54 ± 27.36 after ischemia, 88.41 ± 7.34 prior to ischemia, $p>0.1$). On the other hand, marked destructive changes in mitochondria, as well as a focal and total decrease in ribosome number, were observed in the rats with the passive type of behavior, especially rats of the middle group, at later stages of brain ischemia (Fig. 1, *b*). Dilated channels of reticulum were detected in the cells, dendrites exhibited fewer tubules, and the cytoplasmic matrix in neuron bodies and processes was lightened. These changes point to residual atrophic processes caused by oxygen deficiency. Foci of thinned neurons in the cortex and in some nuclei of the thalamus revealed by electron microscopy were also indicative of atrophic changes. Atrophic processes in axodendritic (excitatory) SAC synapses were also detected by electron microscopy and were especially pronounced in the rats with the active type of behavior three months after brain ischemia. They were manifested in a decreased number of axon terminals and synaptic vesicles and in a shortening and thinning of synaptic membranes. Spines were diminished in size, many of them were deprived of active zones, and the spiny apparatus was atrophied. The number of tubules in dendrites was fewer than normal, or tubules were absent (Fig. 3, *a*). Dendrites in the vicinity of atrophied spines were deprived of mitochondria, and the number of ribosomes in the neuron cytoplasm in the vicinity of atrophied axosomatic synapses was reduced. Quan-

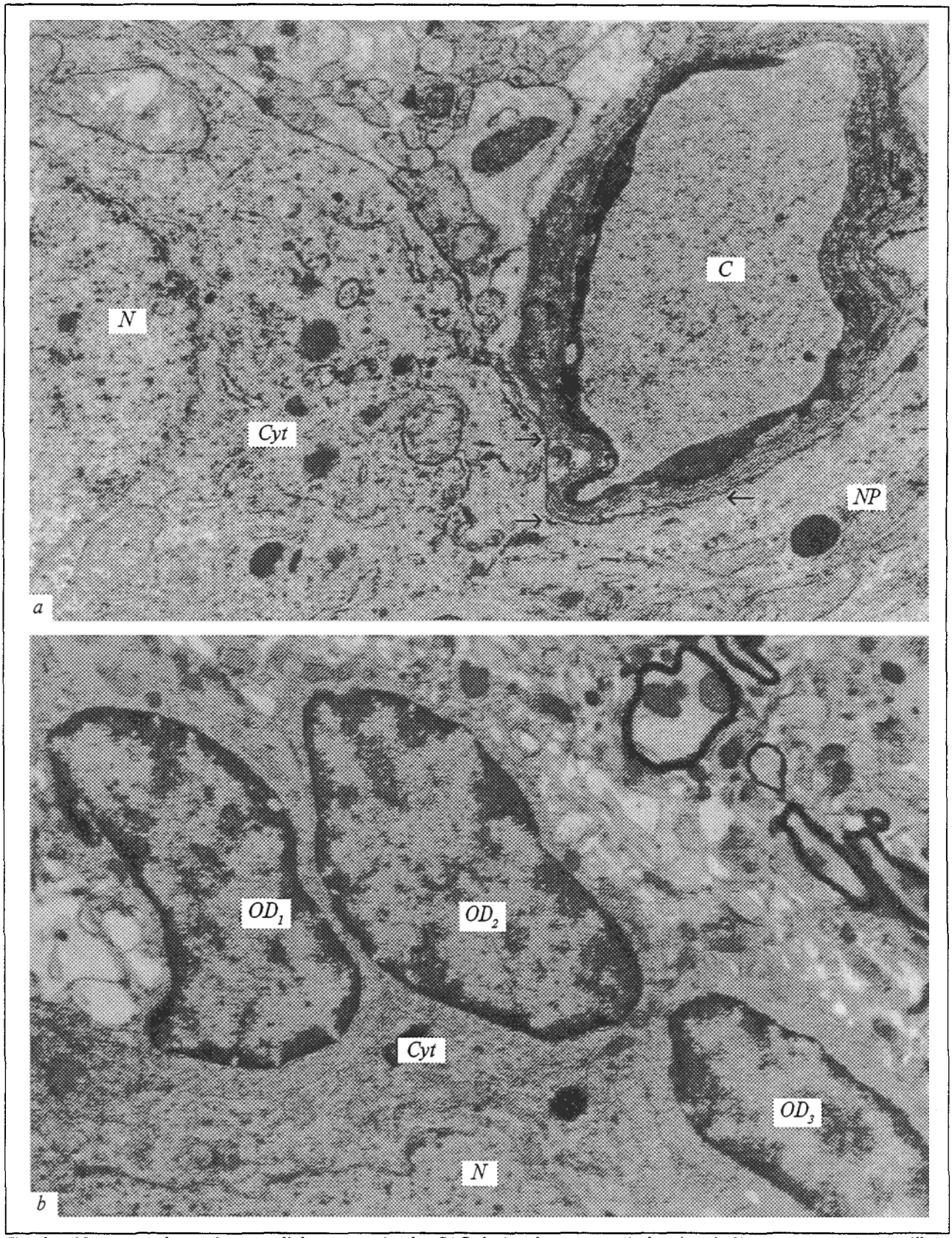

Fig. 2. Neurovascular and neuroglial contacts in the SAC during long-term ischemia. *a*) direct contact of a capillary (*C*) with a body (*Cyt*) and neuron process (*NP*) in a rat with the active type of behavior one month after ligation of the carotid arteries. *N*: nucleus. ×25,000. *b*) contact of three oligodendrogliacytes ($OD_1 - OD_3$) with a neuron body (*Cyt*) in a rat of the middle group three months after ligation of the carotid arteries. *N*: nucleus. ×15,000.

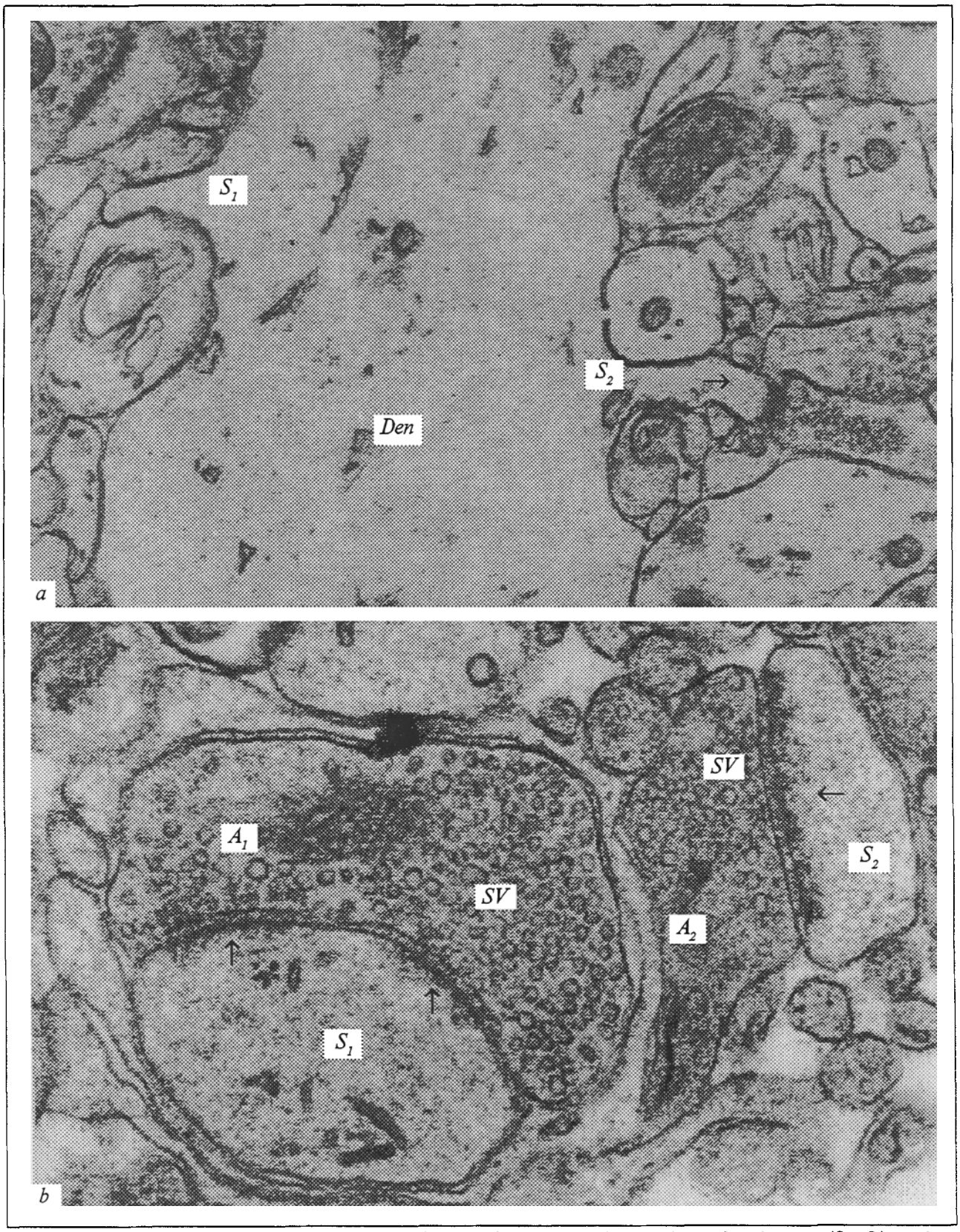

Fig. 3. Ultrastructure of axodendritic synapses in the SAC during long-term ischemia. *a*) atrophy of spines (S_1-S_2) on dendrite (*Den*) of a rat with the active type of behavior three months after ligation of the carotid arteries. The spine on the right has preserved synaptic contact (arrow). Tubules are not found in the dendrite, ×54,000. *b*) two axodendritic synapses on spines (S_1-S_2) in a rat with the passive type of behavior one month after ligation of the carotid arteries. A large number of synaptic vesicles (*SV*) are seen in axon terminals (A_1-A_2). Synaptic membranes are well expressed (arrows), ×64,000.

titative evaluation of the density of axodendritic synapses over an area of 13.5 μ^2 in the 2nd-3rd layers of SAC revealed a tendency towards a decrease in the number of synapses three months after brain ischemia, which was more pronounced in the rats with the active type of behavior (1.62±0.07, as opposed to 2.32±0.14 in the control) than in the rats with passive behavior (1.71±0.06, *vs.* 2.28±0.11 in the control) and the rats of the middle group (1.68±0.08, *vs.* 2.19±0.12 in the control). During this stage of brain ischemia we observed an insignificant decrease in motor activity (48.40±25.07 after ischemia, 88.41±7.34 prior to ischemia, $p=0.14$) and a significant decrease in exploratory activity (6.60±2.29 after ischemia, 38.90±11.23 prior to ischemia, $p<0.01$) in rats with the active type of behavior. This was evidently associated with the decrease in the total functional activity of the axodendritic (excitatory) SAC synapses. Along with this, besides atrophically changed synapses in the cortex we detected synapses with heightened functional activity: these exhibited a large number of synaptic vesicles and a preserved structure of mitochondria (often hypertrophied) with a well-marked and elongated synaptic membranes and a well-developed spiny apparatus. Functionally "hyperactive" synapses were observed in the brain of rats from all three groups, but most often in the animals with the passive type of behavior and in the rats of the middle group (Fig. 3, *b*). In the same period of brain ischemia we observed an increased motor activity (hyperactivity) in the rats with the passive type of behavior (63.62±16.14 after ischemia, 45.10±8.53 prior to ischemia), and especially in the rats of the middle group (97.43±8.81 after ischemia, 62.25±5.10 prior to ischemia, $p<0.05$), which was evidently due to a relative prevalence of functional activity of axodendritic (excitatory) synapses over axosomatic (inhibitory) synapses.

Thus, two different processes were observed at later stages of circulatory hypoxia induced by ligation of the common carotid arteries: compensatory-recovery and destructive-atrophic. We have established a link between behavioral type and the recovery rate of structural and functional brain activity in the postischemic period. Rats with the active type of behavior showed an increased rate of recovery processes in the brain at later stages of ischemia than did the rats of the other two groups. It was earlier established that rats with the active type of behavior (in contrast to the other two groups) exhibit a more pronounced structural and metabolic adaptation of the brain to oxygen deficiency at an early stage of ischemia [6]. Therefore, the rats with the active type of behavior were characterized by a more effective short-term, as well as long-term adaptation to circulatory hypoxia. Atrophic processes in synapses and inhibition of exploratory activity were observed in rats with the active type of behavior three months after the onset of ischemia. This indicated a decrease in the structural and functional activity of the brain, which is considered to be one of the manifestations of long-term adaptation to oxygen deficiency. Diminished functional activity of the brain was also observed by other authors in cases of long-term adaptation to oxygen deficiency caused by anemia of the cortex [1,8]. The formation of direct contacts between capillaries and neurons without an intermediate astrocyte chain and the increase in the number of satellite glia cells making contact with neuron bodies and processes and promoting recovery of nucleoprotein synthesis in cells [9] may be classified as structural changes providing for long-term adaptation [5] to circulatory hypoxia. The appearance of a large number of mitochondria of a new generation, which have undergone "a course of adaptation to hypoxia" and as a result exhibit increased resistance to oxygen deficiency, may also be referred to such structural changes.

Our findings demonstrate a link between the type of animal behavior and the structural and functional changes in the brain observed at later stages of circulatory hypoxia.

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