Recombinations of Neocortical Ultrastructure in the Hibernants During Adaptation to Hypothermia

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The ultrastructure of the sensorimotor cortex of ground squirrel is studied under normal conditions and during artificial hypothermia. It is found that during adaptation to hypothermia the receptor-mediated endocytosis is activated in neurons. Specific synaptoarchitectonic recombinations are revealed: active axo-axon synapses are formed in the synaptic regulatory complexes.

Key Words: *ground squirrel; hypothermia; ultrastructure; neuron; synapse*

The mechanisms responsible for the unique adaptation processes occurring in the brain of hibernating mammals at low temperatures have been extensively investigated [2,7,10,11]. However, as we are aware ultrastructural recombinations [5] in the brain of these animals were not studied. It was demonstrated that stress of any origin induces nonspecific ultrastructural rearrangements in rat sensorimotor neurons and synapses [3,4,6]. Specific mechanisms of adaptation to hypothermia operating in the hibernants may have another morphofunctional basis [2]. In order to reveal specific features of neuronal and synaptic reactions to hypothermia, we examined the ultrastructure of the sensorimotor cortex (SMC) of ground squirrels during experimental adaptation to artificial hypothermia.

MATERIALS AND METHODS

Ten ground squirrels (*Citellus pygmaeus Pallas*) weighing 200-250 g captured in the lowlands of Dagestan were used. The study was carried out in June, i.e., in the middle of the active period of ground squirrels. Five animals were placed in chambers with regulated water-cooling system and sensors for the body temperature monitoring. They were cooled for 20-30 min to 25°C. After the desired level of hypothermia had been attained, the animals were left in the chambers for 2 h and then decapitated. Five animals (control) were kept for the same time in similar chambers under normal conditions (26°C, body temperature 36-38°C).

Material for electron microscopy was collected in the cold, fixed in 2.5% glutaraldehyde and 1% osmium tetroxide, and after standard processing embedded in Epon 812 [8]. Ultrathin sections were cut in a UMTP-6 ultramicrotome and examined under a PEM-100 electron microscope.

RESULTS

Electron microscopy revealed no changes in the SMC of the control ground squirrels. Neuronal cytoplasm contained numerous free and bound ribosomes, mitochondria, and a well-developed granular endoplasmic reticulum (Fig. 1, *a*). The lamellar complex (LC) was represented by a system of cisternae and vesicles varying in size with occasional coated vesicles in a close vicinity (Fig. 1, *b*). In the majority of neurons, the vesicular portion of the LC was

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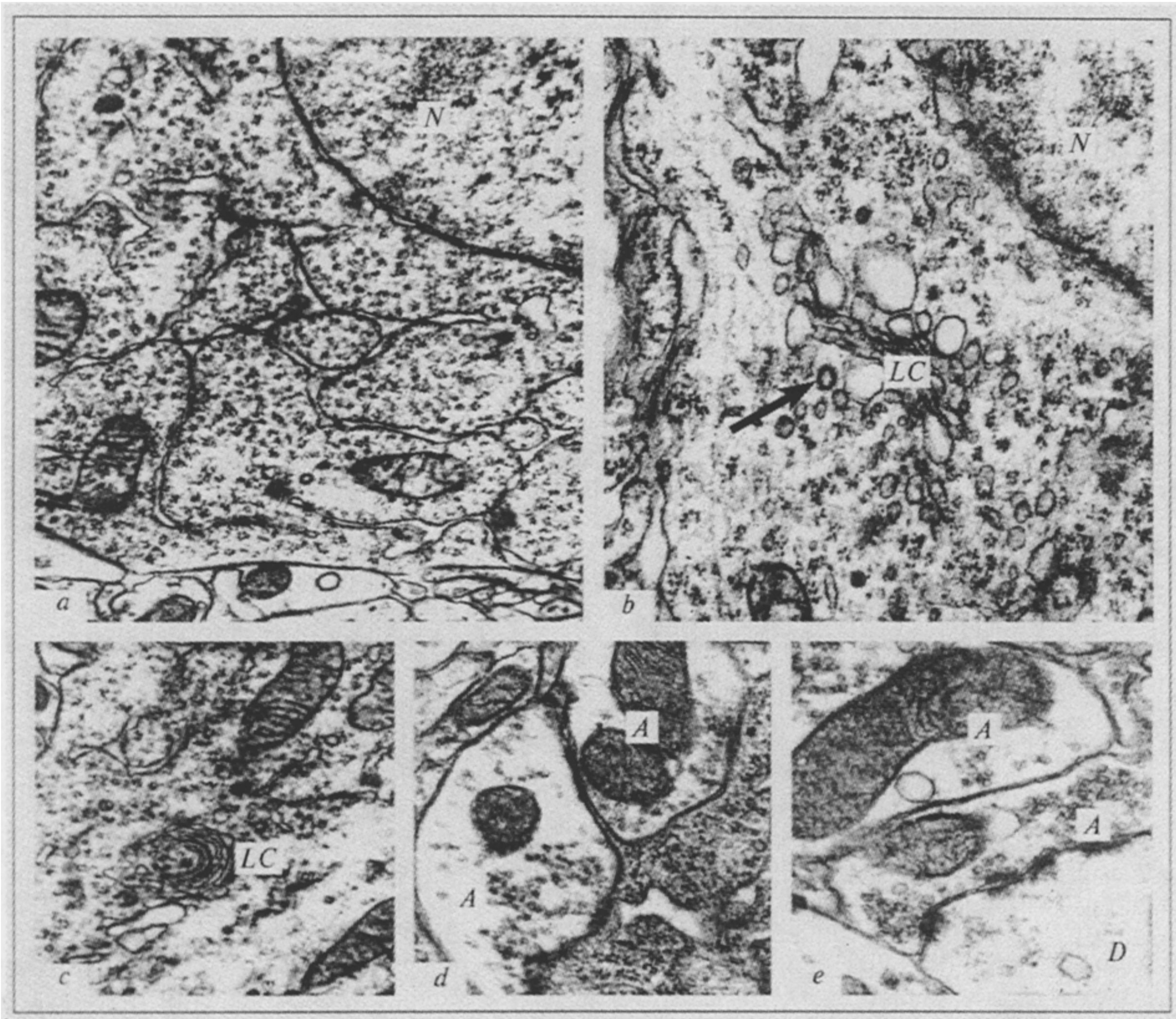

Fig. 1. Ultrastructure of the sensorimotor cortex of a normothermal ground squirrel. *a*) well-developed granular endoplasmic reticulum and numerous ribosomes in neuronal cytoplasm, ×19,000; *b*) lamellar complex with coated vesicle (arrow), ×30,000; *c*) compact horseshoe-shaped lamellar complex. ×18,500; *d*) active axosomal synapses, ×26,500; *e*) asymmetrical axospinous synapse with an extra axon, ×34,500. Here and in Figs. 2 and 3: *N*) nucleus; *LC*) lamellar complex; *A*) axon; *D*) dendrite.

underdeveloped; the LC was compact and had the shape of a horseshoe (Fig. 1, *c*). Symmetrical axosomal synapses were active and contained numerous synaptic vesicles near the active zone (Fig. 1, *d*). Asymmetrical axospinous synapses with several active zones, pronounced postsynaptic thickening, and groups of synaptic vesicles were located near the active zone (Fig. 1, *e*). Generally, an extra axon terminal contacted with presynaptic axon of the asymmetric synapse (Fig. 1, *e*). Under normal conditions, the axo-axonal contacts had an atypical ultrastructure: active zones and the adjacent groups of synaptic vesicles were absent.

After a 2-h hypothermia, the LC neurons were hypertrophic, and dilated cisternae of the complex contained amorphous material of medium electron density. Primary and secondary lysosomes, multivesicular corpuscles, and coated vesicles appeared in close vicinity of the LC (Fig. 2, *a*). Several coated vesicles fusing with multivesicular corpuscles were seen (Fig. 2, *b*). The coating was still discernible on the membrane of a multivesicular corpuscle at sites where coated vesicles adhered. This ultrastructure implies a pronounced activation of the receptor-mediated endocytosis: binding of the ligand molecules to the neuron plasma membrane receptors and internalization of the ligand—receptor complexes as coated vesicles. Coated vesicles fuse with multivesicular corpuscles or with endosomes and are transported to the LC, where the ligand—receptor complexes are degraded by lysosomal enzymes [1]. Recombinations of the organelles participating in receptor-mediated endocytosis suggest that the receptor mechanisms play a certain role in brain adaptation to hypothermia.

An increase in the expenditure of energy by neurons during adaptation to hypothermia causes swelling of the mitochondria and emergence of the dumb-bell-like structures. Organelles were accumulated in the neuronal processes, with elongated mitochondria predominating in the dendrites (Fig. 2, *c*) and round mitochondria with electron-dense matrix and concentric cristae in the axons (Fig. 2, *d*).

It is noteworthy that recombinant transformations of the axo-axonal contacts are formed in the neocortical neuropile. These contacts looked inactive in normothermia, while in hypothermia the number of mitochondria in the pre- and postsynaptic area increased (Fig. 3, *a*). Clusters formed of 2-3 axons contacting with each other were seen. In the contact zone, the ultrastructure of membranes was similar to that of the active zone of a symmetric synapse (Fig. 3, *b*). Groups of synaptic vesicles were seen in the presynaptic axon near the active zone as well as vesicles fusing with the presynaptic membrane (Fig. 3, *c*). Generally, one axon participating in the axo-axonal contact acted as a presynapse in the asymmetric axospinous synapse (Fig. 3, *d*).

Thus, new data on ultrastructural recombinations occurring in the SMC of the ground squirrel during adaptation to hypothermia were obtained. Activation of neuronal organelles participating in the receptor-mediated endocytosis and formation of numerous axo-axonal synapses in the neuropile have been documented. Our results show that synaptic regulation of the stimulation and inhibition processes, along with the neuroendocrine processes, plays an important role in the adaptation of the brain of heterothermal animals to hypothermia.

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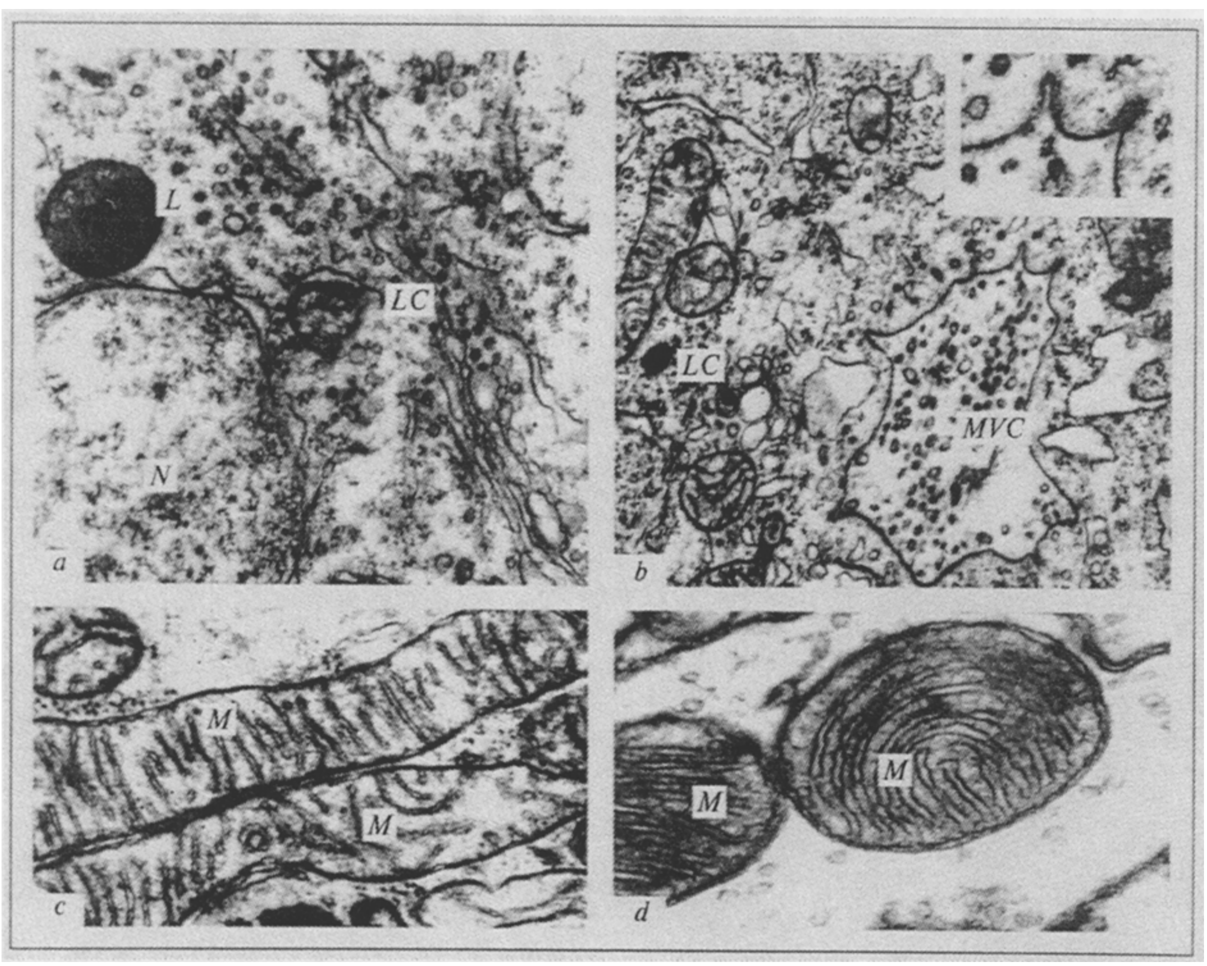

Fig. 2. Ultrastructure of the sensorimotor cortex of a hypothermal ground squirrel. *a*) lamellar complex containing homogeneous substance and numerous coated vesicles, ×27,500; *b*) large multivesicular corpuscle fusing with coated vesicles, ×22,500. The insert is a fragment of intact coating, ×48,500; *c*) elongated mitochondria in the dendrite, ×41,500; *d*) round mitochondria with concentrically positioned cristae in the axon, ×73,500. *L*) lysosome; *MVC*) multivesicular corpuscle; *M*) mitochondria.

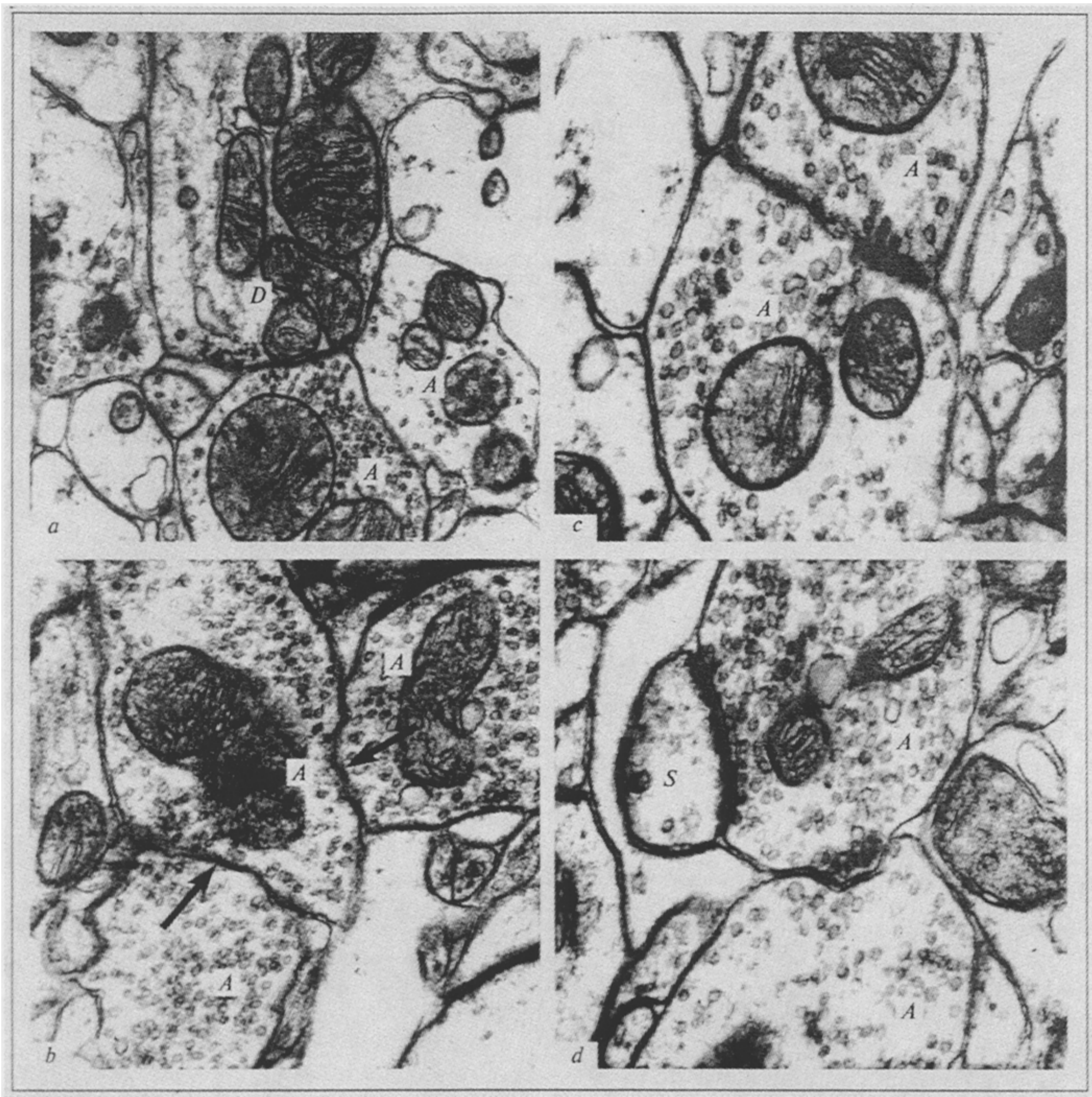

Fig. 3. Ultrastructure of the axo-axonal synapses in the sensorimotor cortex of a hypothermal ground squirrel. *a*) increased number of mitochondria in the pre- and postsynaptic parts of synapses, ×34,000; *b*) complex formed by three axons, arrows indicate the active zones, ×42,000; *c*) fusion of synaptic vesicles with the presynaptic membrane in an axo-axonal synapse, ×62,500; *d*) axo-axonal synapse whose postsynaptic axon acts as the presynapse in asymmetrical axospinous synaptic contact, ×62,000. *S*) spike.

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