Neurosecretory Cells of Brain Amygdaloid Complex

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Neurosecretory cells of the main zones of the amygdaloid complex were studied by Golgi method and light and electron microscopies. Light microscopy demonstrated that these cells have long axons and few branches and possess the properties of karyochrome and light cells.

Key Words: *brain amygdaloid complex; neurosecretory cells; neuronal and electron microscopic organization of the brain*

Neurons with morphological signs of secretory activity were found in the amygdaloid complex (AC) of rat brain. The goal of this study was to characterize these cells using light and electron microscopies and to demonstrate their identity to long-axon and low branching neurons (according to classification [6]).

MATERIALS AND METHODS

Experiments were performed on adult male and female Wistar rats weighing 300-320 g. The material was taken using a MBS-9 lens, treated as described elsewhere [4], and fixed in cold 2.5% glutaraldehyde in phosphate buffer. Slices were prepared in an LKB ultratome, contrasted with lead citrate [15], and examined under a JEM 2000 EX transmission electron microscope.

RESULTS

Neurosecretory cells are found in 2 main sexual dimorphism M zones (the dorsomedial and anterior cortical nuclei) described previously [2,5].

Light microscopy and Nissl staining with cresyl violet revealed 3 types of neurons: karyochromic, cytochromic, and light cells. Karyochromic neurons are generally large or middle-sized cells with polygonal bodies, large dark-stained nucleus occupying the most part of the cell body, and practically homogeneously dark-stained cytoplasm. Cytochromic neurons are larger than karyochrome neurons and have round bodies, basophilic cytoplasm, and light nucleus with large nucleolus. These cells are designated as cytochromic neurons because their staining is determined by specific features of the cytoplasm. Light neurons have round or oval middle-sized bodies, light perikarya containing small lumps and granules of basophilic substance, and light euchromatin-rich nuclei. This classification of neurons based on tinctorial properties of Nissl-stained cells is similar to that proposed previously [8], but differs by clearly defined meaning.

The dorsomedial nucleus are formed by karyochromic and light neurons, while the anterior cortical nucleus contains all types of neurons.

Studies of the neuronal organization of these AC zones showed that long-axon and low branching neurons with short dendrites are prevalent in the dorsomedial nucleus. Bodies of short-dendrite neurons lie on the vascular walls, and their processes surround the vessels (Fig. 1, *a*). Short-dendrite neuron network with included pyramidal and spindle-like neurons of the cortical type is found in the anterior cortical nucleus, especially in the medial part of the surface cellular zone. Comparison of Nissl-stained and Golgi-impregnated specimens showed that short-dendrite neurons are karyochromic cells, while cytochromic cells correspond to pyramidal and spindle-like neurons of the cortical type.

Electron microscopy substantiates the proposed classification of neurons and allows us to understand their specific functional features.

Electron microscopy revealed that all neurons can be divided into 2 main groups (dark and light neurons)

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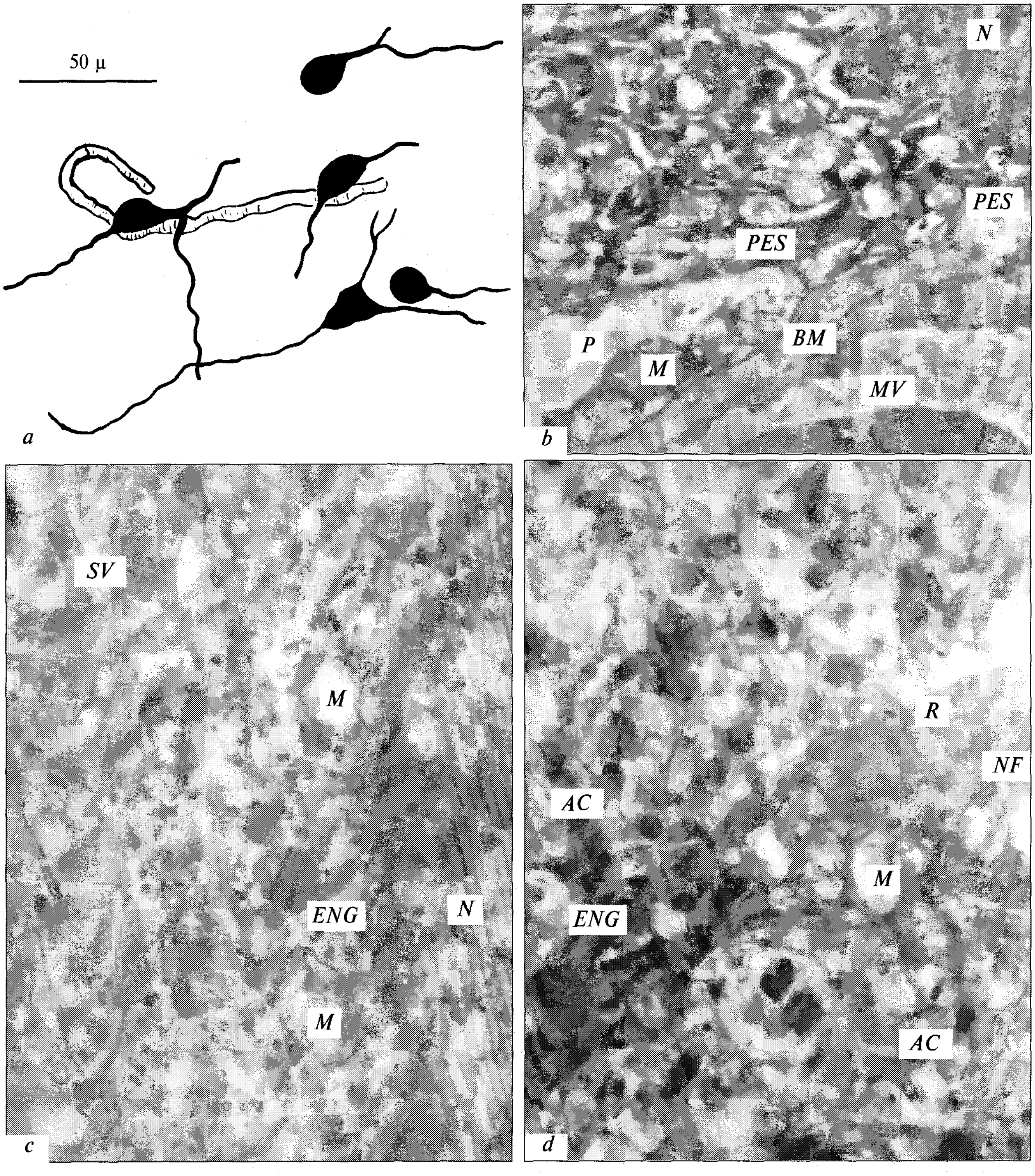

Fig. 1. Neurosecretory cells of the amygdaloid dorsomedial nucleus of rat brain: short-dendrite neurons on vascular surface (*a*, Golgi method), dark neuron in contact with capillary wall (*b*, ×18,000), light neuron with elementary neurosecretory granules (*c*, ×27,000), and nonmyelinated axon (*d*, ×18,000). Here and in Fig. 2: BM, basal membrane; AC, axon collaterals; M, mitochondria; MV, microvilli; SV, small vesicles; NF, neurofilaments; P, pericyte; PES, periendothelial space; R, ribosomes; ENG, elementary neurosecretory granules; and N, nucleus.

differing in their electron density. There are also transient forms of neurons with light nuclei and dark electron-dense cytoplasm.

Dark cells are prevalent in the dorsomedial nucleus (88% neurons) and have spherical, oval, or polygonal small and middle-sized bodies (10-40 μ), nuclei

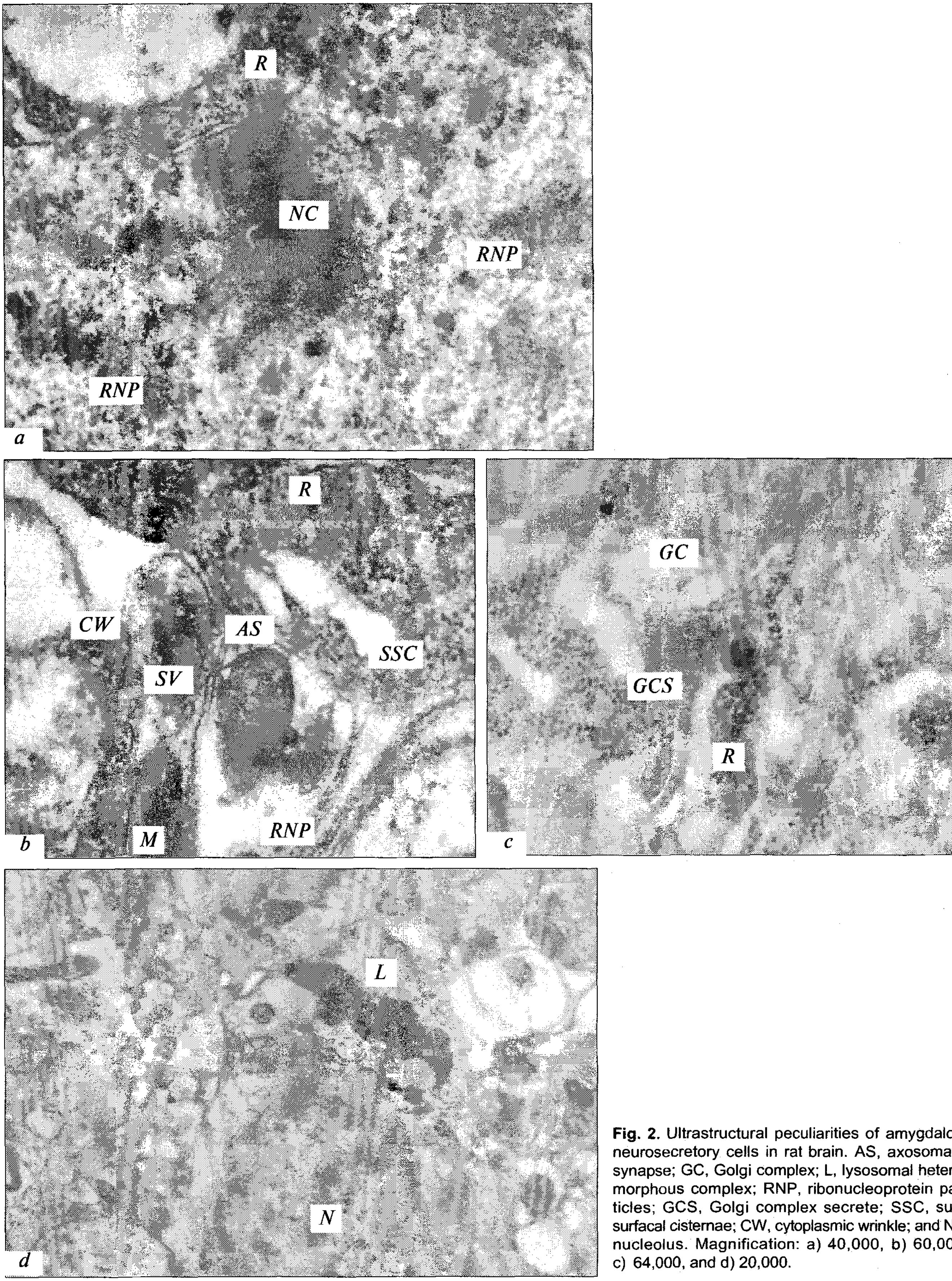

Fig. 2. Ultrastructural peculiarities of amygdaloid neurosecretory cells in rat brain. AS, axosomatic synapse; GC, Golgi complex; L, lysosomal heteromorphous complex; RNP, ribonucleoprotein particles; GCS, Golgi complex secrete; SSC, subsurfacal cisternae; CW, cytoplasmic wrinkle; and NL, nucleolus. Magnification: a) 40,000, b) 60,000, c) 64,000, and d) 20,000.

occupying the most part of the cell, and narrow ring-like cytoplasms. Neuron bodies are in contact with capillaries, wide periendothelial areas form tight junctions in the adjacent neuropil, and the endothelial surface contains a great number of microvilli (Fig. 1, *b*). These features are typical of vessels in neuroendocrine centers [1,3].

Dark neuron nuclei are characterized by irregular shape and have protrusions and invaginations with cytoplasmic processes. A dense plate (marginal chromatin) subjacent to the inner nuclear membrane is enriched with granular material that tends to spread toward the nucleoplasm. Abundant ribonucleoprotein (RNP) particles determine high electron density of the nucleoplasm. Large nucleolus has a gap-like area that indicates division, because some neurons contain 2 nucleoli. RNP particles form focal associations near the nucleolus (Fig. 2, *a*). Granules vary in size and density (from inter- to perichromatin granules).

Dark neuron cytoplasm also has specific features. Some neurons have very little cytoplasm and look like naked nuclei. The cytoplasm is rich in free ribosomes and polysomes (Fig. 2, *b* and *c*). Cytoplasmic channels are enlarged and form cisternae (lake-liked structures containing "islets" of cross-sectioned elements of the granular network). This probably reflects complex spatial interactions of cell membrane components. It can be also assumed that these cells have irregular surfaces with numerous wrinkles, because cytoplasmic processes containing many polysomes are often observed in the neuropil at a distance from the neuron body (Fig. 2, *b*).

Dark neuron cytoplasm contains many mitochondria, highly developed Golgi complex with signs of secretory activity (Fig. 2, *c*), and single lysosomes.

Dark neurons have nonmyelinated axons (Fig. 1, *d*) forming numerous collaterals and varicosities (up to 2 μ in diameter). Mitochondria (small transparent vesicles, 50 nm) and elementary neurosecretory granules (ENG, 220 nm) are seen in varicosities (Fig. 1, *d*).

Dark neurons receive many axosomatic synapses. The presynaptic component is filled with light small vesicles (30-50 nm). Less abundant 75-118-nm vesicles with dense centers are seen in the conical axon end (Fig. 2, *b*) contacting with another axon end with small transparent vesicles. Previous studies of the brain hypothalamic region showed that neurosecretion is typical of giant cell and small cell nuclei. Hence, light vesicles most probably contain acetylcholine, while vesicles with dense centers contain catecholamines. Light vesicles can also contain GABA and glutamic acid. The presence of these bioactive substances in AC neuropil was reported [11-14].

Thus, ultrastructural peculiarities of dark neurons suggest that they are functionally active cells in the secretory phase. Light microscopically, these cells are karyochromic neurons. Studies of neuronal organization showed that they are long-axon and low branching neurons of the short-dendrite type.

The population of light neurons revealed by electron microscopy is heterogeneous. The largest cells with light euchromatin-rich nuclei and clear cytoplasm containing Nissl bodies (rough cytoplasmic reticulum) should be assigned to classic neurons. Light microscopically, they correspond to cytochromic neurons. Golgi impregnation showed that these cells are long-axon and high branching neurons. Structural features of other light neurons suggest that they are neurosecretory cells in the phase of generation and secretion of bioactive substances.

Elementary neuresecretory granules with a diameter of 75-180 nm are found in the cytoplasm of these middle-sized light neurons (Fig. 1, c). They contain more developed cytoplasm than dark neurons and less free ribosomes. Light and spherical nuclei have insignificant deformities. The euchromatin dominates over the heterochromatin, the content of RNP particles is low, and the nucleolus is compact. Mitochondria are less abundant compared to dark neurons, and the Golgi complex is moderately developed and composed of densely packed cisternae and vacuoles with osmiophilic material. There are many lysosomes containing osmiophilically heterogeneous substances. Heteromorphous lysosomal complexes are formed in some light neurons indicating the important role of lysosomes in neurosecretory cells (Fig. 2, *d*). This description of dark and light neurons possessing secretory activity is identical to that of the brain hypothalamic region [3,9].

Thus, studies of cytological peculiarities of neurons in the main sexual dimorphism zones of AC showed that these regions contain classic neurons and neurosecretory cells. At the light microscopy level, classic nerve cells are cytochromic long-axon and high branching neurons. Neurosecretory cells are seen as karyochromic and light neurons (long-axon and low branching neurons). These data suggest that despite increasing complexity of structural organization and integrative properties of nerve cells during brain evolution of long-axon and low branching neurons are still present in the brain, because their neurosecretory properties.

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