ULTRASTRUCTURE OF LAYER 1 OF THE RAT CINGULATE CORTEX

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UDC 611.813.1-08.599.323.4

Our ideas on the functional role of the cingulate cortex have widened considerably as a result of neurophysiological research undertaken in the last three decades, which has demonstrated the organizing role of this part of the cortex in central regulation of visceral systems (Airapet'yants and Sotnichenko, 1967; Suvorov, 1967; Beller, 1977, 1983; etc.). The cingulate cortex is today considered to be responsible for the emotional overlay of sensations and the motor expression of emotions. Pathology of this region of the cortex has been linked with various nervous and mental disorders and memory disturbances (Ballantine, Cassidy, Flanagan, et al,, 1967; Bragina and Dobrokhotova, 1971). The cytoarchitectonics of the cingulate cortex has been studied in detail but there have been few investigations at the electron-microscopic level, and most of them have been devoted to the description of the layer-by-layer distribution of afferent terminals. In the most recent publication by Wenzel, Duwe, and Schulz (1980) on the ultrastructural organization of the anterior limbic cortex of the rat, just as in other investigations of this region, little attention was paid to layer 1 of the cortex. However, layer 1 is known to occupy a special place in the cerebral cortex. The abundance of ascending dendrites, belonging to neurons in deeper layers of the cortex, and the numerous contacts of these dendrites with terminals both of axons of cells in the same area and of afferent fibers, create favorable conditions for integrative activity, as Bekhterev (1898) pointed out originally. It is also considered that general inhibition of the cortex is effected through activation of apical dendrites of layer 1 (Beritov, 1969). Layer 1 of the cortex also is interesting for the researcher as part of the CSF—brain barrier, participating in the regulation of mechanisms of neurohumoral interactions (Otellin, Rybakov, and Baikovskaya, 1981).

The aim of this investigations was to study the ultrastructural organization of layer 1 of the rat cingulate cortex.

MATERIALS AND METHODS

Male Wistar rats weighing 200-220 g were used. Under ether anesthesia the rat was perfused through the ascending aorta with fixing solution as described by Brian (1980). The material was subsequently processed in the usual way and embedded in Epon. To reveal specialized intercellular junctions more clearly, in some cases a 1% solution of tannin was used during processing (Simionescu and Simionescu, 1976). The cingulate cortex is usually subdivided into anterior and posterior divisions (Fig. 1). Pieces of cortex from areas 24b (agranular cortex) and 29c (granular cortex), according to Brodmann's classification (Fig. 1) were investigated. This classification was chosen because it has been used in the majority of neurophysiological and morphological studies. Brodmann's areas 24b and 29c are components of areas La and Lm, according to Svetukhina's classification (1962) and they correlate with areas C_1 and Rg according to the classification suggested by Zilles, Zilles, and Schleichen (1980). Vogt, Rosene, and Peters (1981) suggested subdividing layer 1 of the rat cingulate cortex, which is very wide, into three sublayers: 1a, 1b, and 1c. The division was based on the fact that sublayer 1b contains numerous myelinated fibers. For sharpening the blocks and measuring the width of individual sublayers of layer 1, semithin sections were used as a guide. Ultrathin sections were mounted on grids with square mesh and examined in the JEM-100B electron microscope. The boundaries of the sublayers were defined during survey examination of layer 1, because under higher power the boundaries of the sublayers cannot be identified.

Department of Morphology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. Translated from Arkhiv Anatomii, Gistologii i Émbriologii, Vol. 86, No. 2, pp. 22-28, February, 1984. Original article submitted June 7, 1983.

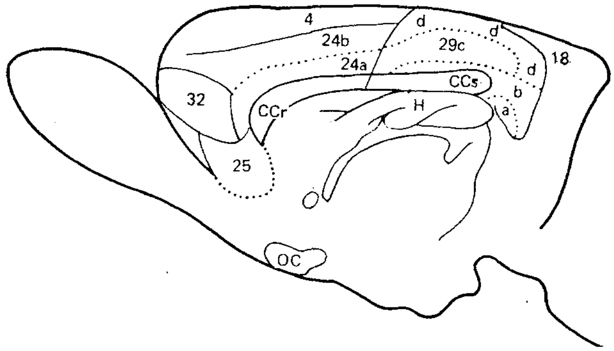

Fig. 1. Topography of cytoarchitectonic areas of rat cingulate cortex after Brodmann. Areas 24a, 24b, 25, and 32 form anterior part of cingulate cortex; areas 29a-d form its anterior part. CCr) Rostrum corporis callosi; CCs) splenium corpus callosi; H) hippocampus; OC) optic chiasma.

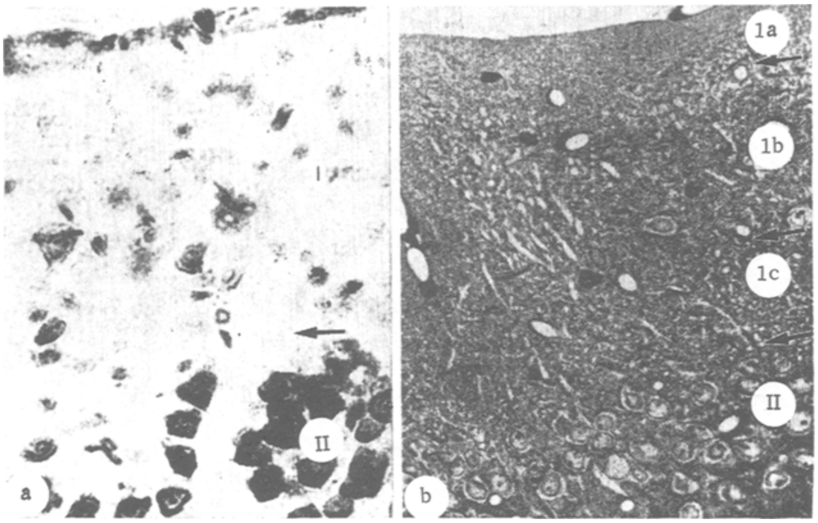

Fig. 2. Structure of layer 1 of cingulate cortex (area 29c) revealed by light-optical methods. I, II) Layers; a-c) sublayers of cortex (arrows indicate boundaries between them). a) Nissl's method, fixation in alcohol; b) semithin section, fixation with glutaraldehyde and OsO_4. Magnification: a) objective 9, ocular 12.5; b) objective 10, ocular 10.

EXPERIMENTAL RESULTS AND DISCUSSION

Sections stained by Nissl's method provide little information about layer 1 of the cerebral cortex (Fig. 2a). A few neurons and glial cells can be seen in these sections in the substance of layer 1, together with a well-marked layer of marginal glia. However, in semithin sections cut from material processed for electron microscopy, sublayers differing in the intensity of their staining and the density of arrangement of their components, can be distinguished (Fig. 2b). The organization of layer 1 can be seen in greater detail on electron-microscopic investigation. Bodies and processes of the marginal glia take part in the formation of the boundary between the cortex and the CSF in the subarachnoid space. In the cingulate cortex, as a rule these cells are fibrous astrocytes with many gliofibrils (Fig. 3). By contrast with layer 1 of the motor cortex (Brodmann's area 4), as described by Rybakov (1972), in the cingulate cortex the bodies of marginal astrocytes lie directly at the surface. This may perhaps explain the absence of a plexus of lamella-like glial processes at the surface in this region of the brain. The number of such processes in regions of the dorsal surface of the brain varies from 10 to 30 (Otellin, Rybakov, and Baikovskaya, 1981). In the cingulate cortex only one or two glial processes often separate processes of nerve cells, synapses, and myelinated fibers located at the surface.

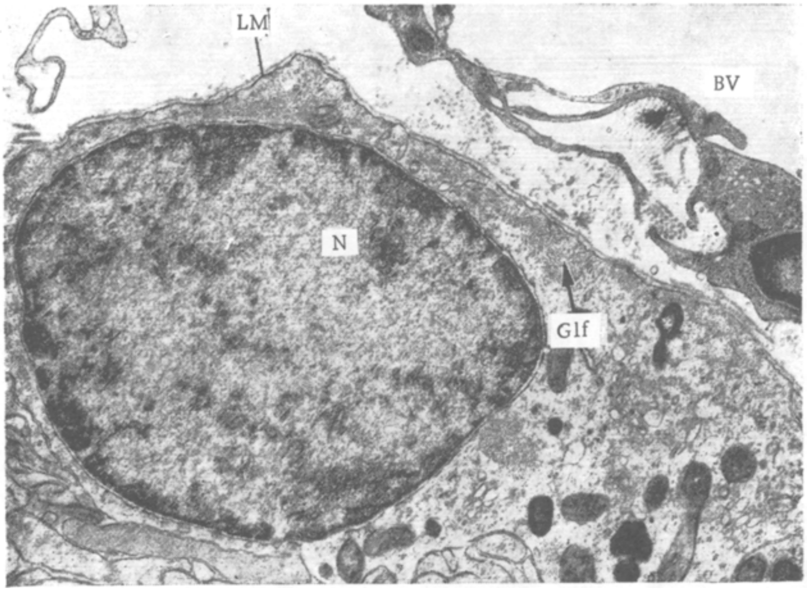

Fig. 3

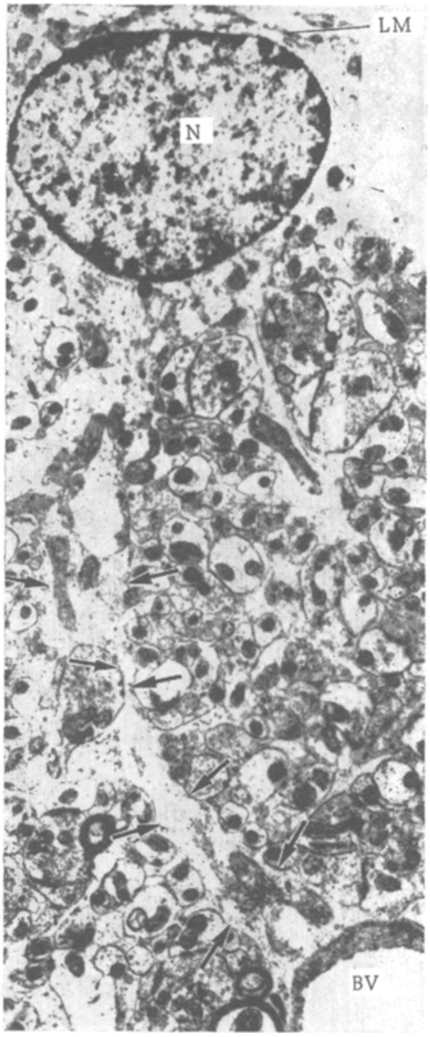

Fig. 4

Fig. 3. Regions of CSF—brain and blood—brain barrier on surface of cingulate cortex (area 24b). Glf) Gliofibrils; BV) blood vessel; LM) limiting membrane; N) nucleus of marginal astrocyte. 12,800 ×.

Fig. 4. Marginal glial cell which at the same time is a perivascular astrocyte. Course of glial process indicated by arrows. Legend as to Fig. 3. 10,800 ×.

The limiting membrane, containing a continuous layer of glycocalyx, and located on the boundary of the media, can be regarded as the substrate of the CSF—brain barrier. Both free areas of the limiting membrane, in contact with CSF, and areas present in the wall of the pial blood vessels (Figs. 3 and 4) can be seen. In the second case the limiting membrane is a component of the blood—brain barrier, which has a more complex structure here than in the substance of the cortex.

Although most cells of the marginal glia are stretched along the brain surface, some of their processes are given off into the depths of the cortex. In one case which we found, the process of an astrocyte penetrates into sublayer la, where it surrounds a blood vessel (Fig. 4). Thus the same cell may be both marginal and perivascular.

Specialized intercellular junctions (tight and gap junctions) are frequently observed between the bodies and processes of surface astrocytes. Sometimes they can be traced from the brain surface to various depths. The contents of the complex system of the intercellular space, which penetrates throughout the thickness of the brain, constitute the internal intercellular medium, and the limiting membrane and intercellular junctions are factors which regulate the entry of various substances into this medium from the CSF. Intercellular junctions are unstable structures. Their number and structure are easily changed by the action of vari-

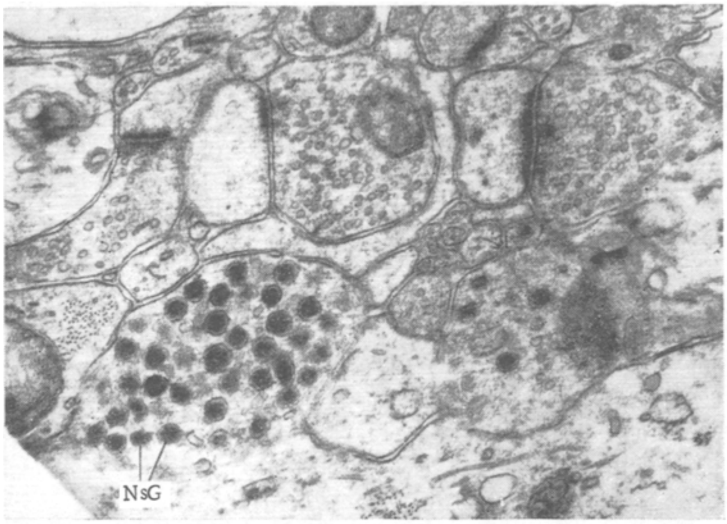

Fig. 5. Axonal profiles with neurosecretory granules in layer 1 of singulate cortex. NsG) Neurosecretory granules. 35,600 ×.

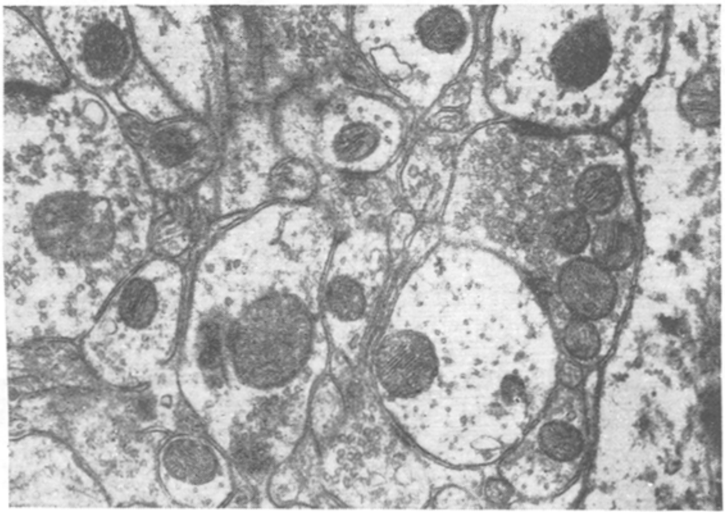

Fig. 6. Axodendritic serial synapses of convergent and divergent types in sublayer 1a of cingulate cortex. 28,000 ×.

ous factors which disturb the permeability of the blood–brain barrier. An increase in the number of specialized junctions is regarded as an indicator of increased permeability, which lies at the basis of the phenomenon of cerebral swelling and edema. Ultrastructural changes in elements of the basement membrane and intercellular junctions, and the swelling of the brain tissue observed under these circumstances, are found in animals treated with bromides (Otellin, Rybakov, and Baikovskaya, 1979). Besides specialized intercellular junctions between astrocytes, gap junctions also are found between glial cells and processes of neurons. Junctions of this kind are considered to be evidence of metabolic relations between the cells forming them. Both ions and amino acids and peptides, with molecular weight up to 1200 daltons, can pass through the channels of the intercellular junctions (Staehelin, 1974; Simpson, Rose, and Loewenstein, 1977).

Profiles (Fig. 5) resembling axons of neurosecretory cells described in the literature (Sterba, 1979; Nemecek, Lodin, Wolff, et al., 1978) were found in layer 1 of the cingulate cortex, more often near the surface.

The close interweaving of axons of different origin with dendrites in layer 1 favors the formation of numerous synapses (Sepp, 1949). Synaptic junctions in layer 1 of the rat cingulate cortex are mainly axospinous and axodendritic synapses. The number of axosomatic synapses and their density on neuron bodies in layer 1 are less than in deeper layers of the cortex.

Near the brain surface (sublayer 1a) synapses are formed by larger pre- and postsynaptic cells. Multifocal synapses of convergent and divergent types are found more frequently, together with serial synapses (Fig. 6). Sometimes divergent synapses are formed by an axon terminal on spines. Unlike in layer 1 of the visual cortex, described by Vorob'eva (1970), in the molecular layer of the cingulate cortex and, particularly often nearer the surface, axon terminals containing many mitochondria (3-9 per section) are observed. As has already been noted, a high concentration of myelinated axons can be seen in sublayer 1b, especially in area 29c, known in light microscopy as Bekhterev's layer.

Layer 1 of the cingulate cortex, sublayer 1c in particular, contains dendrites arranged perpendicularly or at different angles to the brain surface. They are most numerous in the posterior cingulate region, probably because of the high density of cells in layer 2 + 3, which send their dendrites, along with those of neurons in the lower parts of the cortex, into layer 1. In the direction toward layer 2 the caliber of the dendrites and saturation of the dendroplasm with microtubules and mitochondria increased, evidence that these segments belong to the proximal part of the dendrites. In longitudinal sections through dendritic trunks in this region, however, axodendritic synapses are often found: as physiologists have shown, these have a more effective inhibitory action on the postsynaptic neuron than synapses on terminal branches of these same dendrites (Eccles, 1959, 1966; Terzuolo and Araki, 1961).

Layer 1 of the cingulate cortex is thus a heteromorphic formation consisting of nerve and glial cells with their characteristic junctions (synaptic and nonsynaptic), and incorporating structural components of the CSF—brain and blood—brain barriers. Numerous afferents of layer 1 form junctions with processes of cells in layer 1 and also with ascending dendrites of stellate and pyramidal neurons in deeper layers. These distinguishing features of the structural organization of layer 1 suggest that it can be regarded as the morphological substrate of integrative activity and as a unique modulator of activity of the structural components of the cortex. The conclusion that layer 1 of the cerebral cortex plays an integrative and modulating role was based on the results of our own investigations and those of neurophysiologists (Bishop, 1961; Okudzhava, 1963).

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CONNECTIONS OF ZONE AIV WITH ZONES AI, AII, AND Ep OF THE CAT AUDITORY CORTEX

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UDC 611.813.13-018.81-08:599.742.7

To study the complex processes of interneuronal interaction taking place in the auditory cortex we need to know the pathways along which impulses flow between the different parts of the auditory system. An essential link of the system of connections in this system is the association pathways between the separate functional zones of the auditory cortex. This has been demonstrated conclusively in the case of the visual system (Zeky, 1978), for which a unique structural-functional organization of interactions between zones of primary and secondary responses during information processing has been demonstrated at the cortical level. Some information on the organization of pathways between functional zones of the auditory cortex can be found in the morphological literature (Woolsey, 1964; Diamond, Jones, and Powell, 1968). However, it is difficult to judge from it the fine localization of degenerating boutons, for Nauta's method, which was used in these studies, does not allow degeneration of the terminal and preterminal parts of the nerve fiber to be reliably distinguished or the cortical layer in which the system of connections under investigation terminate, to be identified. By electron microscopy the degenerating synapses can be reliably identified and their distribution analyzed not only among layers of the cortex, but also on the surface of components of the neuron. From the precise localization and ultrastructural features, the functional modality of the synapse can be postulated.

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