MORPHOLOGICAL AND BIOPHYSICAL INVESTIGATION OF THE AUDITORY CORTEX AFTER ELECTRODE IMPLANTATION INTO THE INNER EAR IN CATS

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Although the study of the histo- and myeloarchitectonics of the auditory cortex has yielded abundant information, there have been few attempts to study morphological changes in the auditory cortex and, in particular, in area A1, during disturbances of the peripheral part of the auditory system [3, 4]. We have studied the morphological, functional, and structural changes arising in area A1 of the auditory cortex following implantation of electrodes into the chochlea in cats.

EXPERIMENTAL METHOD

Experiments to study responses of cerebral cortical neurons to insertion of an endocochlear prosthesis were carried out on 21 adult cats. Metal electrodes were implanted into the cochlea of all the animals [5, 8]. The opposite ear remained intact. Before the operation the animals' hearing was tested by the evoked potentials (EP) method and only those with normal hearing were chosen. Clinical observation of the experimental animals revealed a reduced response to sound on the ear undergoing the operation. No symptoms were found relative to the animals' behavior, indicating any disturbance of the CNS. Depending on the duration of implantation of electrodes in the inner ear the animals were divided into three groups: 1) 1 month, 2) 3 months, and 3) 5 months. For the morphological investigation and for the spin probe method the brain was removed from the cranial cavity, auditory area A1 was isolated on the side of the experiment (ipsilaterally) and also on the contralateral side, and sections through it were cut in the frontal plane. The contralateral side was the control. Simultaneous histological and microstructural analysis of the control and experimental material is an important technique for the detection of changes in cerebral neurons. The structure of the microsomal membranes in area A1 also was investigated by the spin probe method [1]. The parameter of orderliness was calculated by the usual method [2]. The typical EPR spectrum of the spin probe is illustrated in Fig. 1.

EXPERIMENTAL RESULTS

Light microscopy of the cortex 1 month after implantation of electrodes into the inner ear revealed changes in both neurons and macroglial cells. Vacuolation of the cytoplasm with evidence of marginal edema took place in the neurons (Fig. 2a) and chromatolysis had developed in many of the cells, although in some of them aggregation of the Nissl's substance could be seen, in the form of large clumps. Signs of satellitosis and neuronophagy were visible in circumscribed areas of the auditory cortex. Here and there cell ghosts were observed. Besides many normochromic cells, pycnomorphic neurons also were found (Fig. 2b). The changes observed were diffuse in character although they were more marked in layers III-V of area A1. After 3 months, the signs of edema and swelling in the auditory cortex continued to increase. In most neurons signs of chromatolysis were present, and in some cells this was total in character. Parallel with these changes, the karyoplasm became pale, with the result that the cell nucleoli were distinctly "contrasted" (thus emphasizing their ectopia in some neurons). In the immediate vicinity of the swollen neurons, and also of cells with signs of edema, pycnomorphic neurons could be seen; sometimes these were shrunken, but more often they remained as large pyramidal cells with signs of homogenization of the cytoplasm. The nucleoli

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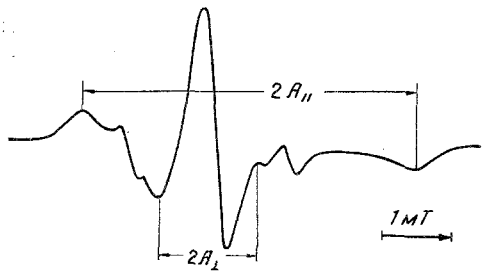

Fig. 1. Typical EPR spectrum of spin probe.

$$S = \frac{A_{\parallel} - (A_{\perp} + C)}{A_{\parallel} + 2(A_{\perp} + C)} \cdot 1{,}723$$

$$C = 1{,}4 - 0{,}053\,(A_{\parallel} - A_{\perp})$$

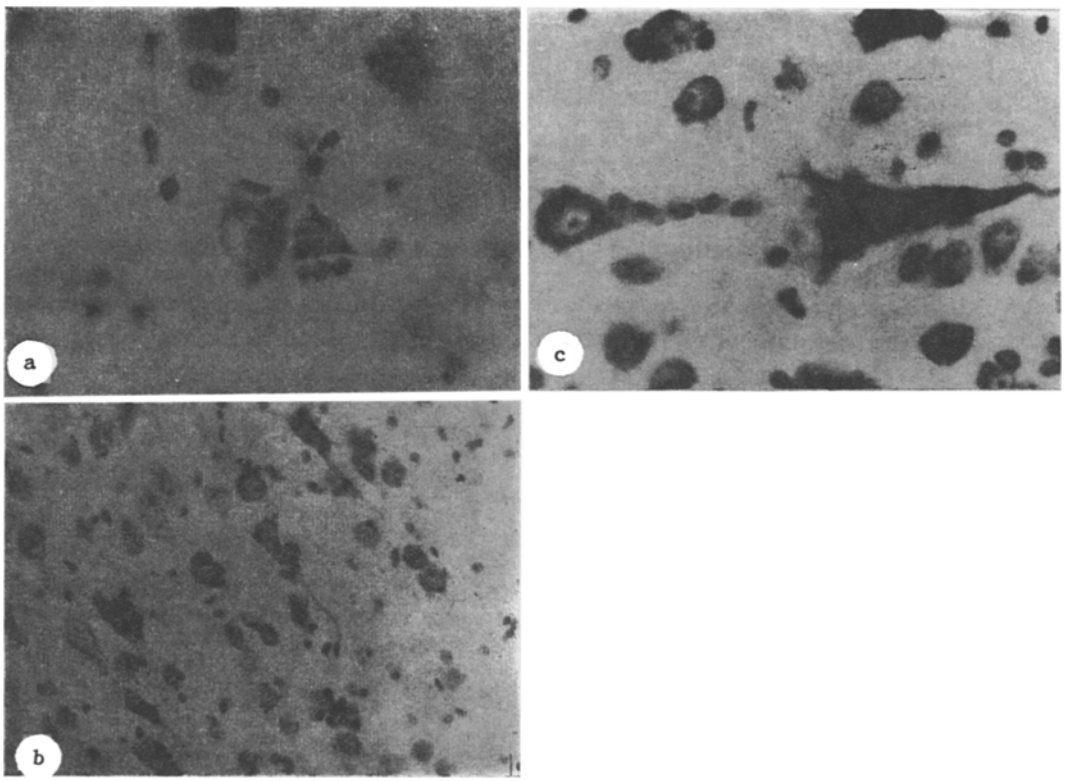

Fig. 2. Auditory cortical neurons in area A1 of the cat brain after implantation of electrodes into inner ear. a) Vacuolation of cytoplasm of neuron (400 ×); b) pycnomorphic neuron (400 ×); c) satellitosis (160 ×). Stained by Nissl's method.

of the shrunken neurons were not easily distinguishable, the cells were changed in shape, the angles of their corners were acute, and the apical process was twisted and clearly defined. Signs of neuronophagy and satellitosis still persisted at this time and were most marked in cortical layer V. Just as in the previous period, solitary ghost cells were seen. During this period a certain quantity of lipofuscin pigment appeared in neurons of layers III-V. The pigment accumulated in form of a small "cap" above the nucleus. As a rule these cells were pyramidal in shape. After 3 months, just as after 1 month, despite the above-mentioned changes in the histoarchitectonics of the auditory cortex, it still preserved its columnar structure.

After 5 months of implantation of electrodes in the inner ear, a change was observed in the character of the cytological disturbances in the auditory cortex. Edema and swelling subsided. Normochromic cells predominated, although hyperchromic neurons also were found, but shrunken forms were rare. Satellitosis was more marked than in the previous period (Fig. 2). In layer V ectopia of the nuclei and nucleoli was found quite often in the neurons, ghost cells appeared here and there, and there were also solitary cells with signs of "severe" damage [6].

TABLE 1. Changes in Parameter of Orderliness (S ± 0.005) in Brain Microsomal Membranes after Different Times of Implantation of Electrodes into the Cochlea

Series of experiments	Duration of implantation of electrodes, months		
	1	3	5
Control	0,830	0,830	0,830
Experimental	0,867	0,850	0,829

The results of the spin probe investigation showed that the microsomal membranes in area A1 were distinguished by their very high degree of molecular orderliness (S > 0.08). This may be connected with the presence of large quantities of saturated fatty acids in these membranes. An even greater increase in orderliness was observed in the cerebral cortex 1 month after implantation of the electrodes. Consequently, mechanical injury at the beginning of the auditory pathway stimulates an essential increase in the microviscosity of the cerebral microsomes. Thus implantation of electrodes into the inner ear not only leads to the morphological changes described above, but also significantly disturbs the internal structure of the microsomal membranes of the brain in area A1 which, as we know, receives the strongest projection in response to auditory excitation [7]. A parallel study of microsomes from the auditory area after the electrodes had remained in the cochlea for 3 months revealed a decrease in the parameter of orderliness of the membranes compared with the previous series of experiments. It will be clear from Table 1 that the parameter of orderliness of the spin probe in specimens obtained after the electrodes had been present in the chochlea for 5 months was virtually the same as in the control. It can accordingly be concluded that complete restoration of the structure of the membrane took place at the molecular level in microsomes of auditory area A1.

Disturbances of water and electrolyte metabolism thus develop in the cerebral cortex after implantation, leading to hydropic lesions of the neurons in the form of edema and swelling, with predominance of cellular edema. By the 3rd month after implantation of the electrodes these disturbances continued to increase, and lead to the appearance of numerous swollen neurons together with pycnormorphic cells. By the 5th month these disturbances subsided, and against this background solitary shrunken neurons with gross irreversible changes, neuron with signs of "severe" damage, and ghose cells could be seen. The latter formed solitary foci of depopulation, which caused no fundamental disturbances of the columnar structure of the cerebral cortex. Besides disturbances of water and electrolyte metabolism, expressed as the types of nerve cell pathology described above, there was a marked response of the cortical glial cells also: astrocytes, oligodendrogliocytes, and macroglia, reflected in phenomena of edema, satellitosis, and neuronophagy. The process continued to develop from the 1st to the 5th month. According to our data, changes in the brain of the experimental animals after 5 months underwent regression.

Morphological and functional analysis of the state of the cerebral cortex showed that a neurotrophic disturbance developed, the main component of which was a disturbance of water and electrolyte metabolism, i.e., hydropic degeneration of the cells. The investigations revealed no morphologically significant changes on the contralateral side, whereas on the ipsilateral surface histological disturbances, which followed the familiar time course at different stages of the experiment, were found in area A1. Three phases can be conventionally distinguished in the development of the underlying hydropic degeneration of the cells: phase 1 (1 month after implantation) — acute edema; phase 2 (after the electrodes had been implanted in the cochlea for 3 months) — edema and swelling; phase 3 (5 months after implantation of electrodes into the ear) — recovery. The results obtained by the spin probe method correlate to a definite degree with the morphological and functional data. Structural changes in the microsomal membranes of area A1, after reaching a peak 1 month after implantation of the electrodes into the inner ear, gradually returned to normal. After 5 months the structure of the membranes returned virtually to normal.

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EXPERIMENTAL REGENERATION OF THE LUNGS

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Following their observations on the time course of healing of lung wounds, many investigators have accepted the view that regeneration of the lung parenchyma with partial or even total resorption of the scar is a possibility [1-4]. Healing of lung wounds was associated with proliferation and migration of the epithelium of those bronchi and alveoli that are damaged as a result of wounding. The bronchial epithelium invades the zone of the defect and takes part in the formation of structures resembling alveoli [2, 12, 14]. It was postulated later that alveoli may also be formed from the alveolar passages in the antenatal period and soon after birth, in response to loss of part of the lung [6, 10, 15]. However, no convincing proof of the existence of this process has yet been obtained.

The aim of this investigation was to study regeneration an incised stab wound of the lung and to identify the most active cellular sources of regeneration.

EXPERIMENTAL METHOD

An aseptic wound of the lungs was produced in 150 male guinea pigs weighing 280-300 g at the age of 5-6 months under sterile conditions and under local anesthesia with 0.25% procaine solution, an incision through the skin, subcutaneous areolar tissue, and spinal and intercostal muscles 1-1.5 cm long was inflicted posteriorly on the right side in the 6th intercostal space. A stab wound of the diaphragmatic lobe of the right lung was then inflicted by puncture with a special scalpel fitted with a guard. The depth of the wound was about 10 mm and its width 5-6 mm. The animals were killed by decapitation after 10 min, 1 h, and 1-7 and 14 days. The lungs were quickly removed from the thorax and investigated by various methods. The role of cyclic AMP (cAMP) in the regulation of many cellular metabolic reactions justified the investigation of this system after lung damage. Fractions of cytoplasmic membranes were obtained from the guinea pigs' lungs. The number of β-adrenergic receptors was determined from the maximal binding of [^{3}H]-dihydroalprenol. Activity of adenylate cyclase (AC) was determined from the quantity of [^{32}P]-cAMP formed. The latter was investigated by the method in [13]. The protein concentration was determined as in [11]. The wound edges, the tissue surrounding the wound, and its symmetrical area of the

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