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An Electron Microscopic Observation on Fine Structure and Acid Phosphatase Activity in Spheroids and Different Types of Axonal Alternations of the Inferior Olivary Complex in Ground Squirrel (*Citellus citellus* L.)

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The fine structure of altered axon and axon terminals, myelinated and unmyelinated axon swellings, so-called "spheroids" and the expression of the enzyme acid phosphatase in the inferior olivary complex of the ground squirrel were studied by electron microscopy. In the well-preserved olivary neuropil, the axon terminals with characteristic fine stricture were accompanied by synapses with altered presynaptic part. These alterations were expressed by the evident changes in synaptic number, hyperplasia of smooth endoplasmic reticulum and by abnormal mitochondria. Most of spheroids contained numerous mitochondria, dense bodies, vesicles and fragments smooth endoplasmic reticulum. Acidic phosphatase-reaction product is present in these altered axonal profiles. These structures were found in normal ground squirrels of both sexes and different ages, predominantly in old animals and during the springtime. The results are discussed in term of plasticity of elements in the neuropil of the inferior olivary complex.

Key words: Axonal alternations, inferior olivary complex, electron microscopy, acid phosphatase, ground squirrel.

Introduction

Connections between neurons in the central nervous system are very dynamic structures and morphological correlates of the synaptic junctions are continuously modified. Historically, the idea that neurons and their connections are able to react during the functional and experimental conditions by remodelling of the axons and synaptic elements belongs to R a m ó n y C a j a l [20]. Various morphological studies reported the occurrence of axonal alternations in the central and peripheral nervous system of apparently healthy animals [1, 2, 5, 11, 12, 18, 22, 23, and 24]. These results suggested that axonal remodelling is a general process within the central neurons system of many vertebrates. Plastic changes can be induced by injury but occur also spontaneously and are based on the same mechanism [10].

In the olivary complex, plastic changes have been shown to be a result from lesions of the cerebellum or the pontine tegmentum [see 21 for references]. Such a phenomenon can be induced by ablation of the cerebellum including the cerebellar nuclei [3,4, 19, and 26]. The aim of the present report was to study the fine structure of axonal alternations and ultrastructural localization of acid phosphatase activity in myelinated and unmyelinated axons and axon terminals of inferior olivary complex in normal ground squirrels of both sexes and different ages in both periods of activity.

Material and Methods

For the electron microscopy, 10 ground squirrels in both periods of activity (the active period and winter sleep) were used. Animals of both sexes were anaesthetized with sodium pentobarbital (40 mg/kg) and fixed by intracardial perfusion with 4% paraformaldehyde and 2% glutaraldehyde in 0.2 M cacodylate buffer containing 0.02% calcium chloride or 0.1 M phosphate buffer, pH 7.2. The perfused animal brains were stored in the same fixative overnight at 4°C. The inferior olivary complex was dissected out and immersed in 1% OsO_4 with 0.2 M cacodylate with 0.02% calcium chloride or 0.1 M phosphate buffer, pH 7.2 for 2 h at 4°C. After embedding in Durcupan ACM, ultrathin sections were cut with a Reichert ultramicrotome or a LKB ultramicrotome. Ultrathin sections were counterstained with uranyl acetate and lead citrate and examined by an electron microscope Hitachi H-500.

For histochemilal observations, ground squirrels in both periods of animal activity, the active (spring and summer) period (×2) and winter sleep (×2) were anaesthetized with sodium pentobarbital (40 mg/kg, i. p.) and fixed by intracardial perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2. Brains were removed from the skull and kept in the same fixative for 5 h at 4°C. Tissue blocks comprising the inferior olivary complex were incubated for acid phosphatase reaction according to the method of G o m o r i [14]. The specimens were prepared for electron microscopy by using a routine procedures that included postfixing with 2% OsO_4 in 0.1 M phosphate buffer, pH 7.2 for two h at 4°C and embedding in Durcupan. Thin sections were cut with a LKB ultramicrotome and stained with lead citrate before examination with an electron microscope Hitachi H-500.

Results and Discussion

The neuropil of the ground squirrel inferior olivary complex is comprised of dendritic profiles, myelinated and unmyelinated axons, axon terminals or varicosities and many glial cells and processes. In all subnuclei of the normal inferior olivary complex two main classes of axon terminals or axon varicosity are observed. Both classes included terminals with various sizes (from less than 1 mm up to 4.5 mm in diameter).

These axon terminals contain round or pleomorphic synaptic vesicles (a mixture of round, oval and slightly flattened vesicles), which range in size. In addition to round or pleomorphic synaptic vesicles, they contain populations of dense-core vesicles (diameter 60-80 nm). They mainly make up synaptic junctions with dendrites of all size, dendritic spines and neuronal somata. Some axon terminals and boutons *en passant* contain a large number of dense core vesicles but not form any specializations with other elements in apposition. In the complex synaptic clusters, which can

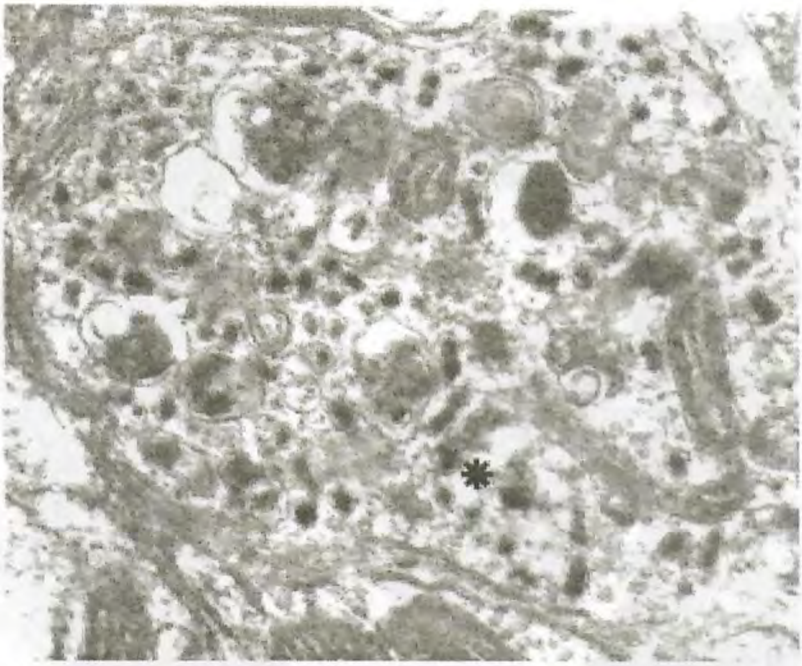

Fig. 1. Altered axon (asterisk) with numerous lysosome-like bodies and increase in number of abnormal dense-core vesicles (× 28 300)

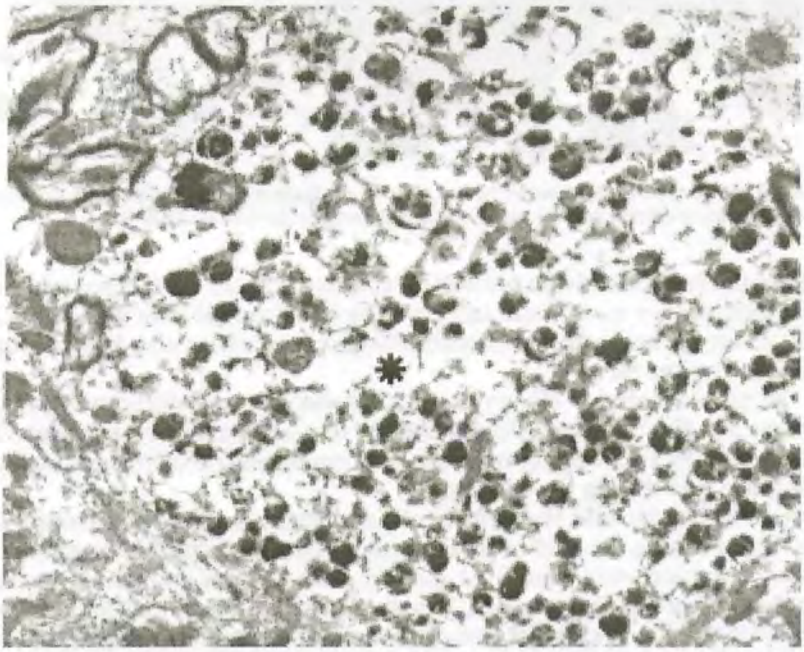

Fig. 2. A spheroid (asterisk) filled with numerous lysosome-like and pleomorphic dense bodies, which are marked with reaction product for acid phosphatase (× 14 030)

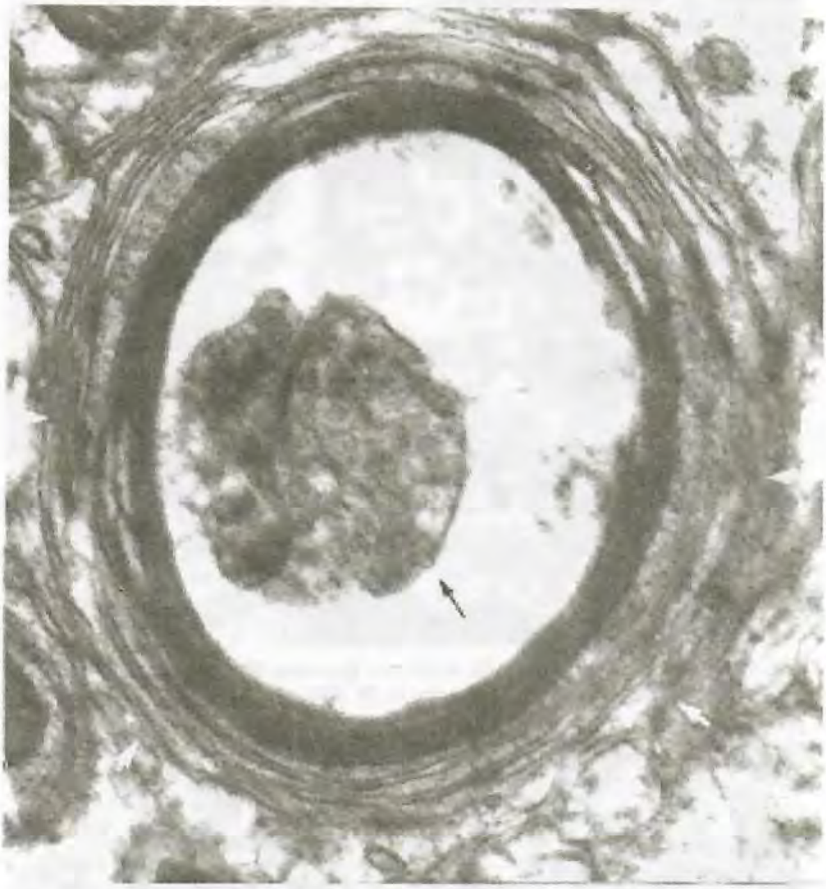

Fig 3. Astrocytic encapsulation (white arrows) of degenerate mylitated axon containing a pyknotic mass with clumped vesicular profiles (arrow) (× 41 400)

be called glomerulus, dendritic profiles form a central core for the assemblage of several axon terminals or varicosities and covering by astrocytic lamellae. These synaptic junctions in the ground squirrel inferior olivary complex are also known from previous publications [see 9].

In the normal inferior olivary complex, spheroids and different type of axonal alternations are distributed randomly and are in all respects well preserved by aldehyde fixation. They are most common in the ground squirrel inferior olivary complex of old animals and during the springtime.

Based on the size and the density of axoplasmic organelles altered axons and their terminals can be devided into two groups. The first group is composed of only moderately enlarged, measured 3-14 mm^2 in cross section and varied in shape and fine structure altered axonal profiles. To sum up, these alternations include hyperplasia of cisternal profile accumulations of mitochondria, numerous lysosome-like and dense bodies (Fig. 1). Another occasional finding are profiles full of numerous mitochondria and glycogen granules.

The second group of axonal alternations includes large spheroid bodies or spheroids. The size of the largest one reached 60 - 80 mm^2. Myelinated and unmyelinated spheroids exhibit dense proliferation of the neurofilaments, numerous mitochondria and lysosomses. Another type of spheroid bodies are packed with numerous lysosome-like and pleomorphic dense bodies. Acidic phosphatase reaction product is detected on the surface of these profiles and is diffusely distributed on dense material between them (Fig. 2). Electron-opaque degenerating axon terminals may also, on rare occasion, occur common in the inferior olivary complex of the ground squirrel. Each of these is sequestrated within a concentric sheath (Fig. 3), which is composed of astrocytic lamellae.

Data presented in this study were provided by some details of thin sectioning analyses of changes in the fine structure of axonal profiles such as: spheroids and other types of axonal alternations, which are marked with reaction product for acid phosphatase in the inferior olivary complex of normal ground squirrel, predominantly in old animals and during the springtime.

The axonal alternations, which have been observed in the well-preserved olivary neuropil, may be accepted as plastic changes, which can be explained in several ways. Spheroids and different types of axonal alternations are present in the inferior olivary complex of healthy animals and are probably example of spontaneous degeneration [5, 10, 11, 23,24], a result of age-related changes [8], but are also found in the inferior olivary complex of neonate child with neuroaxonal dystrophy [17]. The responses to the injury or primary aging in the inferior olivary complex are olivary hypertrophy, loss of neurons and activated astrocytes [6, 13, 15, 16]. Astrocytic multilamellar capsules surround the altered axon terminals or myelinated axons in the inferior olivary complex [7, 9] as in the other parts of the brain [25].

In summary, these results are important morphological declaration of the adaptive property of the olivary neurons in the ground squirrel.

References

1. Angaut, P., C. Sotelo. Fine structure of the cerebellar central nuclei in the cat II. Synaptic organization. — Exp. Brain. Res., **16**, 1973, 431-454.
2. Berg, B. N., A. Wolf, H. S. Simms. Degenerative lesions of spinal roots and peripheral nerves in aging rats. — Gerontologia, **6**, 1962, 72-80.
3. Boesten, A. J. P., E. Marani. A light- and electronmicroscopical study of olivary hypertrophy in the cat. — Neurosci. Lett., **8**, 1979, 158.
4. Boesten, A. J. P., J. Voogd. Hypertrophy of neurons in the inferior olive after cerebellar ablations in the cat. — Neurosci. Lett., **61**, 1985, 49-54.
5. Bozhilova-Pastirova, A. Ultrastructure of the inferior olivary complex in the ground squirrel (Citellus Citellus L.). Dendrites. Age-related changes (in Bulgarian). — Eksperim. med. i morf., **29**, 1990, No 3, 9-17.
6. Bozhilova-Pastirova, A., K. Ichev, W. Ovtscharoff. Distribution of the glial fibrillary acidic protein in the inferior olivary complex of the cat, ground squirrel and pigeon. In Histochemical methods and their application in the science and diagnosis. — Gegenboum. morph. Jbrb. Leipzig, **135 2S**, 1989, 310.
7. Bozhilova-Pastirova, A., K. Itchev, T. Christova. Morphology of the astrocytes in the inferior olivary complex of the ground squirrel (Citellus citellus L.). — Verh. Anat. Ges., **85** (Anat Anz Suppl 170), 1991, 685-686.
8. Bozhilova-Pastirova, A., K. Ichev. Urltastructure of the inferior olivary complex of the ground squirrel (Citellus citellus L.). Ineuropil. Age-related changes in axons (in Bulgarian). — Eksperim. med. i morf., **30**, 1990, No 4, 8-14.

9. Bozhilova-Pastirova, A., W. Ovtscharoff. The inferior olivary complex. — In: Adv. Anat. Embryol. and Cell Biology (Springer -Verlag, Berlin, Heidelberg, New York), **155**, 2000, 47-66.
10. Calverley, R. K. S., D. G. Jones. Contribution of dendritic spines and perforated synapses to synaptic plasticity. — Brain. Res. Rev., **15**, 1990, 215-249.
11. Chan-Pala, V. Cerebellar dentate nucleus. Organization, cytology and transmitters. New York, Springer Verlag, Berlin Heidelberg, 1977, 158-168.
12. Gavelová, M., T. Badonic, A. Mitro. Altered axons and axon terminals in the nucleus gracilis of the dog. — J. Hirnforsch., **24**, 1983, 399-404.
13. Gioccone, G., F. Tagliavini, J. S., Street, B., Ghetti, O. Bugiani. Progressive supranuclear palsy with hypertrophy of the olives. An immunocytochemical study of the cytoskeleton of argyrophilic neurons. — Acta Neuropathol., **77**, 1988, 14-20.
14. Gomori, G. Microscopical histochemistry. Principles and practice. Chicago, University of Chicago Press, 1952, 189-194.
15. Grandi, D., M. L. Arcari. Neuronal aspects and plasticity of inferior olivary complex and nucleus dentatus. — Acta Biomed. Ateneo. Parmense, **63**, 1992, 17-25.
16. Ito, K., Y. Ishikawa, R. D. Skinner, R. E. Mrak, M. Morrison-Bogorad, J. Mukawa, W. S. Griffin. Lesioning of the inferior olive using a ventral surgical approach. Characterization of temporal and spetial astrocytic responses at the lesion site and in the cerebellum. — Mol. Chem. Neuropathol., **31**, 1997, 245-264.
17. Janota, I. Neuroaxonal dystrophy in the neonate. — Acta. Neuropathol. (Berlin), **46**, 1979, 151-154.
18. Leonhardt, H. "Axonal spheroids'" in the spinal cord of normal rabbits. — Cell. Tiss. Res., **174**, 1976, 99-108.
19. Marani, E. Topogrophic histochemistry of the cerebellum. — In: Progr. Histochem. Cytochem., 16. Stuttgart, New York, Gustav Fischer Verlag, 1986, 1-196.
20. Ramón y Cajal, S. Notas preventivas sobre la degeneración y regeneración de la las vias nerviosas centrales. — Trab. Labor. Invest. Biol. Madrit., 1906, 295-301.
21. Ruigrok, T. J. H. C. I. de Zeeuw, J. Voogd. Hypertrophy of the inferior olivary neurons: A degenerative, regenerative or plasticity phenomenon. — Eur. J. Morphol., **28**, 1990, 224-239.
22. Saito, K. Spheroids and altered axons in the spinal gray matter of the normal cat. An electron-microscopic study. — Acta Neuropathol. (Berlin), **52**, 1980, 213-222.
23. Sotelo, C., S. L. Palay. Altered axons and terminals in the lateral vestibular nucleus of the rat. Possible example of axonal remodeling. — Lab. Invest., **25**, 1971, 653-671.
24. Sotelo, C., S. L. Palay. The fine structure of the vestibular nucleus of the rat. — J. Cell Biol., **36**, 1968, 151-179.
25. Van Houten, M., J. Brawer. Regional variation in glia and neurons within the hypothalamic ventromedial nucleus. — J. Comp. Neurol., **179**, 1979, 719-738.
26. Verhaart, W. J. C., J. Voogd. Hypertrophy of the inferior olives in the cat. — J. Neuroph. Exp. Neurol., **21**, 1962, 92-104.