Demonstration of Electron-Dense Material within 'Empty' Synaptic Vesicles in the CNS of Rat

W. OVTSCHAROFF

Department of Anatomy, Histology and Embryology, Medical Academy, Sofia 31 (Bulgaria), 21 July 1975.

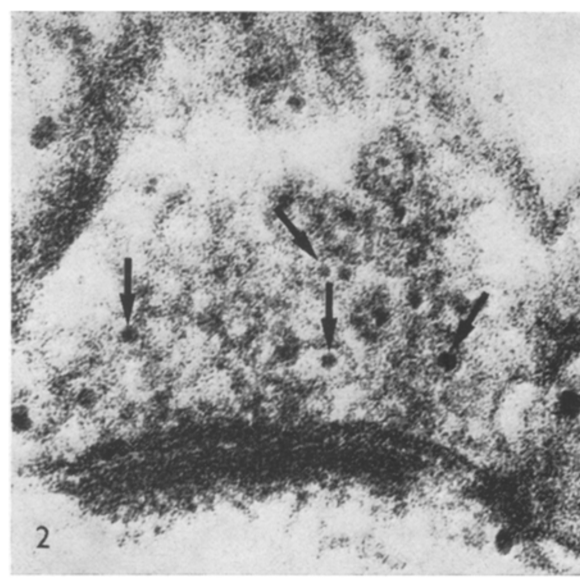
Summary. A method for demonstration of electron-dense particles within electron lucent synaptic vesicles from various regions of CNS of rat is described. On the basis of the controls a visualization of protein substance is detected.

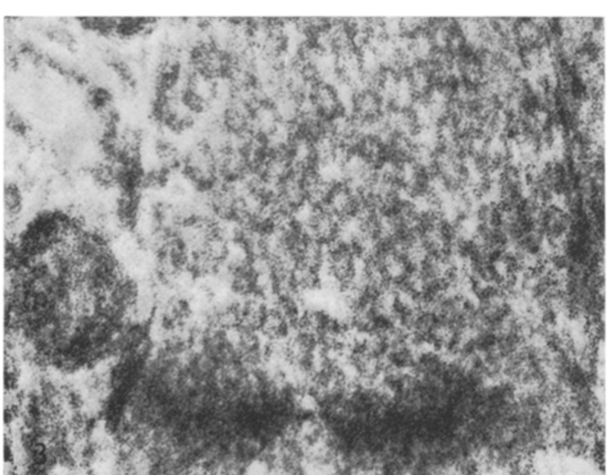
Our studies on synaptic structures have shown the presence of dense particles within the synaptic vesicles of rat brain material, subjected to acid hydrolysis after fixation[1]. Recently, GRAY and PAULA-BARBOSA[2] published results showing dense material within 'empty' synaptic vesicles following fixation in acid-aldehyde. A method is described for demonstration of dense granules within such 'empty' synaptic vesicles from various areas of the rat CNS.

Materials and method. 6 adult rats were used. The animals were perfused via aorta with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. Small pieces of tissue were additionally fixed in 6.25% glutaraldehyde, dissolved in the same buffer and identical pH for 120 min. Thereafter, they were placed for 120 min in 0.5 N acetic acid, or in 0.1 N HCl, or in 1% trichloroacetic acid. The material was transferred in 1% OsO_4 for 60–90 min, and embedded in durcupan after dehydration. Deamination at small tissue blocks: incubation in nitric acid (6 g nitrite of sodium in 35 ml distilled water, plus 5 ml acetic acid) for 24 h at 4°C, as well as enzyme digestion with trypsin before or after acid hydrolysis[3] were used as controls. Unstained and doubly stained, with uranyl acetate and lead citrate, sections (ultramicrotome Reichert) were observed with electron microscope JEM 100 B.

[1] W. OVTSCHAROFF, unpublished (1974).

[2] E. G. GRAY and M. PAULA-BARBOSA, J. Neurocytol. *3*, 487 (1974).





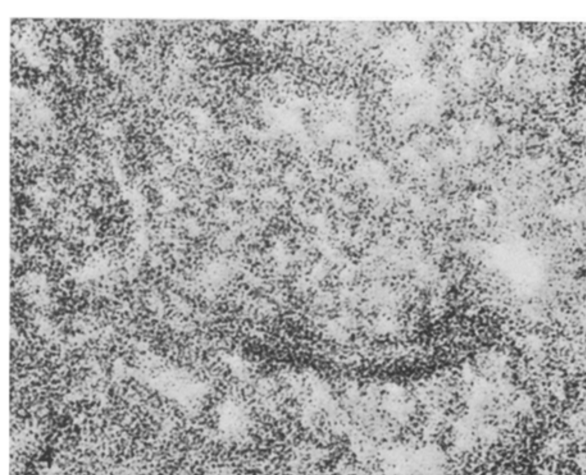


Fig. 1. Electron-dense particles within synaptic vesicles (arrows). Synaptic cleft (big arrow). Unstained section. × 240,000.

Fig. 2. Dense material within synaptic vesicles (arrows) after double staining with uranyl acetate and lead citrate. × 200,000.

Fig. 3. Effect of deamination. Electron-dense particles within synaptic vesicles are no longer visible. × 140,000.

Fig. 4. Enzyme digestion with trypsin removes the electron dense material from synaptic vesicles. × 140,000.

Results and discussion. Electron-dense granules of 50–120 Å are visible in the center of the synaptic vesicles; occasionally they are excentrically situated and bound to the vesicular membrane (Figure 1). Additional staining with uranyl acetate and lead citrate increases the electron-density and the size of the granules (Figure 2). Vesicles with a similar dense center are noted scattered throughout the presynaptic part of the synapses. Treatment of the material with trichloroacetic acid, employed in biochemistry for the precipitation of proteins, leads to formation of larger and denser granules, but considerably damages the structure. Single particles are also found within the synaptic cleft, as well as along the mitochondrial membrane, and within the sacs of the smooth endoplasmic reticulum. The adrenergic vesicles observed in substantia nigra showed their typical dense core. Deamination accounts for a strong reduction of the contrast staining of particles in the vesicles up to their full disappearence (Fig. 3), whereas digestion with trypsin, before or after acid hydrolysis, leads to completely negative reaction for dense granules' demonstration. Based on the above data, it may be assumed that it is a matter of protein structures' visualization. Most probably, the effect of acids is reduced to immobilization of the protein substance within the synaptic vesicles, which makes possible its demonstration with OsO_4. The failure to demonstrate this presumably low-molecular protein[4] using the routine electron microscopic method, may be attributed to the loss of protein during OsO_4 fixation, and during the following dehydration[5]. Recent data reported by WHITTAKER and ZIMMERMANN[6] prove that, in the composition of the acid protein-vesiculin, which plays the role of an acetylcholine carrier substance, a great amount of amino acids participate, such as: glutamine, asparagine, serine, proline and lysine, which react actively with OsO_4[7]. It is presumed that acid hydrolysis accounts for splitting of a number of peptide bonds in this protein or in the transmitters of protein character[8], and the amino acid groups liberated by this way enter into reaction with OsO_4.

[3] W. OVTSCHAROFF, Histochemistry, *44*, 185 (1975).
[4] V. P. WHITTAKER, M. J. DOWDALL, G. H. C. DOWE, R. M. FACINO and J. SCOTTO, Brain Res. *75*, 115 (1974).
[5] M. A. HAYAT, *Principles and Techniques of Electron Microscopy* (Van Nostrand Reinhold Comp., New York, Cincinnati, Toronto, London, Melbourne 1970).
[6] V. P. WHITTAKER and H. ZIMMERMANN, in *Synaptic Transmission and Neuronal Interaction* (Ed. M. V. L. BENNETT, Raven Press, New York 1974), p. 217.
[7] G. F. BAHR, Expl Cell Res. *7*, 457 (1954).
[8] F. E. BLOOM, Brain Res. *62*, 399 (1973).

Cobalt-Staining of Motor Nerve Endings in the Locust (*Locusta migratoria*)

M. PETERS

Institut für Zoologie, RWTH Aachen, Kopernikusstrasse, D-51 Aachen (German Federal Republic, BRD), 23 June 1975.

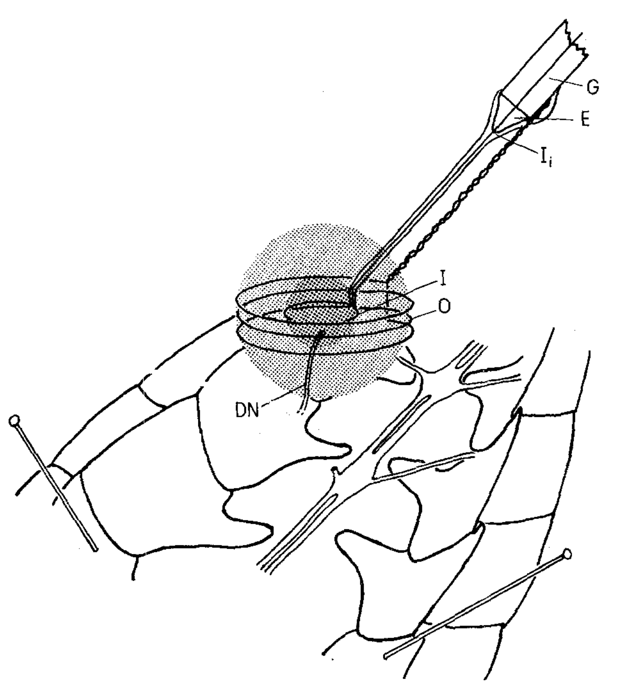
Summary. A method is described for axonal cobalt-staining of peripheral nerve branches. First experiments were carried out on abdominal muscles of the locust.

It is often desirable to confirm electrophysiological results about the peripheral branching of a particular nerve by histological methods. For the analysis of central projections of identified neurones, intracellular staining with Procion Yellow[1] or cobalt[2] and axonal iontophoresis[3] of these dyes have proved most valuable. In the following paper, it is shown that axonal iontophoresis of cobalt is also applicable to peripheral nerve branches.

In order to permit staining of very short and thin nerve stumps, difficult to handle otherwise, a gap-electrode was developed. The dorsal nerve of the second free abdominal ganglion of *Locusta migratoria* was chosen as a test object for optimal staining conditions. USHERWOOD[4] saline, modified by addition of 5 m*M* sodium-acetate and 5 m*M* glucose was used.

Procedure. The abdomen of the locust is opened by longitudinal incisions and pinned out in a chamber containing saline continually bubbled with carbogen. The nerve is sectioned near the respective ganglion. Then, the electrode is positioned above the nerve stump with the aid of an appropriate holder. The electrode basically consists of 2 concentric rings of platinum wire (Figure 1). The outer ring holds a drop of low viscosity liquid paraffin. By means of a fire-polished microcapillary, a drop of distilled water is applied to the inner ring. The nerve

[1] A. O. W. STRETTON and E. A. KRAVITZ, Science *162*, 132 (1968).
[2] R. M. PITMAN, C. A. TWEEDLE and M. J. COHEN, Science *176*, 412 (1972).
[3] J. F. ILES and B. MULLONAY, Brain Res. *30*, 397 (1971).
[4] P. N. R. USHERWOOD and H. GRUNDFEST, J. Neurophysiol. *28*, 497 (1965).



←

Fig. 1. Electrode and preparation. I, inner ring (diameter 1 mm) containing $CoCl_2$; O, outer ring (diameter 3 mm) containing liquid paraffin. Dimensions may be adapted for finer nerve branches. I_i, silicon gum coating to provide electrical insulation of the inner ring in order to allow passage of current; G, supporting glass capillary (diameter 1.5 mm); E, Epoxi-glue; DN, dorsal nerve.