IMMUNOHISTOCHEMISTRY OF SYNAPTIC PROTEINS FOR SYNAPTIC EXOCYTOSIS

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ABSTRACT

The synaptic proteins synaptobrevin/VAMP, SNAP-25, Syntaxin1, NSF and α-SNAP were revealed by means of immunocytochemistry. Materials from the cerebral cortex of adult, newborn and postnatal rats (P6 and P11) were used. Immunostaining for synaptobrevin/VAMP was mainly around the synaptic vesicles, whereas the immunolabeling for SNAP-25 and syntaxin1 was revealed in most cases on the cytoplasmic surface of the presynaptic membrane and to a lesser extend - on synaptic vesicles. Immunostaning for NSF and α-SNAP was found out not only on the axoplasm of axonal endings and varicosities, but also in perikarya and dendrites. In the cerebral cortex of newborn rats, a small number of immunopositive presynaptic parts could be observed. The number of these immunolabeled structures increases evidently with increasing age of rats.

Key words: *synaptic vesicle proteins, presynaptic membrane, transmitter exocytosis, SNARE proteins, immunocytochemistry*

INTRODUCTION

The action potential leads to release of the neuromediator in the synaptic cleft. This occurs after fusion of synaptic vesicle membrane with presynaptic one. The presence of synaptic proteins is necessary to bring synaptic vesicles to the active zone of the presynaptic membrane and to fuse the lipid bilayers of both structures (5). The synaptic vesicles are specific cell organelles and most thoroughly studied by means of biochemical, cytochemical and other methods. In the membrane of synaptic vesicles, several proteins with known and unknown functions are located such as synaptobrevin/VAMP, Rab3, synaptophysin, vesicular transmitter transporter, proton pump, synaptoporin and others (3,4). There are synaptic proteins located in the pesynaptic membrane and axoplasm such as syntaxin, α-SNAP, SNAP-25, NSF and other for realizing the transmitter release and for recycling the processes of these organelles in the presynaptic part of synapses (boutons and varicosities) (5). This effective synaptic machinery includes SNARE (Soluble NSF-Attachment protein Receptor) and SM (Sec1/Munc 18-like) proteins (7). The dissociation of the SNARE complex occurs by means of N-ethylmaleimide sensitive factor (NSF) which is cytosolic ATPase (2). It is possible that SM cytosolic proteins are partners for SNARE in the processes of fusion of synaptic vesicles with the presynaptic membrane. In the presynaptic part, there are numerous partly folded and unfolded SNARE protein molecules. The chaperone systems support the process of folding of these proteins - cysteine string protein α (CSPα), glutamate- and threonine-

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rich protein (SGT), Hsc70 and synuclein (7). The proteins connected with the active zone of the presynaptic membrane such as Munc13's, RIMMs (Rab3-interacting molecules), α-liprins and RIM-BPs (RIM-binding proteins) take also part in the synaptic exocytosis processes.

The goal of this study was to visualize by means of immunostaining procedures several synaptic proteins which are involved in the synaptic exocytosis machinery, e. g. synaptic vesicle membrane proteins synaptobrevin/VAMP, synaptic proteins present on presynaptic membrane and in to lesser extend on synaptic vesicles, i.e. syntaxin1 and SNAP-25, as well the cytosolic proteins - NSF and α-SNAP. Another objective of our investigation was to follow-up the postnatal development of the immunopositive patterns of these synaptic proteins.

MATERIAL AND METHODS

Adult Spague-Dawley rats of both sexes as well as newborn and postnatal rats (P6 and P11) were used for this study. The animals were anaesthetized (4% w/v chloral hydrate, 1 mL/g b. w.) and were perfused for 15 min through the ascending aorta with 2% paraformaldehyde, 2% glutaraldehyde in cacodylate buffer at pH 7,4. Subsequently, brains were removed, postfixed in the same fixative at 4oC for four hours and placed in PBS overnight. Coronal sections of the forebrain with thickness of 30 μm were cut on a vibratome and processed for pre-embeding immunostaining employing the ABC technique.

After treatment in 5% goat serum, the sections were incubated for 24 hours at 4°C with monoclonal antibodies to synaptobrevin/VAMP (1:1000), syntaxin1 (1:1000), SNAP-25 (1:1000), α-SNAP (1:1000) and NSF, respectively. Biotinylated horse anti-mouse IgG (1:250) for one hour and avidin-biotin-peroxidase complex (1:200) for two hours were used as second and third steps of immunolabeling. The incubations were carried out at room temperature under gentle shaking. A 0,05% 3,3'-diaminobenzidine-tetrachloride was used as peroxidase substrate. After washing in 50 mM Tris-HCl at pH 7,6 the cerebral cortex was dissected from the sections, postfixed with 1% OsO4 in 0,1 M phosphate buffer at pH 7,2 for 45 min, rapidly dehydrated and flat-embedded in Epon 812 between glass slide and coverslip. The slides were precoated with dimethylhydroxysilane. The ultrathin sections were viewed unstained or counterstained with lead citrate and uranyl acetate. The primary antibody was replaced by BSA as a control for all the immunostainings (1:1000 in PBS). All control samples were negative.

RESULTS

Numerous synaptic boutons in neurophil and on neuronal perikarya were immunopositive for synaptobrevin/VAMP (Fig. 1).

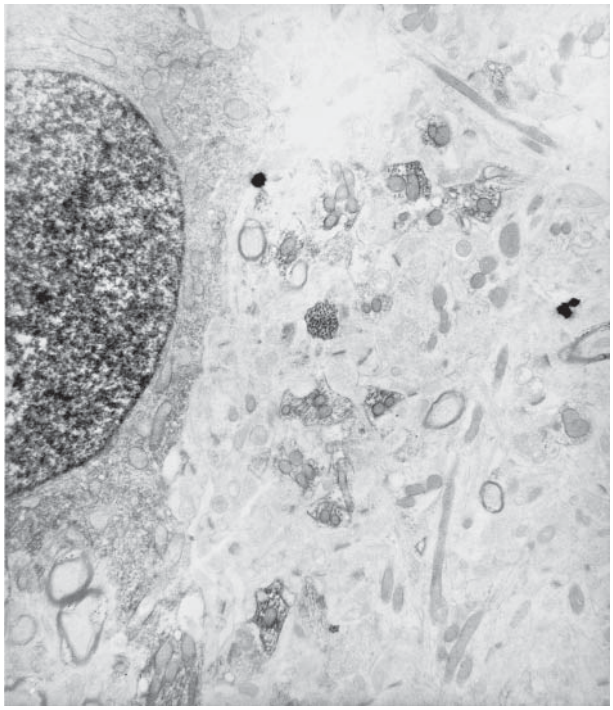

Fig. 1. Synaptobrevin/VAMP, cerebral cortex. Immunopositive axonal endings. Adult rat. Primary magnification 6300.

The reaction product was predominantly located around synaptic vesicles. In the cerebral cortex of newborn rats a few immunostained boutons were observed which number increased in postnatal rats (P6 and P11). Immunolabelled vesicle profiles were seen in some myelinated and unmyelinated axons (Fig. 2).

Syntaxin1 immunostaining was revealed in the membrane of some synaptic boutons and to a

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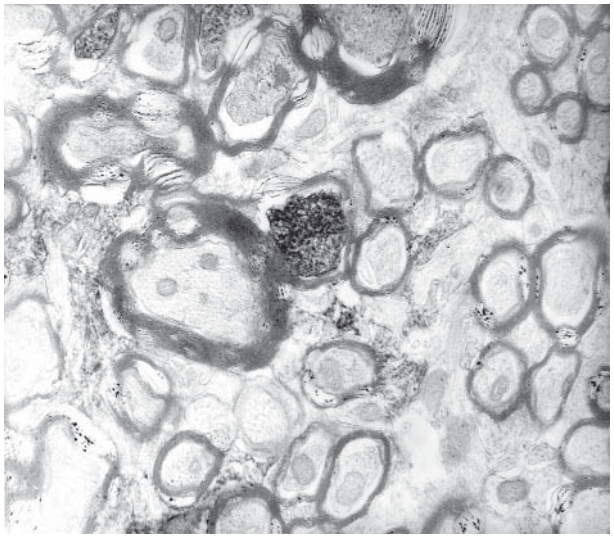

Fig. 2. Synaptobrevin/VAMP, cerebral cortex. Immunolabeled vesicle profiles in the myelinated axon. Adult rat. Primary magnification 16000.

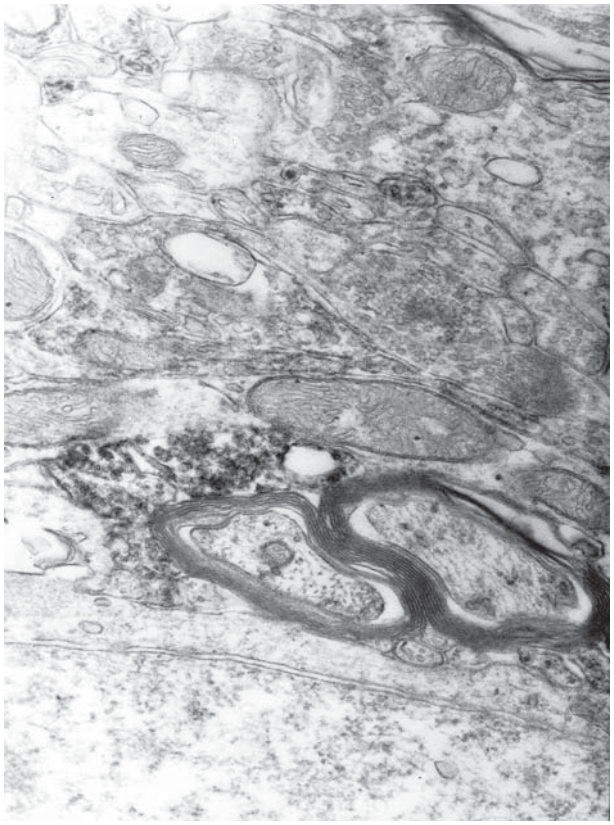

Fig. 3. Syntaxin1, cerebral cortex. Immunopositive axonal ending. Adult rat. Primary magnification 20000.

lesser extend in single synaptic vesicles (Fig. 3). A slight immunolabeling on the vesicle profiles of the Golgi apparatus was established. Only a few axonal

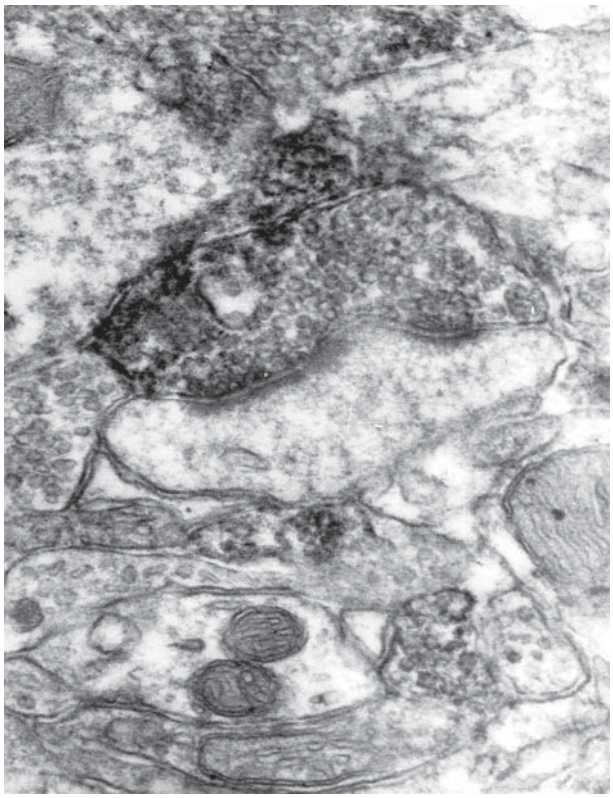

Fig. 4. SNAP-25, cerebral cortex. Immunolabeling of the presynaptic membrane and a lesser extend synaptic vesicles. Adult rat. Primary magnification 30000.

endings were observed in the cortex of newborn rats. These immunostained endings and varicosities became more numerous in the postnatal rats. The immunopositive staining for SNAP-25 was better expressed when compared with the immunopositive product for syntaxin1.

The immunolabeling for SNAP-25 was found out on cytoplamic surface of the presynaptic membrane (Fig. 4) as well as on the synaptic vesicle membrane (Fig. 5). The immunopostive reaction product was evident on the vesicles and sacs in myelinated axons (Fig. 6).

The immunostaining for NSF was revealed in the axoplasm of the presynaptic part between the synaptic vesicles (Fig. 7) as well as in perikarya and postsynaptic parts (Fig. 8). Such immunopositive structures were observed in the cerebral cortex of newborn and postnatal rats (Fig. 9).

The immunolabeling for α-SNAP was evident in the cytoplasm of axonal endings and varicosities (Fig. 10) as well as in perikarya (Fig. 11) and dendritic

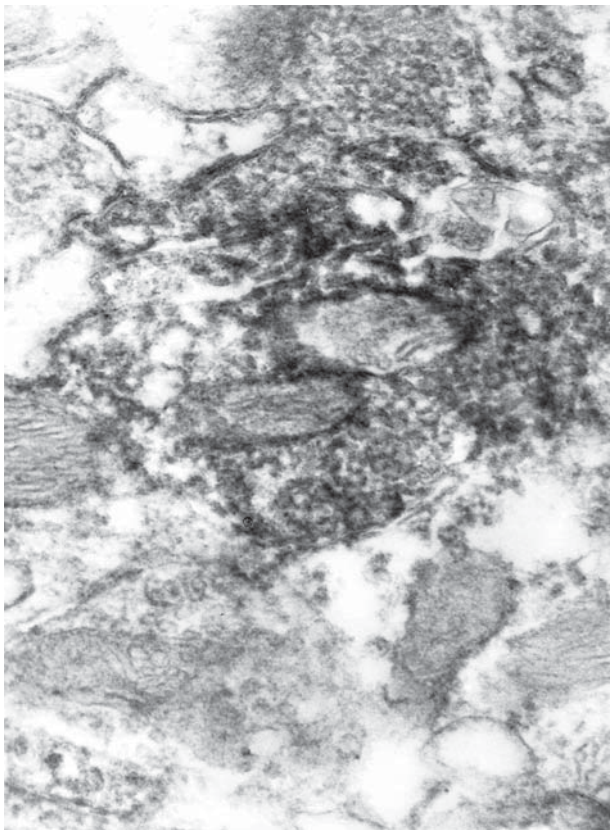

Fig. 5. SNAP-25, cerebral cortex. Immunolabeling of synaptic vesicles. Adult rat. Primary magnification 30000.

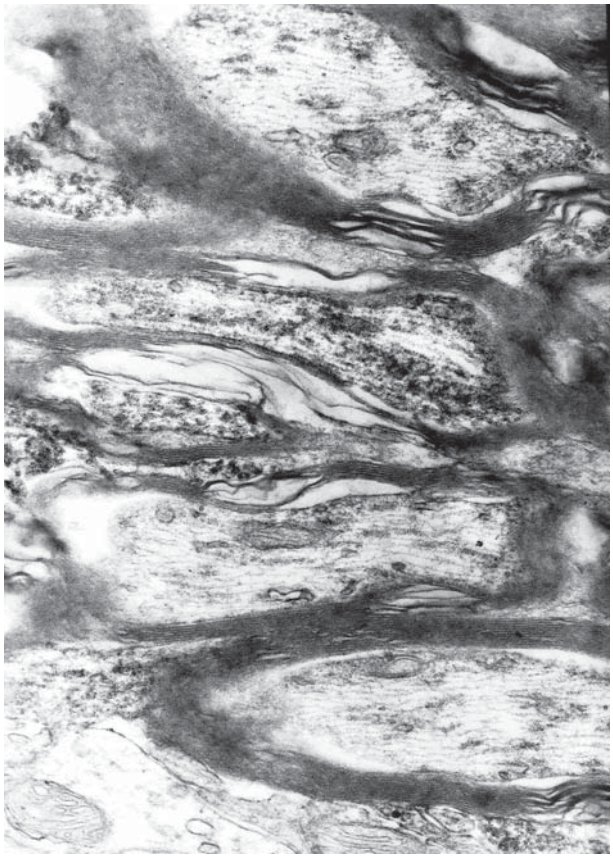

Fig. 6. SNAP-25, cerebral cortex. Immunolabeling of sacs and vesicles in myelinated axons. Adult rat. Primary magnification 30000.

profiles (Fig. 12) in the cerebral cortex of newborn and to a greater extent - in postnatal rats.

DISCUSSION

It is known that three synaptic proteins, synaptobrevin/VAMP, SNAP-25 and synataxin forming SNARE (Soluble NSF Attachment Protein) REceptor or core complex (6,7) are extremely important for the synaptic transmission. Their interaction is, actually, the final step of synaptic vesicle exocytosis. We were able to demonstrate immunocytochemically all these synaptic proteins. The number of synaptobrevin immunopositive axonal endings increased with increasing age of postnatal rats. Synaptic vesicles were observed in the axons probably transferred to the axonal endings or vise versa to the perikaryon.

The immunolabeling was mainly around the synaptic vesicle. Both other members of SNARE, SNAP-25, a peripheral, and syntaxin1, an integral membrane protein were visualized as well. The immunostaining was located predominantly on the cytoplasmic surface of the presynaptic membrane and to a lesser extent on the synaptic vesicle membrane (5).

The immunopositive reaction product at the visualization of NSF and α-SNAP is predominantly the cytosolic localization in presynaptic endings and varicosities. Alpha-SNAP binds to SNARE complex, which leads to the recruitment of NSF cytosolic ATPase dissociating the SNARE complex (1). The intensity of the immunostaining for these two cytosolic synaptic proteins increases with increasing age of postnatal rats. There is an increasing number of immunopositive axonal endings and varicosities, perikarya and other parts of neurons.

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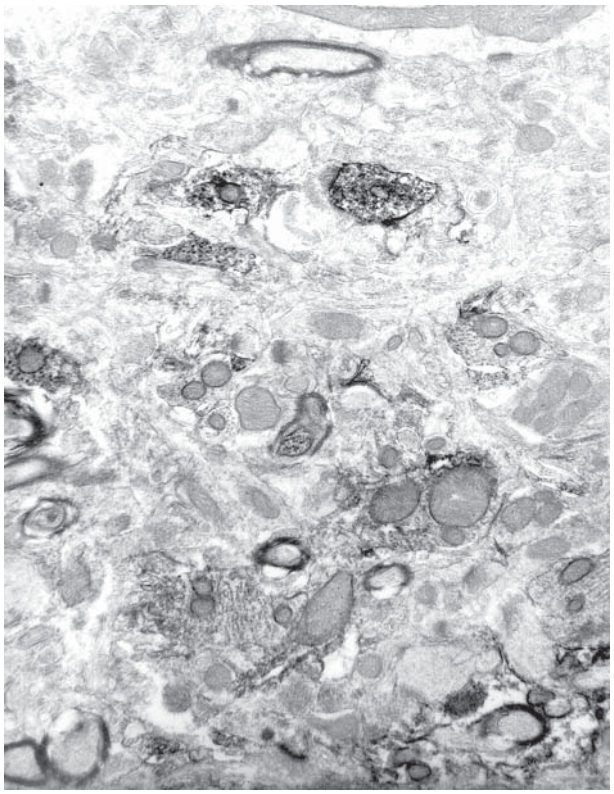

Fig. 7. NSF, cerebral cortex. Immunopositive material in the cytoplasm of neuropil profiles. Adult rat. Primary magnification 10000.magnification 30000.

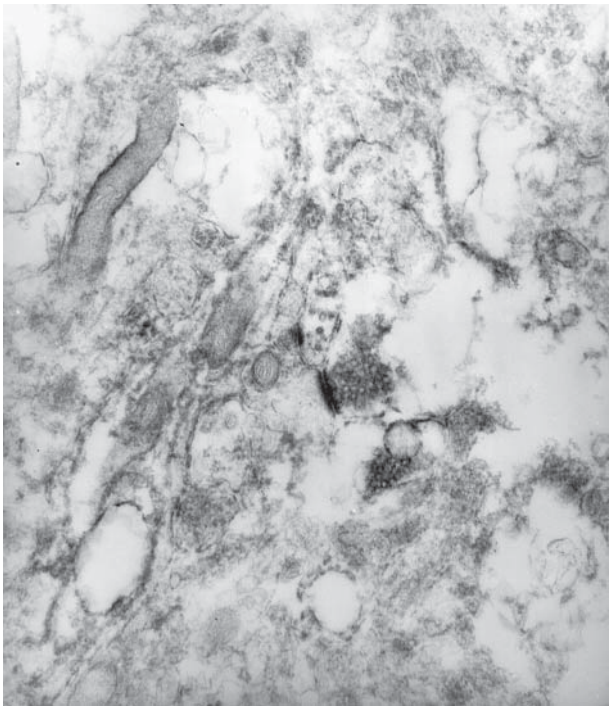

Fig. 9. NSF, cerebral cortex. Immunolabeling of the cytoplasm of presynaptic parts. 11th postnatal day. Primary magnification 25000.

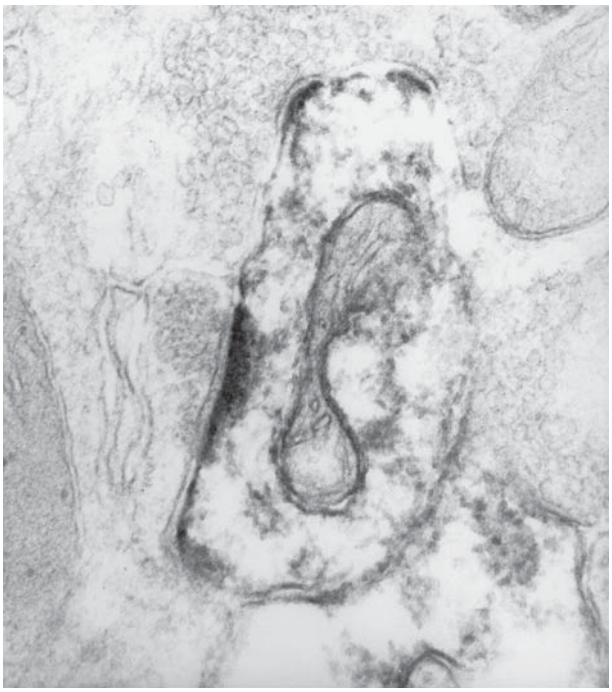

Fig. 8. NSF, cerebral cortex. Immunolabeling of the postsynaptic part. Adult rat. Primary magnification 40000.

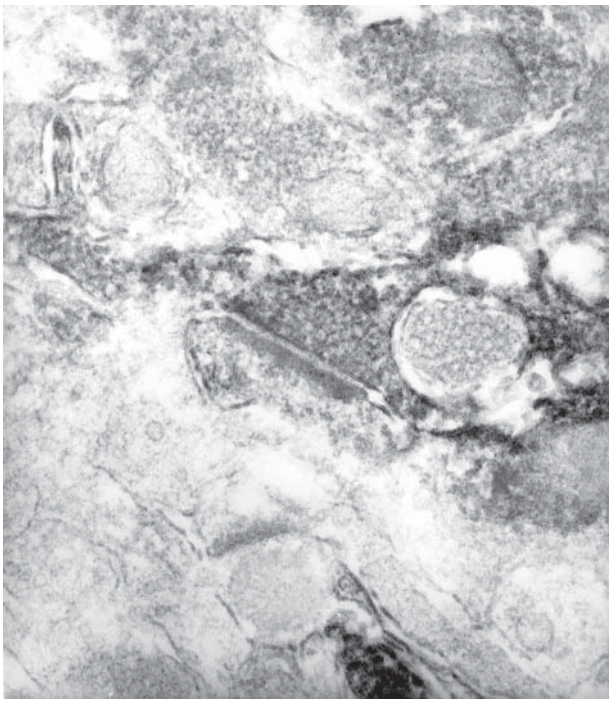

Fig. 10. α-SNAP, cerebral cortex. Immunolabeling of the axoplasm of axonal ending. Adult rat. Primary magnification 50000.

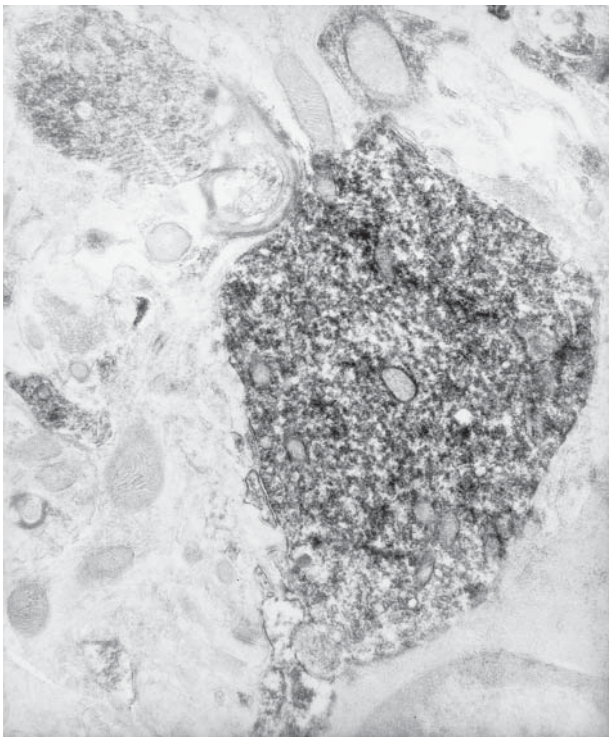

Fig. 11. α-SNAP, cerebral cortex. Immunolabeling of the cytoplasm in the perikaryon. Adult rat. Primary magnification 12500.

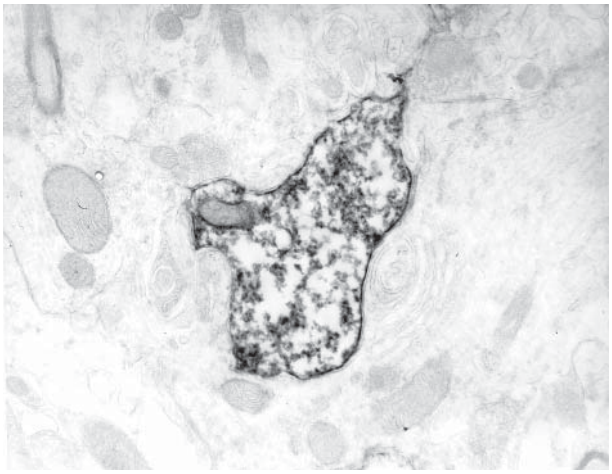

Fig. 12. α-SNAP, cerebral cortex. Immunolabeling of the cytoplasm in the denditic profiles. Adult rat. Primary magnification 16000.

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