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Terminals of Antennal Receptor Cells in the Antennal Lobe of the Butterfly, *Pieris rapae* L.(Insecta, Lepidoptera)

Bong-Hee Lee and Woo-Kap Kim*

Department of Biology, Soonchunhyang University, Onyang, Chungnam 336-600;
**Department of Biology, Korea University, Seoul 136-701, Korea*

Five types of synapses were differentiated in the antennal lobe of *Pieris rapae*. They are in general axo-dendritic synapses and have symmetrical contacts. The type I synapses contain the uniformly round vesicles of medium size, and consist of the 11% of all synapses in the antennal lobe. The type II synapses are filled with the small flat and round vesicles which are densely arranged (19%). The type III synapses possess medium-sized round vesicles and dense core ones(24%). The type IV synapses are characterized by such three kinds of vesicles as small round, small flat and dense core vesicles(33%). The type V synapses exhibit the presence of medium-sized round, large round and dense core vesicles(13%).

The removal of the left antenna on its proximal portion caused the type IV boutons of the above five types in ipsilateral antennal lobe to turn into dark or semidark degenerative changes. Therefore, it was concluded that many of the antennal receptor cells projected into the brain terminate in the antennal lobe to form type IV synapses together with the dendrites.

KEY WORDS: Axo-dendrite synapses, Antennal receptor, Butterfly.

In insects, some of axon fibers of the receptor cells which enter the brain from the antenna have been known to terminate in the ipsilateral antennal lobe (Strausfeld, 1976; Camazine and Hildebrand, 1979), while others run across antennal lobe to terminate in areas out of it (Ernst *et al.*, 1977; Mobbs, 1982). According to a report in *Apis mellifera* (Mobbs, 1982), an axon fiber complement of the antennal receptor cells can be divided into six major bundles on entry into the brain. Of six antennal receptor fiber bundles, three bundles terminate in the 100 or more glomeruli of the antennal lobe that represent their destinations. It has been also reported that in addition to the above-mentioned antennal receptor cells, the intrinsic interneurons, the extrinsic output and feedback cells organize the structural architectures of many glomeruli in the antennal lobe (Mobbs, 1985).

These reports have indicated that in the antennal lobe the complicate synapses must be formed between various nerve cells including the antennal receptor cells. Although the synaptic organizations in the antennal lobe of the insects were described in *Manduca sexta* (Tolbert and Hildebrand, 1981) and *Locusta migratyoria* (Schürmann and Weschler, 1970), they are not almost known in the other insects.

Furthermore, in spite of a report on light microscopical structure of deutocerebrum including antennal lobe with experimental degeneration of the antennal receptor terminal in *Locusta migratoria* (Ernst *et al.*, 1977), investigation regarding electron microscopical structure of synaptic organization formed by antennal receptor terminal in the antennal lobe was not available besides a brief suggestion by Kim and Lee (1986).

The present investigation was undertaken first to observe different types of synaptic organizations in

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the antennal lobe of *Pieris rapae,* then to examine the ultrastructure of degenerated antennal receptor terminals in the ipsilateral antennal lobe following the removal of an antenna on its proximal portion, and finally to identify which type of synapse is constituted in the antennal lobe by the axon terminal of the receptor cell projected from the antenna.

Materials and Methods

The insects used for the present work were one-day-old cabbage butterflies (*Pieris rapae* L.), including both males and females, which were collected from the stock colonies maintained at the laboratory of Korean Entomological Institute in Korea University.

For electron microscopy of normal synaptic organizations in the antennal lobe, the general anesthesia of the butterflies was performed in keeping them at a cool place of 1-4°C for about 30 min. Thereafter, the heads were cut off from cabbage butterflies with a razor blade and prefixed in 1% paraformaldehyde-1% glutaraldehyde at 4°C for 1 hr. After being prefixed for 1 hr., the heads were dissected under the steroscope for getting the brains. The dissected brains continued to be kept in a new prefixative at a cool place overnight for completing the fixation, followed by being washed in 0.4M phosphate buffer, pH 7.4, containing 8% glucose and 0.5% $CaCl_2$, and postfixed in 2% OsO_4 in phosphate buffer at 4°C for 2 hrs. After being washed in 0.4M phosphate buffer, they were dehydrated in a graded concentration of ethanol and in acetone, and embedded in araldite mixture. The fourteen brains were trimmed with a LKB ultrotome V so that only antennal lobe was cut into the ultrathin sections and stained with uranyl acetate and lead citrate.

For electron microscopy of the degenerated boutons in the ipsilateral antennal lobes following the removal of left antennae on its proximal portion, these were experimentally cut off left antennae on its proximal portion, these were experimentally cut off from one-day-old cabbage butterflies. The butterflies, whose left antennae were experimentally cut off, survived one day. After that, the butterflies are treated as descrived above. The seventeen brains were trimmed with a LKB ultrotome V so that only ipsilateral antennal lobes were cut into ultrathin serial sections, which then stained with uranyl acetate and lead citrate. The stained ultrathin sections were finally examined with Jeol CX-II transmission electron microscope at 80 kv.

Results

Five Types of Synapses in the Antennal Lobes

We used the following 5 bases for discriminating many synaptic organizations into five types of synapses in the antennal lobe: (1) the size and (2) the shape of the clear synaptic vesicles and (3) the frequency of occurrence of dense core vesicles in the presynaptic frequency of occurrence of dense core vesicles in the presynaptic terminals. (4) The symmetrical or asymmetrical thickening of the synaptic membrane and (5) the length of the pre- and postsynaptic membranes were also evaluated.

The type I synapse has the uniformly round vesicles of medium size (average 31.3 $\pm$4.8 nm in diameter) which are densely arranged in the bouton. The type I bouton is medium-sized or small and undergo symmetrical contacts either with only dendrite or with both dendrite and axon as shown in Figure 2. The type I synapses make up the smallest ratio in all synapses which occupy the antennal lobe (about 11%).

The type II synapsis is axo-dendritic synapse formed by a large or medium-sized bouton which is filled with the flat (long axis $53.2\pm6.5\pm$short axis 15.7 ± 6.8 nm) and round vesicles(20.4 ± 5.6 nm) of small size (Figs. 2,3). The synaptic contacts of the type II bouton are undergone with a single small or a few dendrites. The type II synapses constitute approximately 19% of all synapses in the antennal lobe.

The axo-dendritic type III synapse is characterized by large or medium-sized boutons containing medium-sized round vesicles (29.8 ± 5.6 nm) and dense core vesicles which are densely arranged (Fig. 4). The type III bouton makes symmetrical contacts with more than one small dendrite. The type III boutons have dense core vesicles in a small number, compared with many clear vesicles included. The frequency in the antennal lobe of type III synapses occurs approximately 24%.

The type IV synapse is formed by symmetrical contacts between a medium-sized or small bouton and more than a small dendrite except in Figure 4 in which two small type IV boutons undergo symmetrical contacts with each single small dendrite. The type IV bouton is filled up with three kinds of small round (20.3±5.2 nm), small flat (53.5±5.4X15.8±5.7 nm) and dense core vesicles (Figs. 4,5,6). The dense core vesicles are included in a small number. The type IV synapses constitute the largest ratio in all synapses which occupy the antennal lobe (approximately 33%).

The type V synapse is axo-dendritic synapse fomred by a medium-sized bouton in which three kinds of medium-sized round (30.7±7.2 nm), large round (56.4±7.4 nm) and dense core vesicles are densely packed in the axoplasm (Figs. 5,6). The type V bouton makes symmetrical contacts with a few small dendrites. Within this bouton the medium-sized round vesicles are in general greater in number than the large round vesicles. The frequency in which type V synapse occurs in the antenhal lobe is approximately 13%.

Axon Terminal Degeneration in the Antennal Lobe after Removing the Antenna

In order to determine which type of synapse out of the five synapses escribed above is formed by the axon terminals of the receptor cells that terminate in the antennal lobe from the antenna, the seventeen brains were fixed one day after being experimentally cut off the left antennae from the butterflies. The ipsilateral antennal lobes consist ultrastructurally of the neuropiles in which many boutons show degenerative alteration. The degenerated boutons, if discriminated according to the classification of synapses mentioned above, belong to type IV boutons.

Some of the degenerated type IV boutons show dark degeneration. Figure 8 exhibits such a darkly degenerated type IV bouton undergoing symmetrical contacts with two small dendrites which are not altered. Many of the clear synaptic vesicle have degeneratively altered to amorphous substance in dark axoplasm, although some of them are still preserved without alteration, especially near the presynaptic membranes. The dense core vesicles which are sparsely distributed in dark axoplasm of this bouton are undergoing a degenerative change, with dense core remaining almost unchanged but limiting membranes already changed to vanish from the vesicles.

The mitochondria embedded in dark axoplasm show degenerative changes in their crista structures, indicating that they should be in an earlier stage of degenerative alteration. The darkly degenerated type IV boutons. The remaining degenerated type IV boutons excluding the darkly degenerated type IV boutons in the ipsilateral antennal lobe have degenerated to a weakly-dark or semi-dark extent.

Several semi-darkly degenerted axons appear around a darkly degenerated axon in the center of Figure 8. Figure 9 shows a type IV bouton which has also undergone semi-dark degeneration. But the two type II boutons are preserved without alteration.

The weakly degenerated boutons of type IV are shown in Figure 10 and 11. The type IV boutons which have undergone weak degeneration in Figure 10 and 11 have increased in number synaptic vesicles in electron dense axoplasm. But the synaptic vesicles occupying these boutons are at the beginning of degenerative change, because three kinds of small round, small flat and dense core vesicles can be still distinguished in semi-dark axoplasm. A mitochondrion which appears in the right weakly-dark type IV bouton in Figure 11 have been degeneratively altered to such an extent that the normal crista structure could not be easily identified within it. It is also found that a weakly degenerated type IV bouton of Figure 11 undergoes symmetrical contacts with seven small dendrites along the synaptic membrane of it.

Discussion

The antennal lobe of the insects into which many of antennal receptor cells project their axons have complicate neuronal pathways as described in some insects (Tolbert and Hildebrand, 1981; Schürmann and Weschler, 1970). All the synapses which make complicate neuronal connections in the antennal lobe of *Pieris rapae* could be classified into five types on several morphological criteria, which means that the five types of neurons including antennal receptor cells innervate the antennal lobe. It may be regarded that there is a difference in the number of neurons which innervate the antennal lobe among the species of insects, although the four kinds of neurons have been reported to innervate the antennal lobe in *Periplaneta americana* (Ernst and Boeckh, 1983).

Both the axo-somatic synapses and the axo-spinous synapses (Chung *et al.*, 1976; chung *et al.*, 1977; Hassler *et al.*, 1978), which can be frequently found in the brains of the vertebrates, were not found in the antennal lobe of *Pieri rapae*. Therefore, many of the synapses observed in the antennal lobe of it were axo-dendritic synapses which were in general made between an axon terminal and a small dendrite in a synapse. The synaptic contacts are characterized by not only the symmetrical thickenings between presynaptic and postsynaptic membranes but also their short length in almost all synaptses.

Therefore, the decisive criteria to classify all the synapses into the five types in the antennal lobe were made in terms of (1) the size and (2) the shape of the clear synaptic vesicles, (3) the presence of dense core vesicles within the axon terminals and (4) the size of axon terminals with the synaptic vesicles.

A large number of the interneurons in the antennal lobe have been reported to have numerous dendrites in *Manduca sexta* (Matsumoto and Hildebrand, 1981) and *Aceta domestica* (Schildberger, 1982). Ernst and Boeckh (1983) reported that there are three kinds of interneurons in the antennal lobe of *Periplaneta americana*. It is assumed from these reports that many of the dendrites in the five types of synapses in the antennal lobe of *Pieris rapae* are the ones which project from the interneurones in the antennal lobe, and that most of the complicate neuronal pathways in the neuropiles of antennal lobe are fomed by projections from these interneurons. This assumption is in accordance with an interpretation of Boeckh *et al.* (1984) on the central antennal pathway in *Periplaneta americana*.

We found that only one type of boutons out of the five types undergoes degenerative changes in the ipsilateral antennal lobe following the experimental removal of a left antenna in *Pieris rapae*. The type of boutons which showed degenerative alteration is type IV in this experiment. Therefore, an antennal receptor terminal projecting from the antenna into the ipsilateral antennal lobe forms type IV synapses with a few dendrites. But we could not find origins of the dendrites which form type IV synapses. Although Ernst and Boeckh (1983) proposed in schematic representation of central antennal pathway in *Periplaneta americana* that some of the dendrites which undergoes synaptic contacts with antennal receptor terminals should project from three kinds of interneurons, we must more efforts to determine the exact origins of the dendrites which undergo synaptic contacts with antennal receptor terminals in the antennal lobe of *Pieris rapae*. It can not be also excluded that an antennal receptor terminal may have a functional relationship with more than one nuron in the ipsilateral antennal lobe, because all the dendrites which form the five types of boutons show similarities in their morphological features.

It was evidenced from this experiment that antennal receptor cells terminate sparsely in the neuropiles of antennal lobe as shown in Figures 9, 10 and 11, but in groups in some areas of the neuropiles as shown in Figures 8. This evidence agrees with a report of Ernst *et al.* (1977), who observed the light microscopical structures of degenerated axon terminals in the ipsilateral antennal lobe after the experimental removal of an antenna in *Locusta migratoria*. It could be also evidenced with observation of degenerative alteration of many axon terminals from antennal receptor cells in this experiment that type IV synapses formed by antennal receptor terminals make up the largest ratio in all the synapses which occupy the antennal lobe of *Pieris rapae*.

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배추흰나비 뇌의 촉각엽에 뻗은 촉각지각신경세포의 축색종말

이봉희 · 김우갑*(순천향대학 이학부 생물학과; *고려대학교 이과대학 생물학과)

배추희나비 뇌의 촉각엽에 있는 신경세포들은 5가지의 신경연접을 형성하는 것으로 관찰되었다. 이 신경연접들은 모두 축색과 수상돌기간의 연접으로 대칭연접이었다.

第1型 神經連接은 축색종말내에 中等度크기의 圓形 連接小胞를 가지고 있었고, 촉각엽에 있는 5가지의 신경연접중 11%의 출현빈도수로 관찰되었다. 第2型 神經連接의 축색종말에는 長形과 圓形의 2가지 연접소포가 포함되어 있었는데, 모두 小形이었으며 이같은 연접소포들이 축색종말내에서 조밀하게 관찰되었다. 제2형 신경연접의 출현 빈도수는 19%이었다. 第3型 神經連接은 축색종말내에 中等度크기의 圓形 連接小胞와 DENSE CORE VESICLE을 포함하고 있었고 그 출현빈도수는 24%이었다. 第4型 神經連接은 축색종말내에 小形의 圓形, 小形의 長形 連接小胞 및 DENSE CORE VESICLE의 3가지 연접소포를 가지고 있었고 출현빈도수는 촉각엽에서 가장 큰 33%이었다. 第5型 神經連接은 축색종말내에 中等度크기의 圓形, 大形의 圓形連接小胞 및 DENSE CORE VESICLE을 포함하였고 13%의 출현빈도수로 관찰되었다.

배추흰나비의 촉각에 있는 지각신경세포가 뇌의 촉각엽으로 뻗어 들어가 위의 5가지 신경연접중 어느 형을 형성하는지를 관찰하기 위하여 좌측 촉각의 기부를 제거하여 지각신경세포를 절단하였는데 그 결과, 좌측 촉각엽에서 제4형의 신경연접이 퇴행성 변화를 나타내었다. 그러므로 촉각의 지각신경세포는 뇌의 같은 쪽 촉각엽에 뻗어와 제4형 신경연접을 형성한다고 결론되었다.

Fig. 1. Semithin section of an antennal lobe from the normal cabbage butterfly's brain showing many glomeruli(gl) compactly arranged with the nerve fibers. The antennal receptor cells are known to be projected into the antennal lobe. Bar: 10 μm.

Fig. 2. Electron micrograph of the antennal lobe containing type I and II synapses. Type I synapses have the uniformly round vesicles of medium size which are densely arranged in axoplasm, while type II synapse has the flat and round vesicles of small size. Type I boutons undergo symmetrical contacts with the dendrites(D). Bar: 1 μm.

Fig. 3. Three type II synapses undergoing symmetrical contacts with the dendrites(D) located between the three boutons. Bar: 1 μm.

Fig. 4. Type III and IV synapses in the antennal lobe containing dense core vesicles. Type III synapse has round vesicles of medium size including dense core vesicles, while type IV synapses possess three kinds of small round, small flat and dense core vesicles. Take a note of the strong symmetrical contacts of type III and IV boutons with the small dendrites(D). Bar: 1 μm.

Fig. 5. Electron micrograph of the antennal lobe showing type IV and V synapses. The type V synapse has three kinds of medium-sized round, large round and dense core vesicles in the upper part. The type IV and V boutons make symmetrical contacts with the dendrites(D). Bar: 1 μm.

Fig. 6. Type IV and V synapses in the antennal lobe containing many dense core vesicles. The type IV synapse has large dense core vesicles including the clear vesicles which are loosely arranged, while type V synapse has small dense core vesicles as well as clear vesicles which are densely packed. D, dendrites. Bar: 1 μm.

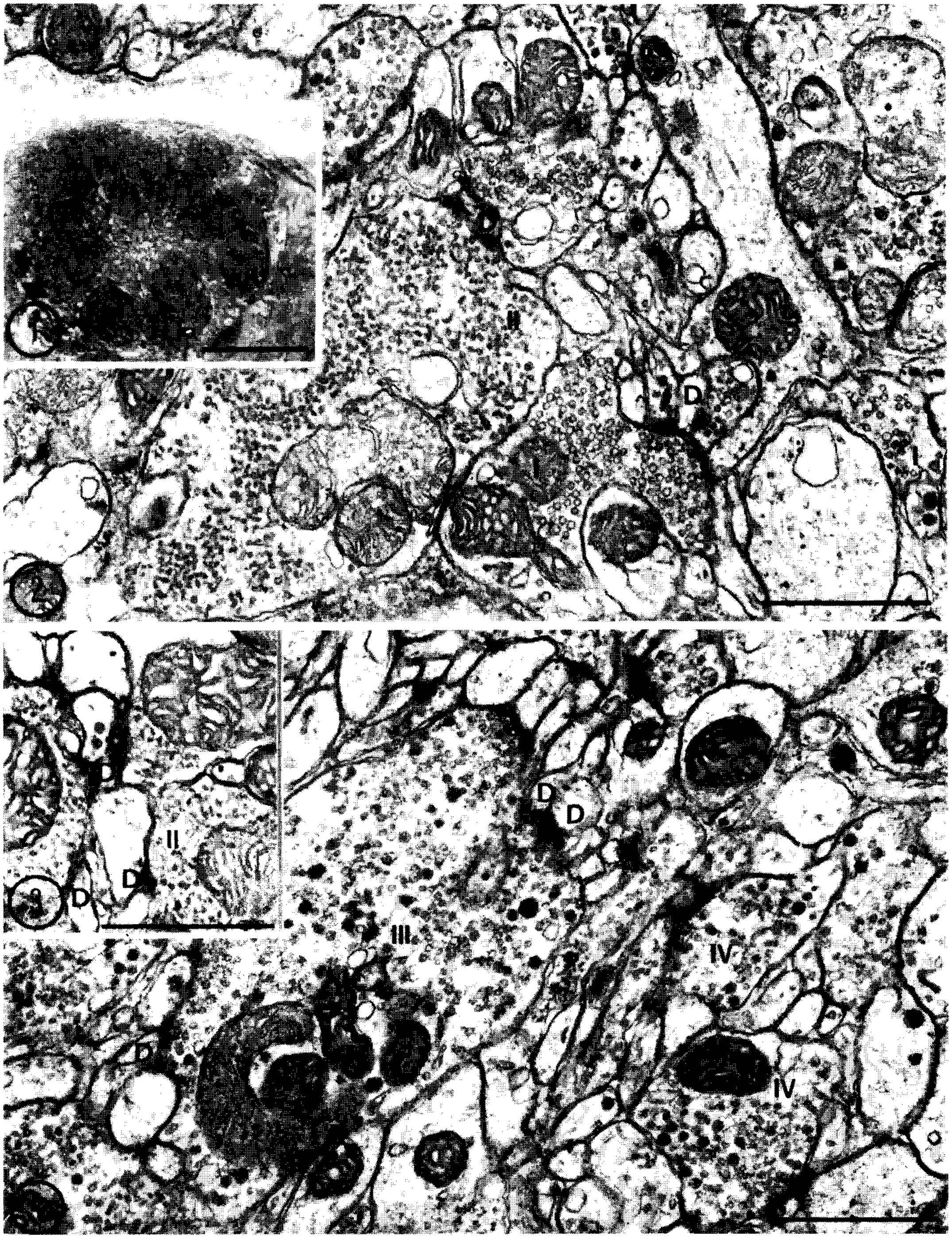
Fig. 7. Semithin section of the ipsilateral antennal lobe from a cabbage butterfly with the experimental removal of the left antennal on the level of its proximal portion. One day after the left antenna was cut off, the brain was fixed to be examined with a electron microscope. Bar: 10 μm.

Fig. 8. A darkly degenerated type IV bouton undergoing symmetrical contacts (small arrows) with the dendrites. A small number of the celar synpatic vesicles which appear near the synaptic contacts of the darkly degenerated bouton are still preserved without alteration in the dark axoplasm. Around the darkly degenerated axon the semi-darkly degenerated nerve fibers, perhaps the antennal receptor fibers, are located. III, type III bouton; V, type V bouton. Bar: 1 μm.

Fig. 9. Electron micrograph of the ipsilateral antennal lobe following the removal of the left antenna. The type IV bouton, which makes symmetrical contacts (small arrow) with a dendrite, was semi-darkly degenerated, but the two type II boutons are preserved without alteration. II, type II boutons. Bar: 1 μm.

Fig. 10. A weakly degenerated type IV bouton undergoing several contacts with the dendrites. A profile of the other weakly degenerated bouton, which is assumed to be type IV, appear in the left part. V, type V bouton. Bar: 1 μm.

Fig. 11. Electron micrograph of the antennal lobe containing a weakly degenerated type IV bouton which undergoes symmetrical contacts with several lined dendrites and the other weakly degenerated type IV bouton which makes symmetrical contact with a dendrite. V, type V bouton. Bar: 1 μm.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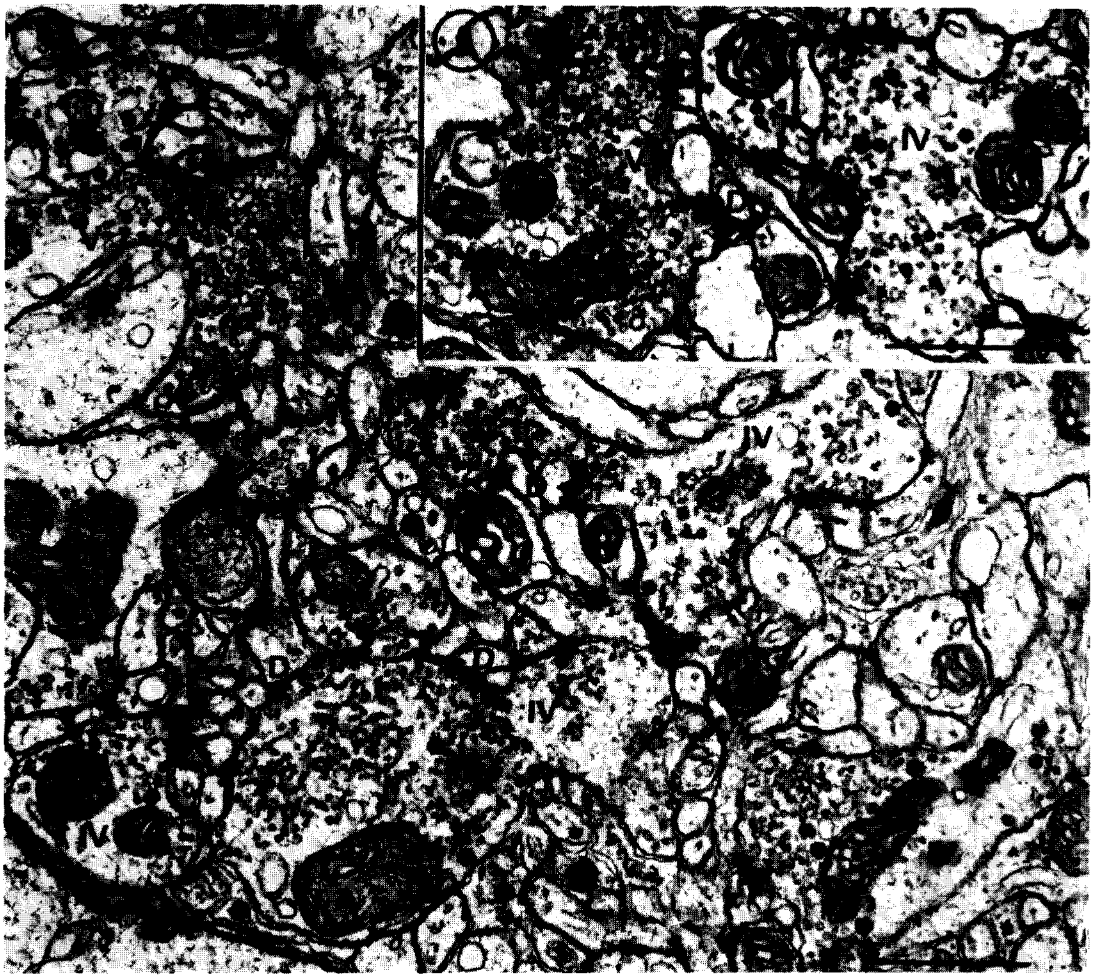

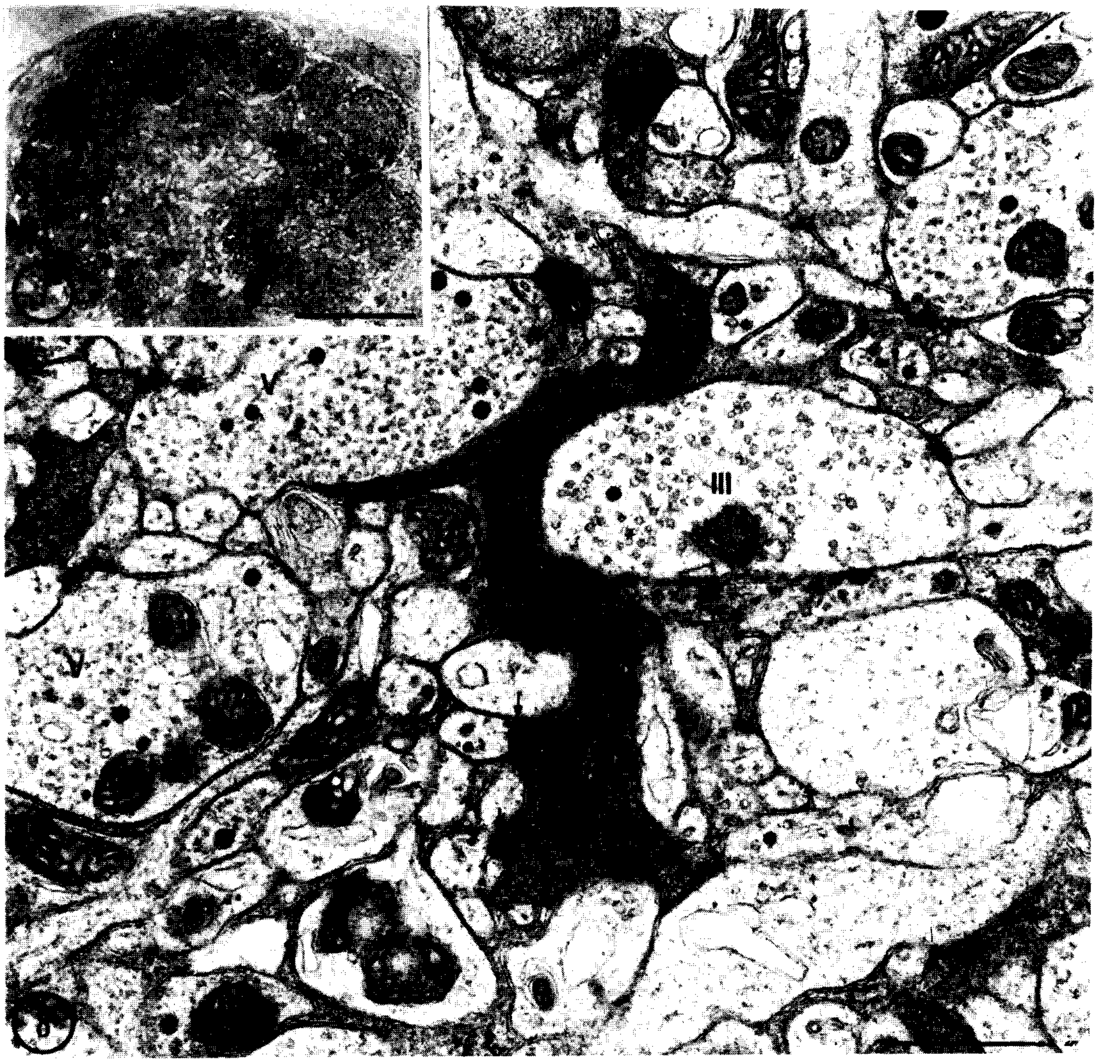
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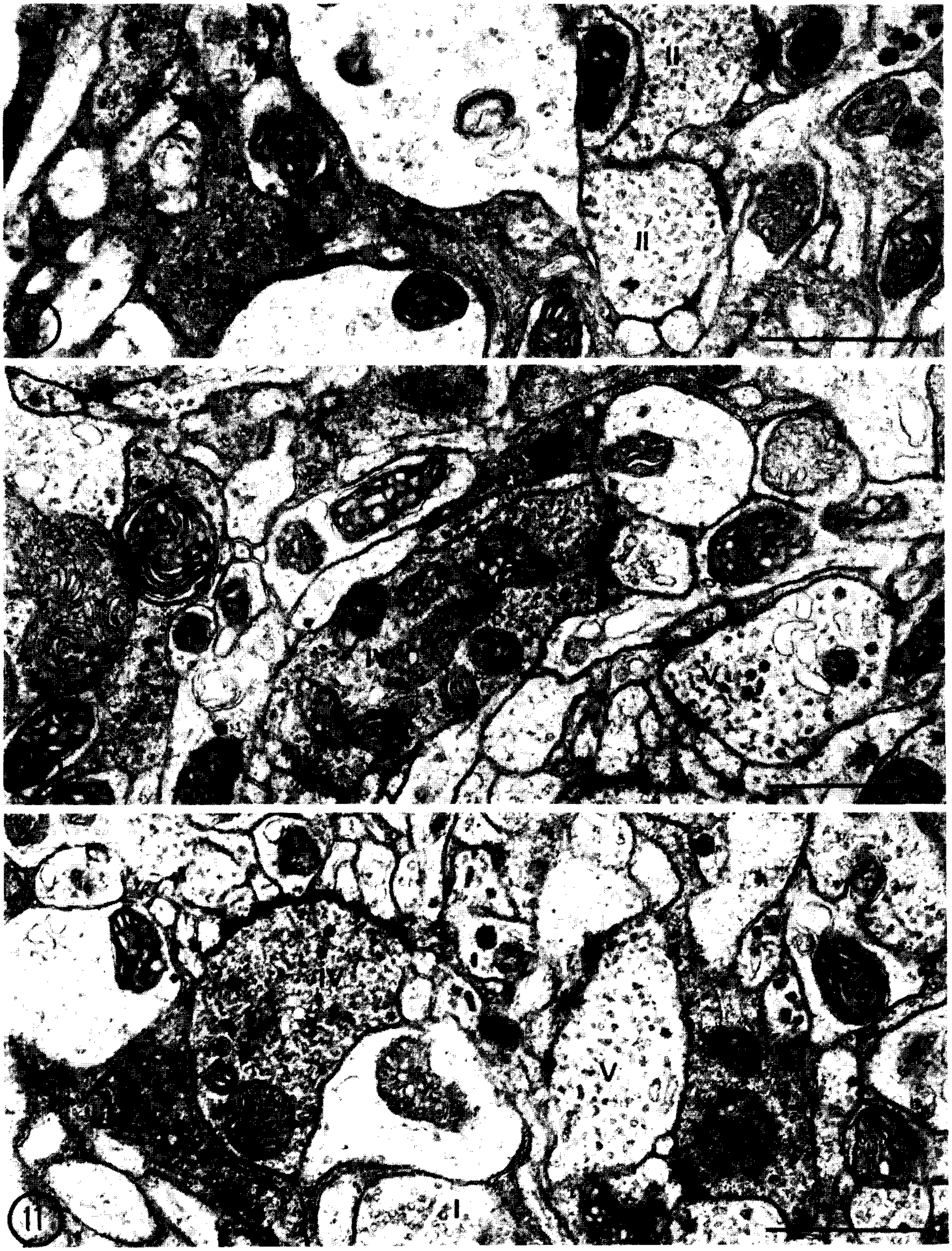
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