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Termination of Antennal Receptor Cells in Subesophageal Ganglion of Cabbage Butterfly *Pieris rapae* (Insecta, Lepidoptera)

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This study has been performed to investigate normal synaptic organizations in the subesophageal ganglion and termination of antennal receptor cells in the ipsilateral subesophageal ganglion of *Pieris rapae*. The various normal synaptic organizations in subesophageal ganglion could be differentiated into the five types. The proximal removal of a left antenna resulted in the weakly-dark, semidark and dark degenerations in the type I boutons of the ipsilateral subesophageal ganglion. Therefore, it was concluded that the axon terminals of the receptor cells projecting from the antenna into the brain form the type I synapses together with the dendrites in the ipsilateral subesophageal ganglion.

KEY WORDS: **Antennal receptor cells, Subesophageal ganglion, Synaptic organization, Experimental degeneration, Neuronal connection**

Suzuki (1975) and Mobbs (1982) reported that the antennal receptor fiber complement divides into a series of strands upon entry into the brain in honeybee. Suzuki (1975) also described three groups of fibers (Tracts 1-3) that can be traced into the glomeruli of the antennal lobe.

It was also reported in cabbage butterflies that some of the antennal receptor fibers terminate in the antennal glomeruli (Lee and Kim, 1988). According to the description of Suzuki (1975) and Mobbs (1982), the antennal receptor fibers innervated to the antennal glomeruli were often assumed to represent the chemosensory receptors of the antennae.

In the honeybee (Suzuki, 1975) three other groups of the antennal receptor fibers (T4-5) bypass the antennal lobe ventrally. The two bundles (T5 and T6) project to the dorsal lobe while one bundle (T4) terminates separately behind the lobe. Kim *et al.* (1990) also reported that some of the antennal receptor fibers are innervated to the dorsal lobe in cabbage butterfly. Suzuki (1975) indicated that in the honeybee some fibers of the T6 division leave the dorsal lobe and project into the subesophageal ganglion.

This study was performed first to investigate various types of synapses in the subesophageal ganglion of the cabbage butterfly, then to find presence of degenerated antennal receptor terminals in the ipsilateral subesophageal ganglion one to six days after the removal of an antenna, and finally to identify which type of synapsis is formed in the ipsilateral subesophageal ganglion by the axon terminal of antennal receptor cell.

Materials and Methods

The one-day-old cabbage butterflies, *Pieris rapae* L. collected from the stock colony of Soonchunhyang University, were used in this experi-

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ment. At temperatures of 28–30°C and relative humidities of 50–60%, the fifth instar larivae became adults ten days later.

For electron microscopy of normal synaptic organizations in subesophageal ganglion, the general anesthesia of the one-day-old cabbage butterflies was performed in keeping them at 4°C for 30 min or more. The heads of cabbage butterflies were cut off with the sharp scissors and prefixed in 1% paraformaldehyde – 1% glutaraldehyde at 4°C for 30 min to 1 hr. The heads were dissected under the stereoscope to isolate the intact brains. The isolated brains were continually put in new prefixative solution at 4°C overnight to complete the prefixation. They were washed three times in 0.4 M phosphate buffer, pH 7.4, containing 7% glucose and 0.5% $CaCl_2$, followed by postfixation in 2% OsO_4 in phosphate buffer at 4°C for 2 hr. After being washed three times, they were dehydrated in graded concentration of ethanol and in acetone, and embedded in araldite mixture. The embedded brains were trimmed with an ultramicrotome so that only subesophageal ganglion could be cut into the ultrathin sections. The sections were stained in uranyl acetate and lead citrate and finally examined with the electron microscope.

For electron microscopy of the degenerated boutons in the ipsilateral subesophageal ganglia following the removal of the left antennae, the proximal portions of the left antennae of one-day-old cabbage butterflies were experimentally cut. The cabbage butterflies, in which the left antennae were removed, were maintained in rear cage to survive one to six days. Thereafter, the butterflies were treated as described above in order to examine ultrastructures of the ipsilateral subesophageal ganglia.

Results

Normal Synaptic Organizations in Subesophageal Ganglion

The normal synaptic organizations in the subesophageal ganglion could be classified into five types on the bases of size and shape of the synaptic vesicles, occurrence of dense core vesicles in presynaptic terminal, and the symmetrical or asymmetrical thickenings of the synaptic membranes, etc.

The type I synapses contained medium-sized (average 30 nm) round, large (average 45 nm) round and dense core vesicles in medium-sized boutons (Figs. 2, 3). They occupied about 18% of all the synapses in subesophageal ganglion. The type I boutons made symmetric contacts with the dendrites. As shown in Figs. 4 and 5, the type II synapses had small (average 20 nm) round and medium-sized round vesicles, and small number

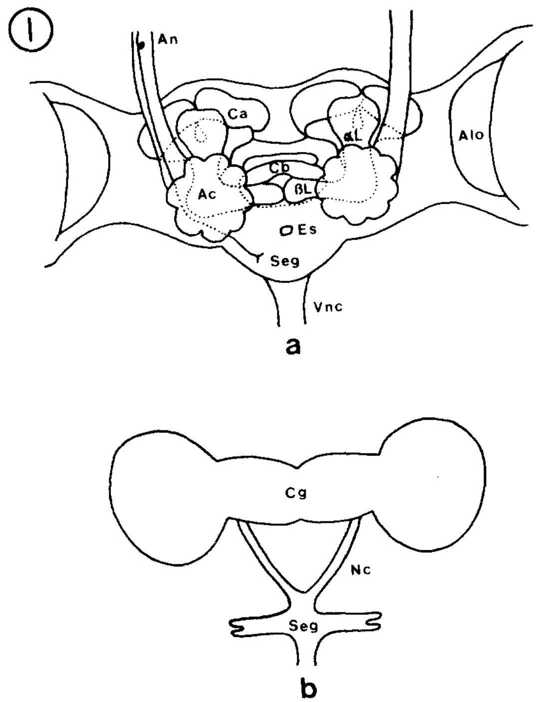
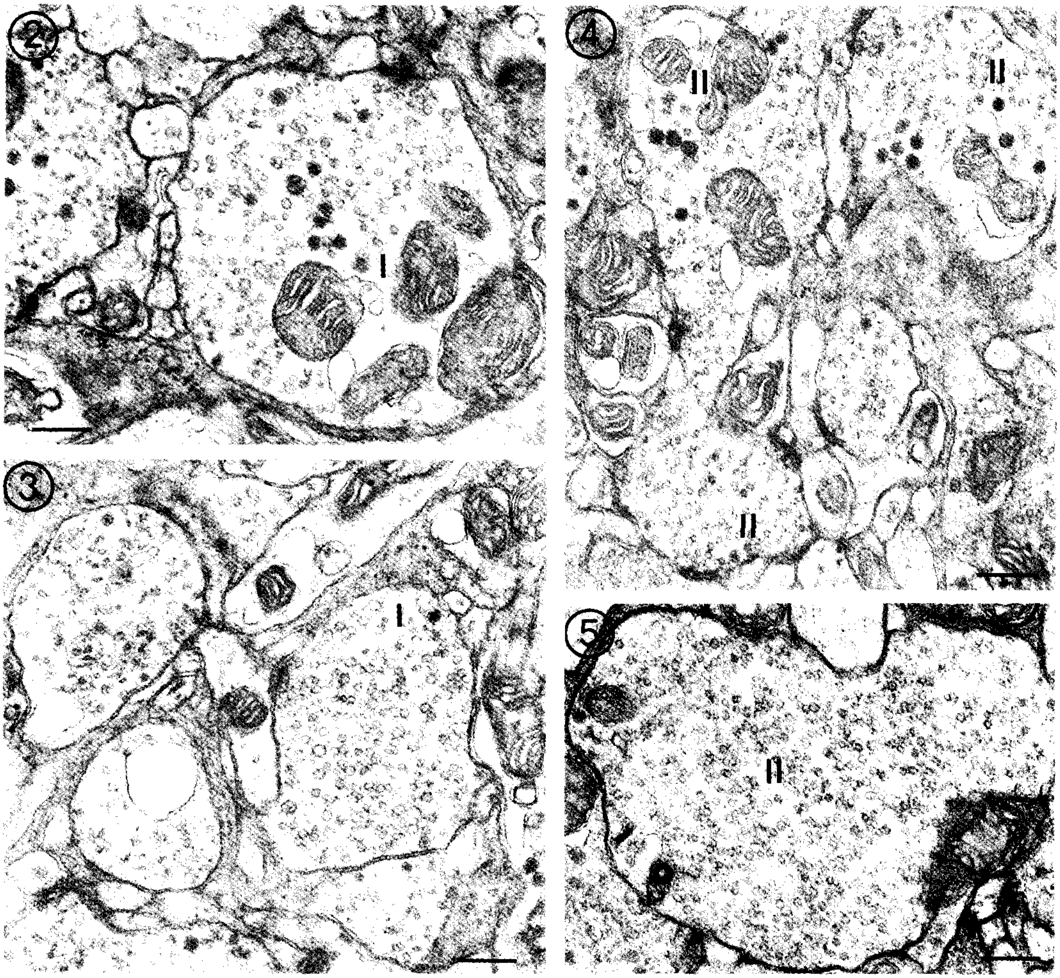


Fig. 1. The brains of the adult and larva of cabbage butterflies, *Pieris rapae*. (a) The internal brain structure of the adult indicating the projection of the antennal receptor fiber into the ipsilateral subesophageal ganglion (Seg). (b) The subesophageal ganglion of the larva lying ventral and posterior to the cerebral ganglion (Cg). When the larva enters the prepupal state, the nerve connectives (Nc) between cerebral ganglion and subesophageal ganglion gradually shorten. The subesophageal ganglion assumes a more anterior position, eventually incorporated into the ventral portion of the cerebral ganglion during metamorphosis. Ac, antennal center; αL, alpha lobe; Alo, anterior lobula; An, antennal nerve; βL, beta lobe; Ca, calyx of corpus pedunculatum; Cb, central body; Es, foramen for esophagus; Vnc, ventral nerve cord.

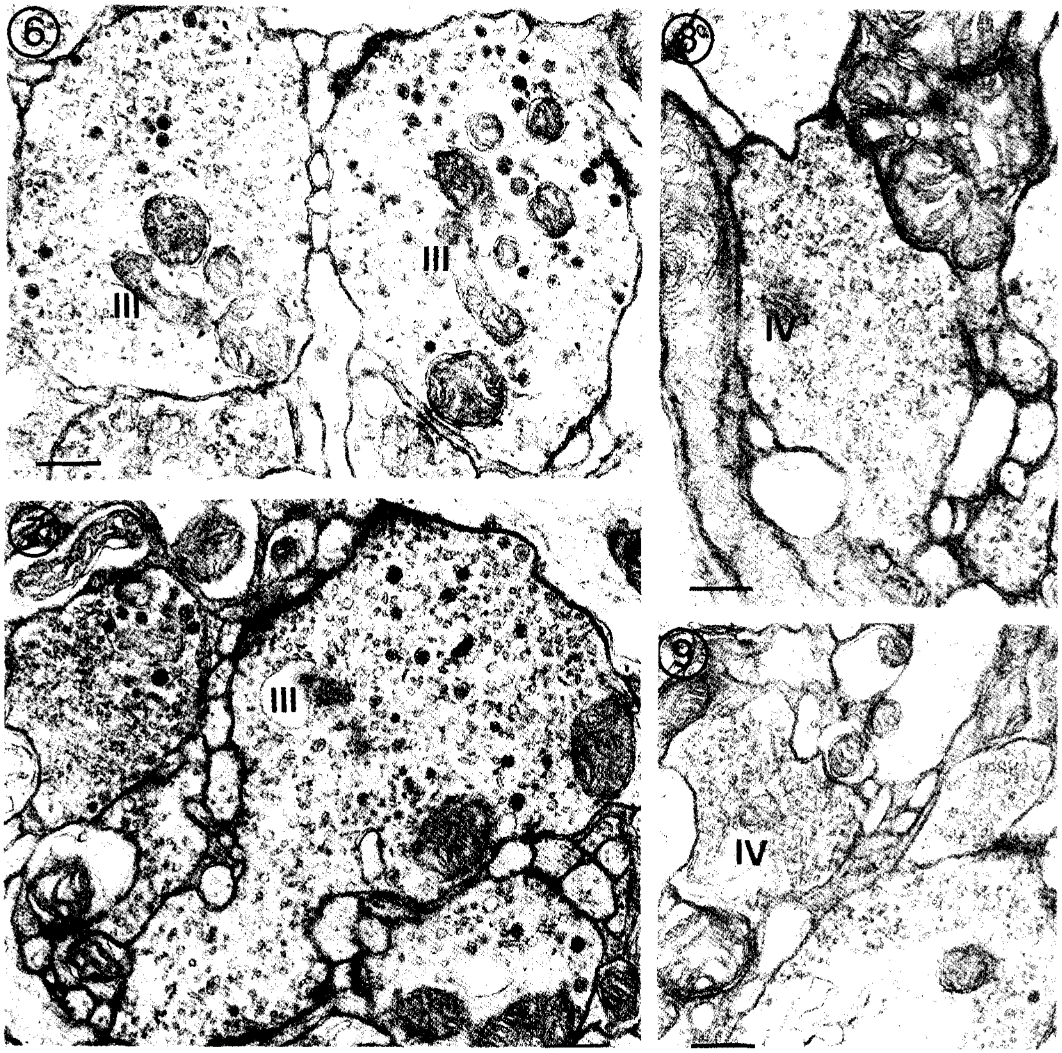


Figs. 2 and 3. Type I synapses in subesophageal ganglion containing medium-sized round, large round and dense core vesicles. The type I boutons undergo symmetric contacts with the dendrites. Bars indicate 2.8 μm.
Figs. 4 and 5. Electron micrographs of type II synapses in subesophageal ganglion showing small, medium-sized and dense core vesicles. A type II bouton in Fig. 5, which makes symmetric contacts with the dendrites, includes a few dense core vesicles. Bars indicate 2.8 μm.

of dense core vesicles in medium-sized boutons (about 11%). The type II boutons also formed symmetrical contacts with the dendrites (Fig. 4). The type III boutons, which underwent symmetric contacts with dendrites, included small and medium-sized round vesicles, small flat vesicles, and dense core vesicles (Figs. 6, 7). The type III synapses constituted the largest ratio of all the synapses which occupied the subesophageal ganglion (about 51%). The type IV synapses possessed medium-sized round and flat vesicles, as shown in Figs. 8 and 9 (about 13%). The type IV boutons made symmetric contacts with the dendrites. The type V synapses contained smaller number of synaptic vesicles within less electron-dense boutons, which underwent the asymmetric contacts with the dendrites (Figs. 10, 11, 12). The type V synapses occupied the smallest ratio (approximately 7%) of all the synapses.

Antennal Receptor Terminal Degeneration in Ipsilateral Subesophageal Ganglion after the Removal of the Left Antenna.

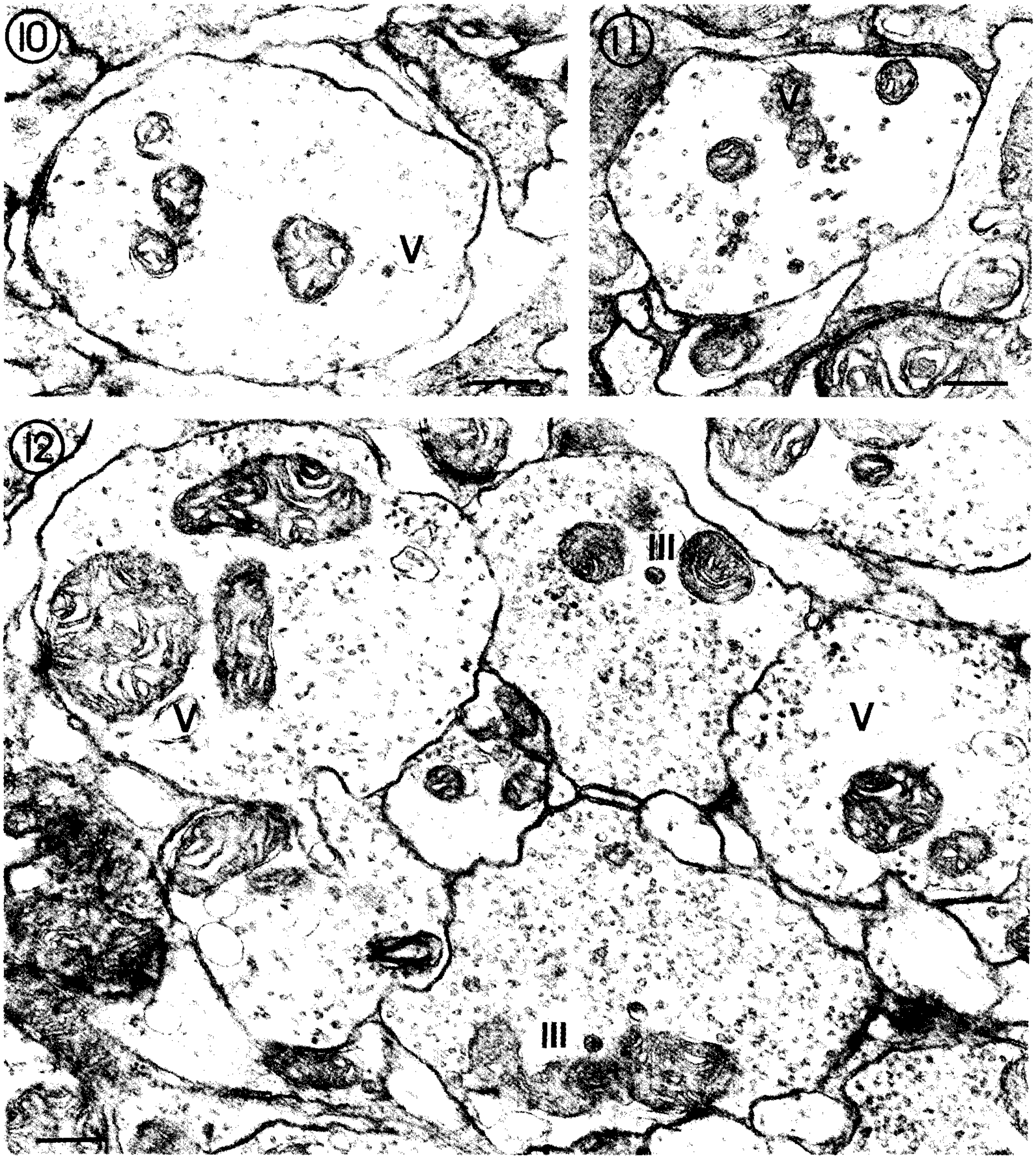
The ipsilateral subesophageal ganglia of eleven



Figs. 6 and 7. Type III synapses in subesophageal ganglion including small and medium-sized round, small flat, and dense core vesicles. The type III boutons undergo symmetric contacts with the dendrites. Bars indicate 2.8 μm.
Figs. 8 and 9. Electron micrographs of type IV synapses in subesophageal ganglion containing many flat vesicles. The type IV boutons have also medium-sized round vesicles. The medium-sized type IV boutons make symmetric contact with the dendrites. Bars indicate 3 μm in Fig. 7 and 2.8 μm in Fig. 8.

brains following the removal of the left antennae were examined with the electron microscope to determine whether the antennal receptor fiber terminals were innervated to the ipsilateral subesophageal ganglion or not, and also which type of synapsis out of the five synapses described above was formed by the antennal receptor fiber terminals. The ipsilateral subesophageal ganglia contained various types of degenerative alteration in many boutons. All the degenerated boutons, if discriminated according to the classification criteria mentioned above, belonged to the type I.

Many type I boutons showed weakly-dark degeneration four days after the removal of the antennae (Figs. 13, 14). These type I boutons, which exhibited weakly-dark degeneration, were filled up with medium-sized round, large round and small number of dense core vesicles. The type I boutons made symmetric contacts respectively with three dendrites in Fig. 13 and with

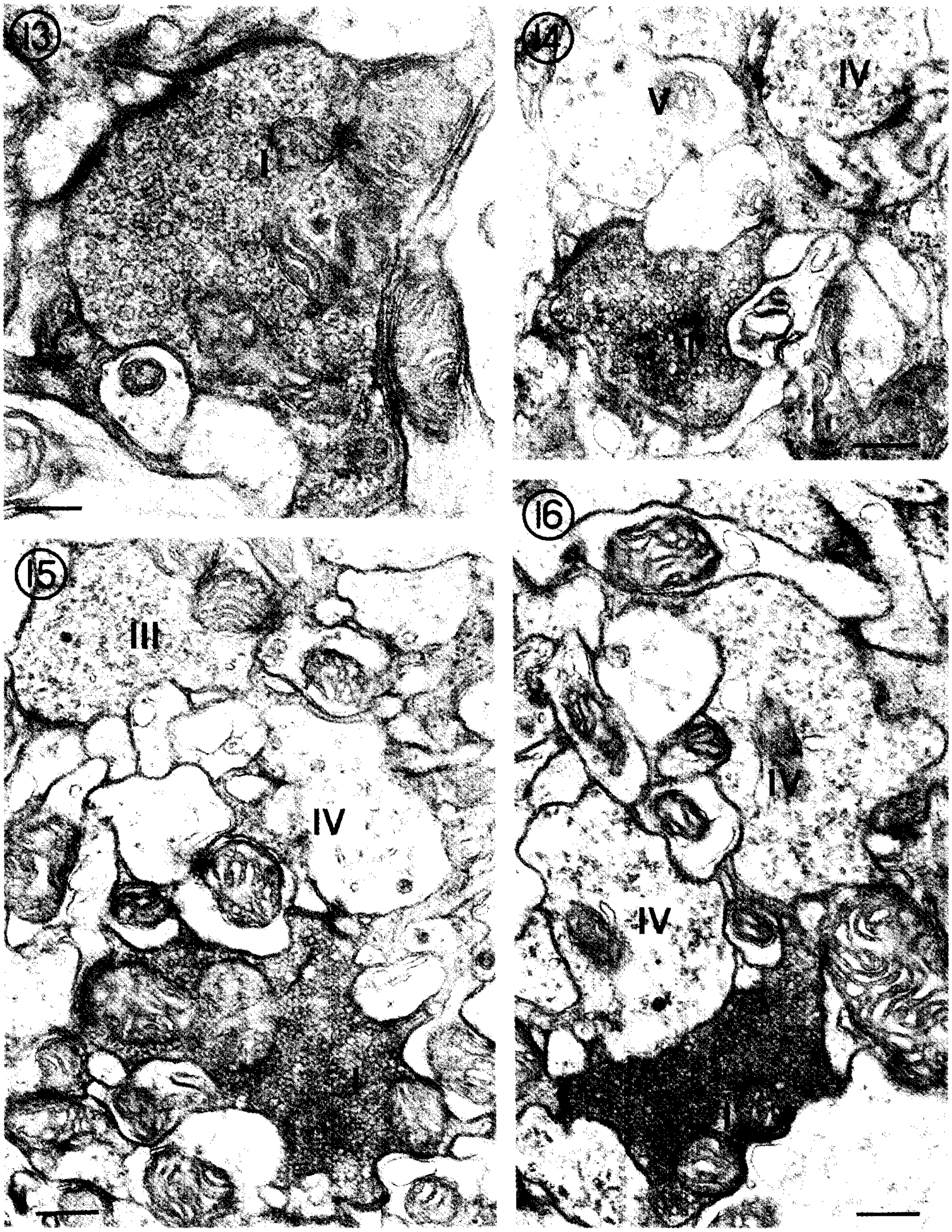


Figs. 10, 11 and 12. Type V synapses in subesophageal ganglion containing a small number of synaptic vesicles. The medium-sized type V boutons, which undergo asymmetrical contacts with the dendrites, include small round and dense core vesicles in their less electron-dense axoplasm. Bars indicate 2.8 μm.

several dendrites in Fig. 14. But the type IV and V synapses were not altered in Fig. 14.

As shown in Fig. 15, some of type I synapses were altered to the semidark degeneration. This degenerated bouton had very increased number of the synaptic vesicles in electron-dense axoplasm. Most of clear synaptic vesicles are presumed to have been shrunken in the bouton, perhaps in the process of fixation. The medium-sized clear synaptic vesicles constituted large number of the synaptic vesicles in the degenerated type I bouton.

Five days after the removal of the antennae, the ipsilateral subesophageal ganglia included many



Figs. 13, 14, 15 and 16. Type I synapses in the ipsilateral subesophageal ganglion four or five days after the proximal removal of a left antenna. The type I boutons show weakly-dark (in Figs. 13 and 14), semidark (in Fig. 15) and dark (in Fig. 16) degeneration. The other types of boutons are preserved without degenerative alterations. Bars indicate 3.2 μm in Fig. 13, and 2.8 μm in Figs. 14, 15 and 16.

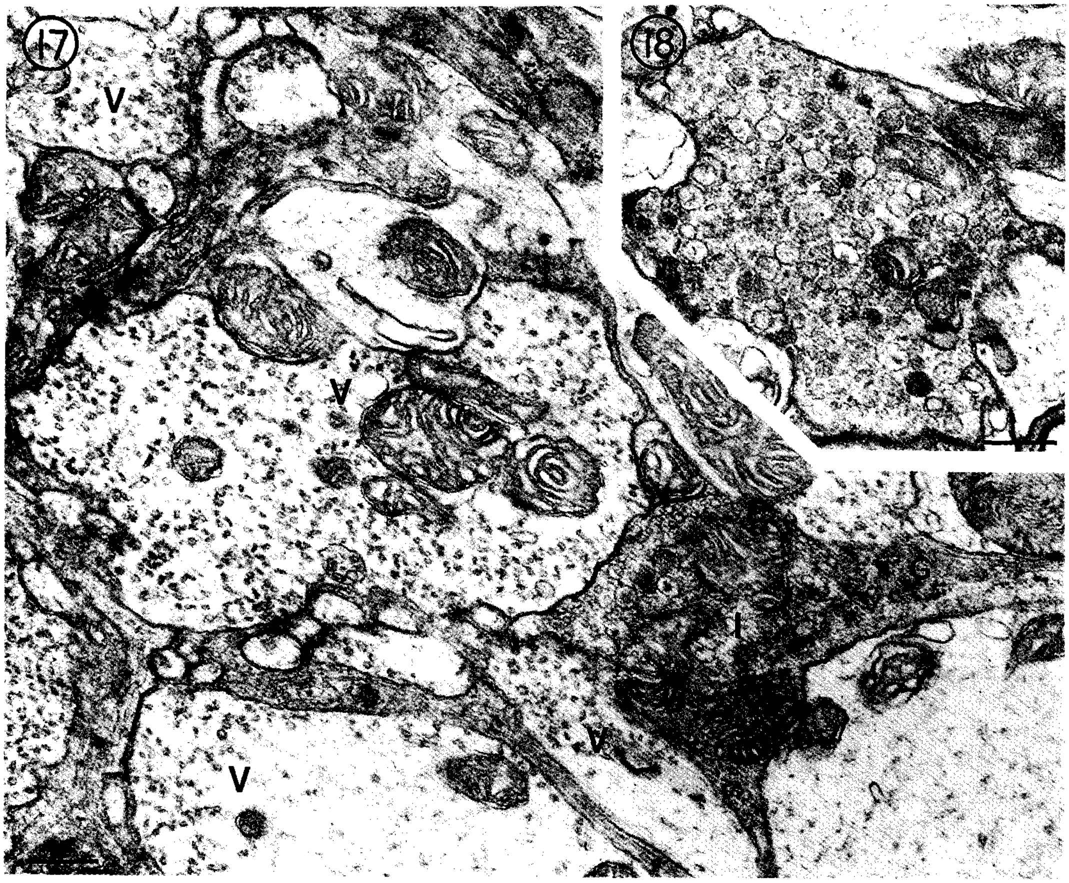


Fig. 17. Electron micrograph of ipsilateral subesophageal ganglion after the removal of the left antenna. A degenerated bouton (I) is surrounded by the glial process (G). The degenerated bouton is thought to be the type I, because of presence of medium-sized round, large round and dense core vesicles within the bouton. Bar indicates 2.8 μm.
Fig. 18. The degenerated bouton in the subesophageal ganglion containing a number of enlarged synaptic vesicles. This type of degeneration in the bouton could be unusally found in ipsilateral subesophageal ganglion five days after the removal of a left antenna. Bar indicates 2.8 μm.

darkly-degenerated type I boutons, which were filled up with medium-sized round, large round and dense core vesicles in very electron-dense axoplasm (Fig. 16). These synaptic vesicles were also shrunken to the smaller size, perhaps also in the process of fixation. The synaptic contact of the type I bouton with the dendrite was preserved without degenerative alteration.

The degenerated type I boutons were sometimes surrounded in part by glial processes, as shown in Fig. 17. The limiting membranes between the degenerated bouton and the glial processes, were already changed in part to the unclear state. This degenerated type I bouton is presumed to be phagocytosed by the adjacent glial cells.

Sometimes, the degenerated bouton, which was filled with the swollen clear and dense core vesicles, could be found in ipsilateral subesophageal ganglion five or six days after the removal of the left antenna (Fig. 18). Although it was not easy to determine the type of the synapsis, it could be presumed to be the type I.

Discussion

The subesopahgeal ganglion, like all segmental ganglia, has a scaffold of longitudinal tracts and transverse commissures (Altman and Kien, 1987). A detailed investigation of these longitudinal tracts and transverse commissures in subesophageal ganglion was performed in locust by Tyrer and Gregory (1982). All the synaptic ultrastructures organized by the nerve processes in the subesophageal ganglion of the cabbage butterfly can be classified into the five types. The subesophageal ganglion contains motor neurons projecting into the muscles of the mouthparts and the neck, and related sensory inputs (Altman and Kien, 1987). It has been long reported that, in addition to its segmental motor functions, the subesophageal ganglion plays an important role in regulating the motor activity of the whole insect which has the complicate neuronal composition. In view of these complicate structural architecture and physiological function of subesophageal ganglion, the five types of synaptic organizations can be identified in the subesophageal ganglion.

Four to five days after the proximal removal of a left antenna the ipsilateral subesophageal ganglion contains many degenerated axon terminals. The degenerated axon terminals show mostly weakly-dark, semidark and dark alterations. In the one-day-old cabbage butterfly following the removal of the left antenna the ipsilateral antennal lobe (Lee and Kim, 1988) and the ipsilateral dorsal lobe (Kim *et al.*, 1990) exhibited the same degenerative alterations as found in the subesophageal ganglion.

Some fibers of the receptor cells projected from the antenna can be concluded to be innervated to the ipsilateral subesophageal ganglion, running through the lateral and ventral margins of antennal lobe and the dorsal lobe. This pathway of the antennal receptor cell was also evidenced in the brain of the honeybee (Suzuki, 1975; Mobbs, 1982).

It was reported that in the subesophgeal ganglion of the locust there is an ipsilateral projection from the antennal mechanoreceptors (Aubele and Klemm, 1977; Gewecke, 1979), including fibers from pedicellar companiform sensilla and scapal hair plates (Bräunig *et al.*, 1983). From these reports it can be assumed also in cabbage butterfly that the antennal receptor fibers terminated as the type I in the ipsilateral subesophageal ganglion have information about antennal movement from the ipsilateral antenna. In addition to the ipsilateral projection from antennal mechanoreceptors, it was known that the subesophageal ganglion receives primary afferents from mechanoreceptors and proprioceptors in other body segments, carrying information about head movement, head orientation, body movements, and position (Altman and Kien. 1987). These results seems to be similar in the subesophageal ganglion of cabbage butterfly.

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배추흰나비 식도하신경절에 종지하는 촉각 지각신경세포에 관하여

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배추흰나비의 식도하신경절에 분포하는 신경연접기구를 관찰하고 촉각의 지각신경이 뇌를 거쳐 식도하신경절에 종지하는지를 밝히기 위하여 전자현미경을 이용해서 본 실험을 수행하였다.

식도하신경절의 모든 신경연접기구는 연접소포의 크기, 형태 등에 의하여 5가지로 구분될 수 있었다. 촉각의 기부를 잘라내어 촉각 지각신경을 절단한지 수일 후에는 같은 쪽의 식도하신경절에서 많은 축색종말이 전자밀도가 높게 나타나는 퇴행성 변화를 하였다. 이같은 퇴행성 변화를 한 축색종말은 모두 제 1 형 축색종말이었다. 그러므로 촉각 지각신경은 식도하신경절에서 수상돌기와 제 1 형 신경연접을 형성하는 것으로 결론된다.