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Projection of Antennal Receptor Cells into Dorsal Lobe of Brain in *Pieris rapae* (Insecta, Lepidoptera)

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The ipsilateral dorsal lobe of the brain one or two days after cutting a left antenna in *Pieris rapae* has been examined with electron microscope to investigate the connection of the receptor cells between antenna and dorsal lobe. The proximal removal of the left antenna leads to the weakly-dark, semidark or dark degeneration of antennal receptor terminals in ipsilateral dorsal lobe. Therefore, it is concluded that some of antennal receptor cells which project into the brain terminate in ipsilateral dorsal lobe located immediately behind the antennal lobe.

KEY WORDS: Antennal receptor cells, Axon termination, Dorsal lobe in brain, *Pieris rapae*.

In *Apis mellifera*, three bundles of receptor fibres (T1-T3) which project into brain from antenna terminate in the 100 or so glomeruli of antennal lobe (Suzuki, 1975; Mobbs, 1982; Rospars, 1988). According to a report of Suzuki (1975), two bundles (T1 and T2) pass into the central network, whereas a third (T3) penetrates the glomeruli from the periphery of antennal lobe without entering into the central network.

However, three other bundles of antennal receptor fibres (T4-T6) bypass the antennal lobe ventrally. Some of antennal receptor fibre bundles terminate in the dorsal lobe located behind the antennal lobe, while the others enter the dorsal lobe, then leave it, and finally project into a few areas out of the dorsal lobe in the brain.

Ernst *et al.* (1977) also described the termination of antennal receptor cell axons in the ipsilateral dorsal lobe of *Locusta migratoria*. It was later reported that the antennal receptor fibres terminating in the dorsal lobe of brain consist of presumed mechanosensory fibres (Mobbs, 1985).

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Kim and Lee (1986) recently reported that some of the antennal receptor cells which project into brain terminate in the ipsilateral antennal lobe from *Pieris rapae*. However, they did not describe where to be terminated by the other antennal receptor fibres which passed around the ventral margin of antennal lobe.

In this report, the authors clarify the termination of antennal receptor cells and simultaneously their connection with other neurons in ipsilateral dorsal lobe from *Pieris rapae*.

Materials and Methods

The one-day-old cabbage butterflies (*Pieris rapae*), which were collected from the stock colonies maintained at a laboratory of Soonchunhyang University, were used in this experiment. It took ten days for five instar larva to become an adult in rear cage at temperature of 28-30°C and relative humidities of 50-60%.

The left antennae of one-day-old cabbage butterflies were experimentally cut off on their proximal portions. The cabbage butterflies, whose the

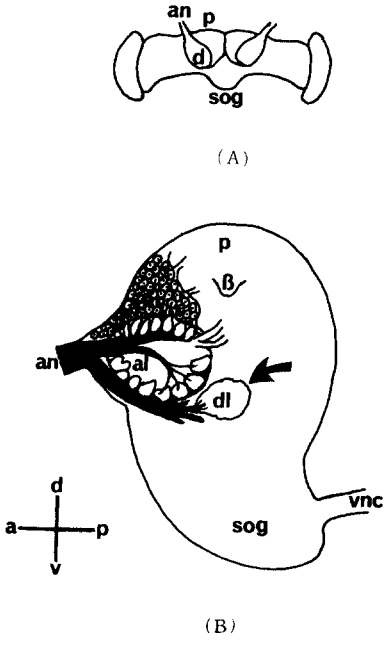

Fig. 1. (a) Schematic representation of the cabbage butterfly brain with deutocerebra (d) and antennal nerves (an) located anteriorly to protocerebrum (p). sog, subesophageal ganglion.
(b) Diagrammatic sagittal view through antennal lobe (al) and dorsal lobe (dl) of the brain from the cabbage butterfly. The antennal receptor fibre bundles project into several regions of the brain including the antennal lobe. But there is no evidence in cabbage butterfly that an antennal receptor fibre bundle, which bypasses the antennal lobe ventrally, terminate in dorsal lobe (large arrow) behind the glomerular neuropil of antennal lobe. β, β-lobe of portocerebrum (p); sog, subesophageal ganglion; vnc, ventral nerve cord; a, anterior side of brain; d, dorsal side; p, posterior side; v, ventral side.

left antennae were cut off, were kept to survive one or two days in rear cage.

Thereafter, the heads of the cabbage butterflies were cut off with a sharp scissor and prefixed in 1% paraformaldehyde-1% glutaraldehyde at 4°C for 30 min to 1 hr. The cuticle layers of the heads were removed under the stereoscope for isolating the whole brains. The removal of cuticle form the brains was always performed in cool prefixative. For completing the fixation of isolated brains, they continued to be put in new prefixative solution at a cool place overnight. They were washed three times in 0.4 M phosphate buffer, pH 7.4, containing 8% glucose and 0.5% $CaCl_2$. The brains were then postfixed in 2% OsO_4 in phosphate buffer at 4°C for 2 hrs. They were dehydrated in graded concentrations of ethanol and in acetone, and embedded in araldite mixture.

The embedded brains were trimmed with a LKB ultrotome V so that only ipsilateral dorsal lobes were cut into ultrathin sections. After being stained with uranyl acetate and lead citrate, the ultrathin sections of ipsilateral dorsal lobe were finally examined with Jeol CX-II electron microscope at 80 kv.

Results

The ipsilateral dorsal lobe of the brain from the cabbage butterfly, which was fixed one day after removal of left antenna, includes many weakly-dark boutons (Fig. 2, large asterisk) which show initiation step of degenerative alteration. The weakly-dark boutons contain relatively increased number of clear synaptic vesicles and a small number of dense core vesicles. Within some of these boutons, a small electron-dense lysosomal particle is also included (small arrow). Most of weakly-dark medium-sized boutons undergo symmetrical contacts with small dendrites in dorsal lobe.

In the ipsilateral dorsal lobe of the brain which was fixed two days after removal of the left antenna, semidark boutons (Figs. 3 and 4, large asterisk) which exhibit moderate electron-density in their axoplasm can be frequently found. These boutons also undergo symmetrical contacts with the small dendrites (small asterisk). Most of them are also medium-sized, except for a semidark bouton located in upper part of Fig. 3. The semidark boutons include both a greatly increased number of clear synaptic vesicles and a number of dense core vesicles. Within the semidark boutons in Figs. 3 and 4, many of clear synaptic vesicles and some of dense core vesicles do not clearly show their limiting membranes. As shown in Fig. 4, the semidark boutons are partly surrounded by the glial cell processes (two arrows). Therefore, some of these boutons do not partly expose their axolemma, as indicated by three arrowheads in Fig. 4.

The darkly degenerated boutons are included in

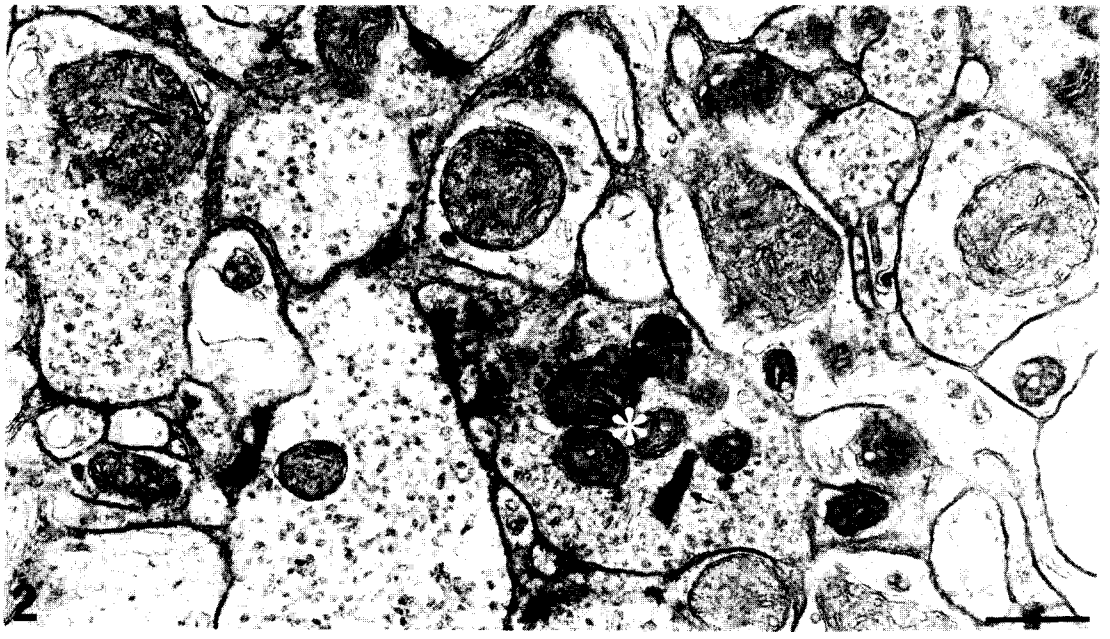

Fig. 2. Electron micrograph of ipsilateral dorsal lobe one day after the cutting of left antenna. An weakly-dark bouton(large asterisk) contains increased number of synaptic vesicles and an electron-dense lysosomal particle(small arrow). Most other boutons are preserved without sign of degenerative alteration. Bar indicates 0.5 μm.

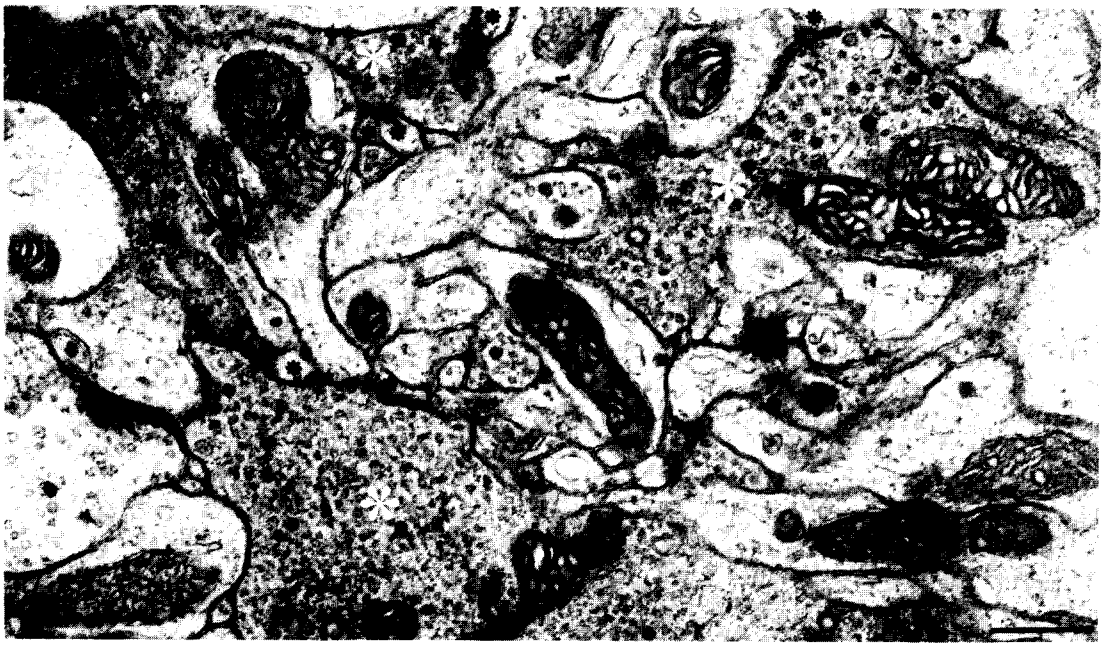

Fig. 3. Three semi-darkly degenerated boutons(large asterisks) in ipsilateral dorsal lobe one day after removal of left antenna showing symmetrical contacts with small dendrites(small black asterisks). The limiting membranes of most dense core vesicles disappeared in the lower bouton. Bar indicates 0.5 μm.

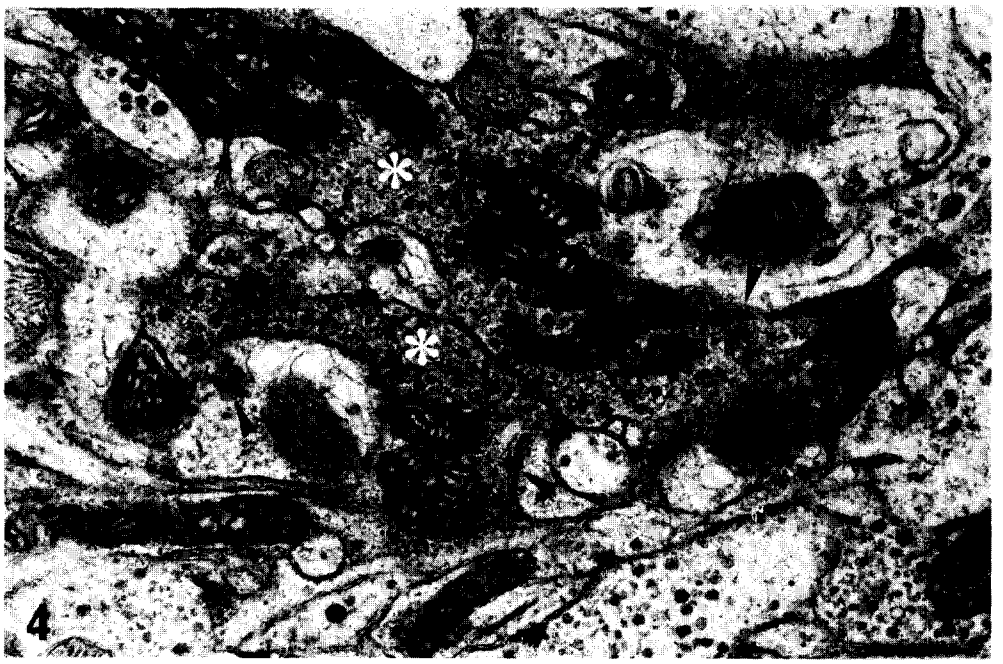

Fig. 4. Two degenerated boutons(large asterisks) in ipsilateral dorsal lobe two days after left antenna removal showing semidark axoplasm and partial disappearance of axolemma in the boutons (arrowheads). The two small arrows in upper part indicate the axolemma of degenerated bouton immediately contacted with glial cell process (G). Bar indicates 0.5 μm.

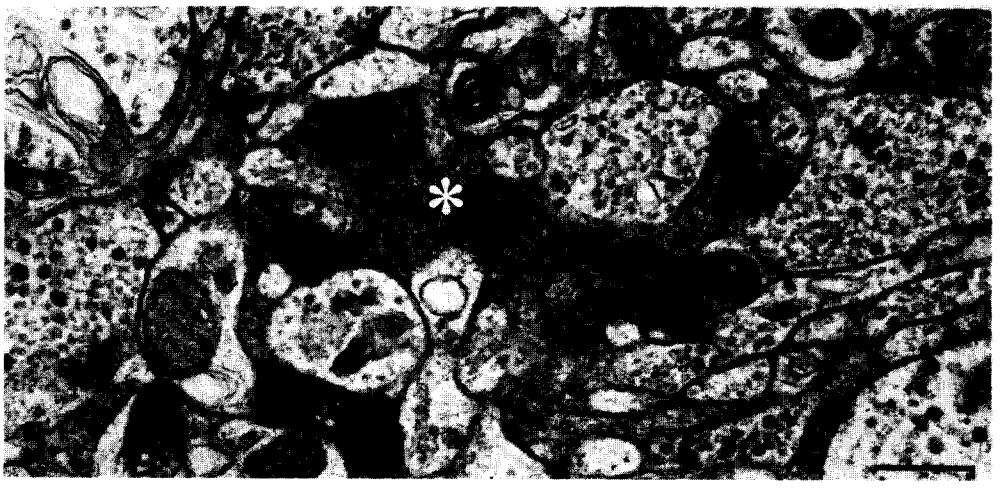

Fig. 5. One darkly degenerated bouton(large asterisk) in ipsilateral dorsal lobe two days after removal of left antenna showing dense core vesicles in dark axoplasm. Most of clear synaptic vesicles are degeneratively altered into amorphous substance in axoplasm. Bar indicates 0.5 μm.

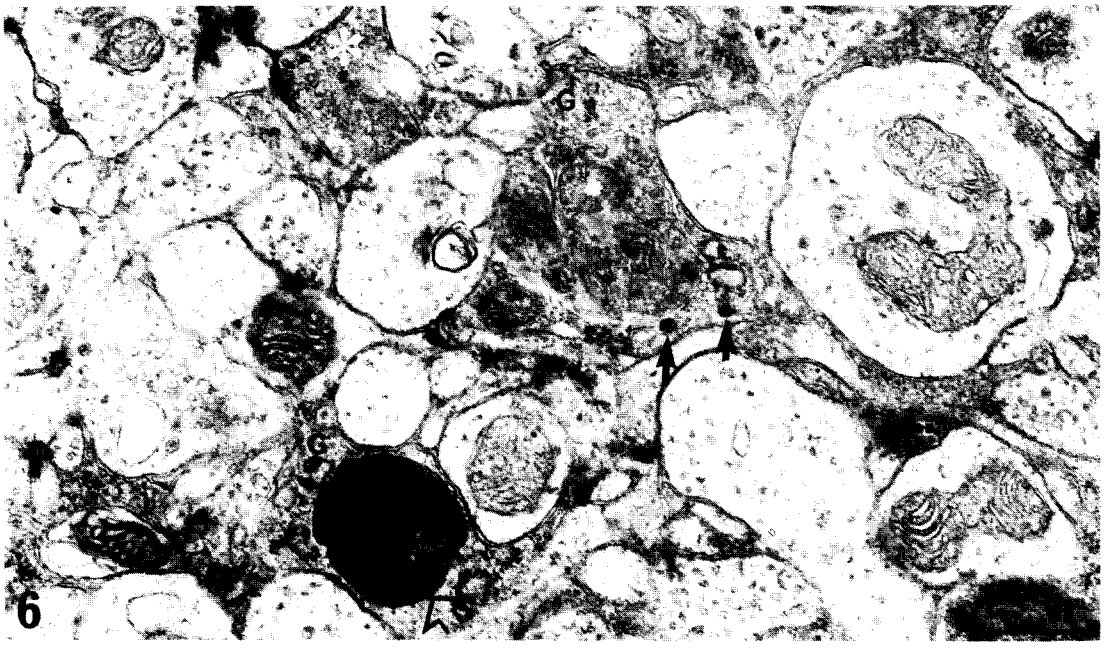

Fig. 6. Electron micrograph of ipsilateral dorsal lobe two days after removal of left antenna containing an weakly-dark bouton(large asterisk). The two glial cell processes(G) contain moderately-electron-dense irregularly-shaped structure(small asterisk) and electron-dense round structure(arrowhead) respectively. It is presumed that irregularly-shaped or round structures might be derivatives from greatly degenerated boutons engulfed by glial cells. This assumption is partly supported by the presence of some inclusions, such as weakly altered dense core vesicles(arrow), within glial cell process. Bar indicates 0.5 μm.

the ipsilateral dorsal lobe of the cabbage butterfly two days after cutting a left antenna (Fig. 5, large asterisk). In these darkly degenerated boutons which are mostly medium-sized the dense core vesicles are clearly found. However, the clear synaptic vesicles are not clearly exposed in dark exoplasm.

As shown in Fig. 6, within ipsilateral dorsal lobe of the brain fixed two days after the removal of the left antenna sometimes the glial cell processes contain either an electron-dense and round structure (open arrow) or a moderately-electron-dense and irregularly-shaped structure (small asterisk). One of the glial cell processes contains a few electron-dense round structures which bear a striking resemblance to dense core vesicles. Therefore, these structures are assumed to be derived from the degenerated boutons which were engulfed by the glial cell process.

Discussion

It could be evidenced in this experiment that some of receptor cells projecting into brain from the antenna terminate in the ipsilateral dorsal lobe, with an observation of axon terminals in dorsal lobe following the cutting of antennal nerve in *Pieris rapae*. The projection of antennal receptor cells into the ipsilateral dorsal lobe has been also described in *Apis mellifera* (Pareto, 1972; Mobbs, 1982, 1985) and *Locusta migratoria* (Ernst *et al.*, 1977) by light microscopic observations.

Lee and Kim (1988) earlier reported that the neuronal connections in antennal lobe of *Pieris rapae* consist of five types of synapses and the type IV synapses formed by antennal receptor terminals make up the largest ratio in all the synapses which occupy the antennal lobe.

Two days after the removal of a left antenna, the axon terminals of antennal receptor cells in

ipsilateral dorsal lobe showed weakly-dark, semidark and dark degenerations. The degenerated structures which are presumed to be degenerated boutons could be also found in the glial cell processes which sparsely occurred in the dorsal lobe. It was not easy to regard these structures as strongly degenerated boutons engulfed by the glial cell processes in this experiment. However, Lee *et al.* (1985) reported that many degenerated boutons of damaged intralaminar thalamic neurons could be found within the glial cell processes a few days after stereotactic coagulation of intralaminar thalamic nucleus in *Saimiri sciureus*. Ernst *et al.* (1977) also reported that twelve hours after the removal of an antenna the glial cells proliferate and envelop axonal material in their processes in the antennal lobe and dorsal lobe of *Locusta migratoria* and *Periplaneta americana*.

The degenerative change is progressive rather than simultaneous in all antennal receptor fibres, as described in *Locusta migratoria* and *Periplaneta americana* by Ernst *et al.* (1977). Thus, axon terminals of all the antennal receptor cells terminating in the dorsal lobe showed different degenerative changes two days after cutting an antenna; weakly-dark, semidark, dark degeneration and so on.

The axon terminals of antennal receptor cells sparsely terminate through the whole ipsilateral dorsal lobe. This structural finding is also in accordance with result on pattern of projection of anternal receptor cells into the dorsal lobe of *Apis mellifera* (Pareto, 1972). However, many antennal receptor cells terminate in group in some areas of antennal lobe of *Pieris rapae*, although some of them sometimes terminate sparsely (Lee and Kim, 1988). Therefore, the dorsal lobe receives a smaller number of axon terminals of antennal receptor cells than the antennal lobe in *Pieris rapae*.

Almost all the axon terminals of antennal receptor cells terminating in ipsilateral dorsal lobe undergo synaptic contacts only with small dendritic terminals. It could not be clarified in this experiment that what kind of neurons projects small dendrites making contacts with axon terminals of antennal receptor cells in the dorsal lobe. However, it was reported that antennal receptor fibres end on antennal motor fibres in dorsal lobe of *Apis mellifera* (Pareto, 1972). Therefore, it is highly probable that direct synaptic contacts occur between sensory (receptor) and motor fibres in this region without intermediate interneurons, and these contacts permit monosynaptic reflexes.

Therefore, it can be assumed that small dendritic terminals undergoing syaptic contacts with antennal receptor fibres in the dorsal lobe may be those that project from motor neurons also in *Pieris rapae*.

References

Ernst, K. D., J. Boech, and V. Boeckh, 1977. A neuroanatomical study on the organization of the antennal pathways in insect. II. Deutocerebral connections in *Locusta migratoria* and *Periplaneta americana*. *Cell Tissue Res.* **176:** 285-308.

Kim, W. K. and B. H. Lee, 1986. An ultrastructural study of the synaptic organization of deutocerebrum and the antenna-deutocerebrum connection in cabbage butterfly *Pieris rapae*. *Proc. XIth Int. Cong. on Electron Microscopy, Kyoto*, pp. 3181.

Lee, B. H., R. G. Hassler, Y. W. Chung, Y. Hasegawa, and F. M. Nitsch, 1985. Terminals of intralaminar thalamic neurons in the pallidum externum of *Saimiri sciureus*. *Acta anat.* **123:** 1-8.

Lee, B. H. and W. K. Kim, 1988. Terminals of antennal receptor cells in the antennal lobe of the butterfly, *Pieris rapae* L. (Insecta, Lepidoptera). *Korean J. Zool.* **31:** 334-343.

Mobbs, P. G., 1982. The brain of the honeybee *Apis mellifera*. I. The connections and spatial organization of the mushroom bodies. *Phil. Trans. Roy. Soc. Lond.* **B298:** 309-354.

Mobbs, P. G., 1985. Brain strucutre. *In*: Comprehensive Insect Physiology, Biochemistry and Pharmacology. (Kerkut, G. A. and L. I. Gilbert eds.), Vol. 5, *Pergamon press*, Oxfod, pp. 299-370.

Pareto, A., 1972. Die Zentrale Verteilung der Fühlerafferenz bei arbeiterinnen der Honigbiene, *Apis mellifera* L. *Z. Zellforsch.* **131:** 109-140.

Rospars, J. P., 1988. Structure and development of the insect antenno-deutocerebral system. *Int. J. Insect Morphol. & Embryol.* **17:** 243-294.

Suzuki, H., 1975. Convergence of olfactory inputs from both antennae in the brain of the honeybee. *J. Exp. Biol.* **62:** 11-26.

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배추흰나비 뇌 배엽에 종지하는 촉각지각 신경세포에 관하여

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배추흰나비에서 좌측 촉각을 제거한지 1일 또는 2일후 뇌의 좌측배엽을 전자현미경으로 관찰하여 촉각의 촉각지각신경세포가 배엽에 종지하는지를 조사하였다. 촉각지각신경세포는 촉각제거로 받은 큰 손상으로 말미암아 배엽에서는 그 축색종말에 전자밀도가 낮거나, 중등도이거나 또는 높은 퇴행성변화를 나타내었다. 그러므로 뇌로 뻗은 촉각지각신경세포중 일부는 촉각바로 뒤에 있는 배엽에 종지한다고 결론되었다.