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# Architecture of Cerebral Neuroendocrine System in the Larva of Cabbage Butterfly *Pieris rapae*

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**This investigation has been carried out to clarify structural architecture of cerebral neuroendocrine systems in the fifth instar larva of cabbage butterfly *Pieris rapae*. In order to examine the cerebral neurosecretory cell systems the brain and retrocerebral neuroendocrine complex were histochemically stained with the paraldehyde fuchsin. The brain of the fifth instar larva contains three kinds of neurosecretory cells: medial, lateral and tritocerebral neurosecretory cells. The axon bundles of medial and lateral neurosecretory cells form medial neurosecretory pathway(MNSP) and lateral neurosecretory pathway(LNSP) within the brain respectively. Especially, prior to exiting the brain, the axon bundles of medial neurosecretory cells located in both left and right cerebral hemispheres decussate in cerebral medial region and project to contralateral retrocerebral neuroendocrine complexes. Outside the brain the axon bundles of medial and lateral neurosecretory cells form the nervi corporis cardiaca(NCC) I and II respectively. The NCC I and II run together to the retrocerebral neuroendocrine complex, forming the large nerve bundles in both left and right sides. The axon bundles of tritocerebral neurosecretory cells which pass through the brain along the tritocerebral neurosecretory pathway (TNSP) form the NCC III outside the brain. Some of the NCC I and II terminate in the corpus cardiacum, while the others pass through the corpus cardiacum without termination. The nerve bundle which passes the corpus cardiacum forms the nervus corporis allatum(NCA) I which runs between the corpus cardiacum and the corpus allatum. They are finally innervated to the corpus allatum. The NCC III projects to the corpus cardiacum. However, most of NCC III pass through the corpus cardiacum without branching and then run down for another organ.**

**KEY WORDS: Neurosecretory cell, Brain, Retrocerebral neuroendocrine complex, Cabbage worm, *Pieris rapae***

Insect brains have many neurosecretory cells which release their products usually as various kinds of neurohormones. The larval brain of cabbage butterfly, *Pieris rapae*, has medial, lateral and tritocerebral neurosecretory cells (Lee *et al.*, 1990). Most cerebral neurosecretory cells in insects form a specific terminal structure in specific neurohaemal regions in which they discharge their products into the haemolymph (Geldiay and Edwards, 1973; Rowell, 1976; Raabe, 1983; Ichikawa, 1991). The brain is, therefore, a primary source of neurosecretory material, and has important function as regulator of neuroendocrine mechanism in an insect (Mason, 1973; Awasthi, 1975; Singh and Arif,

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1983).

In house cricket *Acheta domestica* some of cerebral neurosecretory cells terminate in a neurohaemal area on the ventral median surface of the brain (Geldiay and Edwards, 1973). However, in most insects including the house cricket the principal neurohaemal sites in which the neurosecretory cells usually terminate were found to be included in retrocerebral neuroendocrine complex, which consists of corpus cardiacum and corpus allatum.In addition to these neurohaemal organs another organ such as stomodeal dilator muscles in silkworm *Bormyx mori* also contain cerebral neurosecretory terminals (Ichikawa, 1991).

The insect retrocerebral neuroendocrine complex is connected to the brain by a few nerves, NCC I and II and NCA I. It has been also reported that in most insects already described the NCC I is formed by the medial neurosecretory cells, while the NCC II is formed by the lateral neurosecretory cells. In addition, the insects which include the tritocerebral neurosecretory cells have the NCC III outside the brain.

The insects have respectively different topographies, axonal pathways and terminations of the cerebral neurosecretory cells. In the present study, the exact topography of all putative cerebral neurosecretory cells and their outputs in the cabbage worm *Pieris rapae* were examined using paraldehyde fuchsin staining of the brain and retrocerebral neuroendocrine complex.

## Material and Methods

The fifth instar larvae of cabbage worms, *Pieris rapae*, collected from the stock colony of Soonchunhyang University were used in this experiment. After general anesthesia of the fifth instar larvae was performed in keeping them at 4°C for 30 min or more, their heads, including the corpora cardiaca and corpora allata, were cut off. They were fixed in Bouin's fluid for 12 hrs, dehydrated in graded concentration of ethanol, and embedded in paraffin. The embedded brains were serially sectioned with the microtome. The serial sections of the brains and the retrocerebral neuroendocrine complex were mounted on the slide glasses and stained in the paraldehyde fuchsin solution(Gabe, 1953).

Prior to histochemical staining of the serial sections, they were initially oxidized in freshly prepared mixture of equal volumes of 0.6% $KMnO_4$ and 0.6% $H_2SO_4$ for 1 min at 20-22°C. Following being rinsed in distilled water, they were bleached in 2.5% sodium metabisulfite for 40 sec. Thereafter, they were washed in running tap water for 5 min or more. After being passed through distilled water and 30% to 70% alcohol, they were stained in paraldehyde fuchsin solution for 2 min. They were washed in 3 changes of 96% alcohol. Finally, they were dehydrated in 2 changes of acetone, cleared in 2 changes of xylene, mounted in Canada balsam, and examined under the microscope.

## Results

The brain is connected to retrocerebral neuroendocrine complex by three kinds of nerves (Fig. 1). The NCC I and II which run together forming the large nerve bundle project out from the ventral central surface of the brain and are connected to the corpus cardiacum of the retrocerebral neuroendocrine complex. The NCC III which projects from the ventral surface of the brain runs down to the corpus cardiacum. In addition, the NCA I connects between the corpus cardiacum and the corpus allatum.

As shown in Fig. 2, the brain in the fifth instar larva of the cabbage worm contains three kinds of neurosecretory cells: medial, lateral and tritocerebral neurosecretory cells. The axon bundle of medial neurosecretory cells decussates in a cerebral medial region before exiting the brain (Figs. 2, 4a) and then run down to the contralateral hemispheres of brain (Fig. 2). This pathway of the medial neurosecretory cells is called the medial neurosecretory pathway (MNSP). The axon bundle of lateral neurosecretory cells which shows no decussation in the brain forms lateral neurosecretory pathway (LNSP) (Fig. 2). The LNSP runs down along the MNSP (Figs. 2, 4b, c). The axon bundle of the tritocerebral

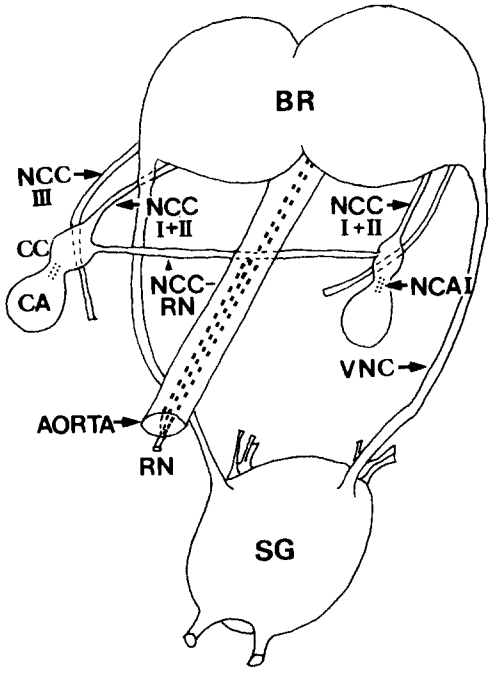


**Fig. 1.** Schematic representation of the cerebral neurosecretory system in fifth instar larva of *Pieris rapae*. The brain is structurally connected with the retrocerebral neuroendocrine complex, corpora cardiaca (CC) and corpora allata(CA), by NCC I+II and NCC III. The NCA I runs between the CC and CA. NCC-RN, nervus corporis cardiaci-nervus recurrens; RN, recurrent nerve; SG, suboesophageal ganglion; VNC, ventral nerve cord.

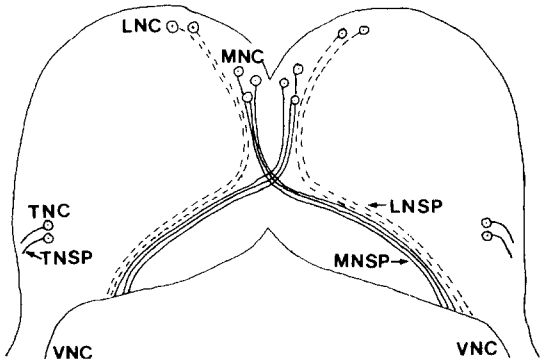


**Fig. 2.** Schematic representation of localization of cerebral neurosecretory cell bodies and their axonal pathways in the fifth-instar-larva brain of *Pieris rapae* which includes three groups of medial, lateral and tritocerebral neurosecretory cells. The medial neurosecretory cells (MNC) form first axonal pathway, medial neurosecretory pathway (MNSP), which decussates in the cerebral medial region, while the lateral neurosecretory cells (LNC) form second axonal pathway, lateral neurosecretory pathway (LNSP), which runs together with the MNSP. The tritocerebral neurosecretory cells (TNC) form third axonal pathway, tritocerebral neurosecretory pathway (TNSP). VNC, ventral nerve cord.

neurosecretory cells runs along the separate pathway from the MNSP and LNSP. This pathway is called the tritocerebral neurosecretory pathway (TNSP).

The somata of medial neurosecretory cells are located in the pars intercerebralis of the brain. As shown in Fig. 3a, the medial neurosecretory cells are sometimes found to transport their product along their axons. The fifth- instar-larval brain includes small number of neurosecretory cells in dorsolateral rind of the protocerebrum, compared with the medial neurosecretory cells (Figs. 3a, b). These cells are called the lateral neurosecretory cells. The experimental data for the presence of the tritocerebral neurosecretory cells in the fifth instar larva of cabbage worm have been reported by Lee *et al.* (1990) who demonstrated localization of two neurosecretory cells in each tritocerebrum. Lee *et al.* (1990) have also demonstrated ca. 27 medial neurosecretory cells and ca. 9 lateral neurosecretory cells in each cerebral hemisphere of the fifth instar larvae.

Sometimes the axons of medial neurosecretory cells which decussate in cerebral medial region show weak reactivities to paraldehyde fuchsin (Fig. 4a). However, the lateral neurosecretory axons in the brain exhibit strong reactivities to paraldehyde fuchsin, indicating that they include abundant neurosecretory materials (Figs. 4b, c). The combination of medial and lateral neurosecretory axons in the brain leads to formation of a large nerve outside the brain, which consists of the NCC I and II (Fig. 5a).

Some of the NCC I and II which project to the corpus cardiacum pass through it and then form the NCA I between the corpus cardiacum and the corpus allatum (Fig. 5b). The NCA I projects to the corpus allatum. But, as shown in Fig. 5c, the NCC III which projects from the brain to the corpus cardiacum appear to pass through it without

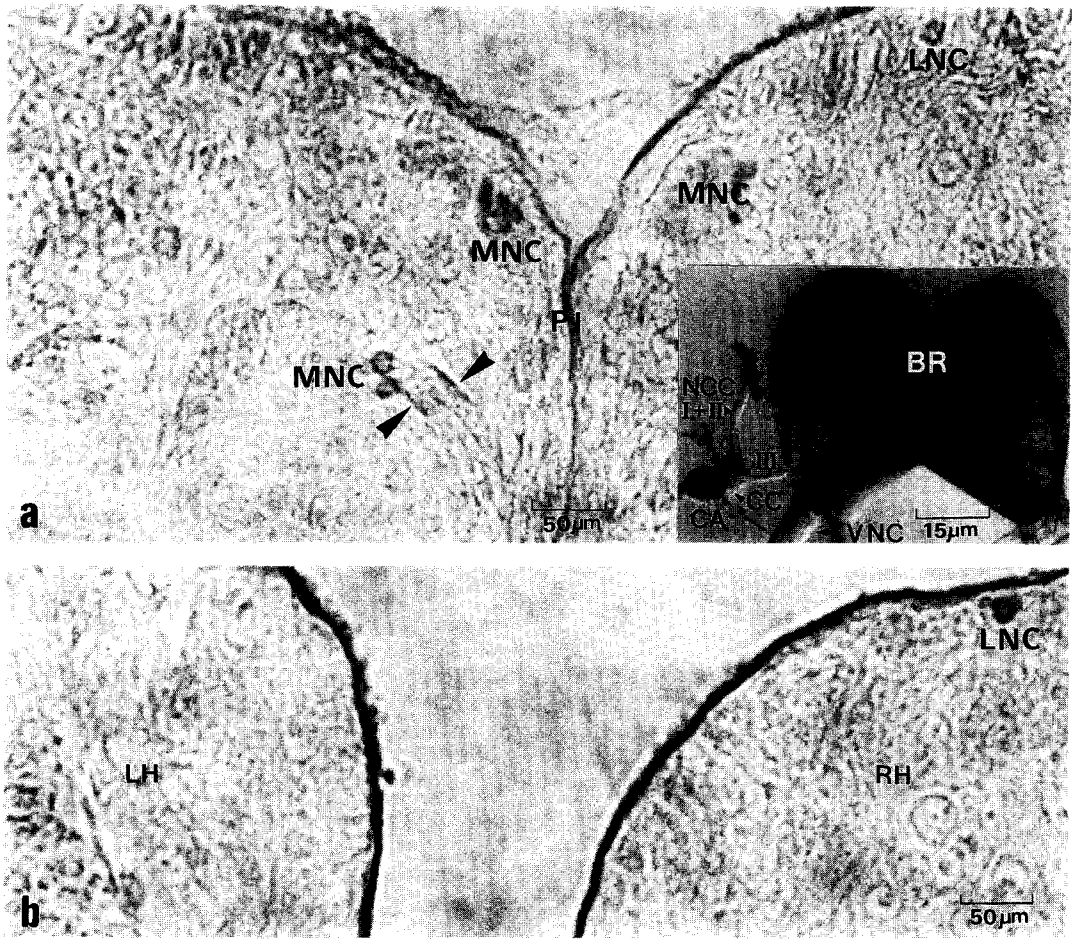


**Fig. 3.** Light micrographs of medial and lateral neurosecretory cells in the fifth-instar-larva brain. (a) Several MNC and a LNC are seen. The axons of medial neurosecretory cells contain paraldehyde-fuchsin-positive neurosecretory material (arrowheads). The inset shows the whole brain(BR) connected with the left retrocerebral neuroendocrine complex(CC and CA) by NCC I+II and NCC III. PI, pars intercerebralis; VNC, ventral nerve cord. (b) A LNC is located in left cerebral hemisphere(LH). The presence of TNC in the brain of the fifth instar larva from *Pieris rapae* was previously conformed by an investigation of Lee *et al.* (1990). RH, right hemisphere of the brain.

branching. The NCC III is finally innervated in another organ. Some of the NCC I and II are innervated in the corpus cardiacum (Figs. 6a, b), the others are innervated in corpus allatum (Fig. 6b).

## Discussion

Histochemical investigation of the cerebral neurosecretory cells using the paraldehyde fuchsin has shown that the brain of the fifth instar larva of *Pieris rapae* contains ca. 54 medial cells, ca. 18 lateral cells and ca. 4 tritocerebral cells as neurosecretory cell (Lee *et al.*, 1990). These cerebral neurosecretory cells may regulate various physiological actions by producing different neurosecretory signals in the form of neurohormones. In *Bombyx mori*, three types of cerebral neuropeptides including bombyxin that was previously called 4K-prothoracicotropic hormone (4K-PTTH), have been demonstrated, and the localization of the neurosecretory cells responsible for production of the peptides has

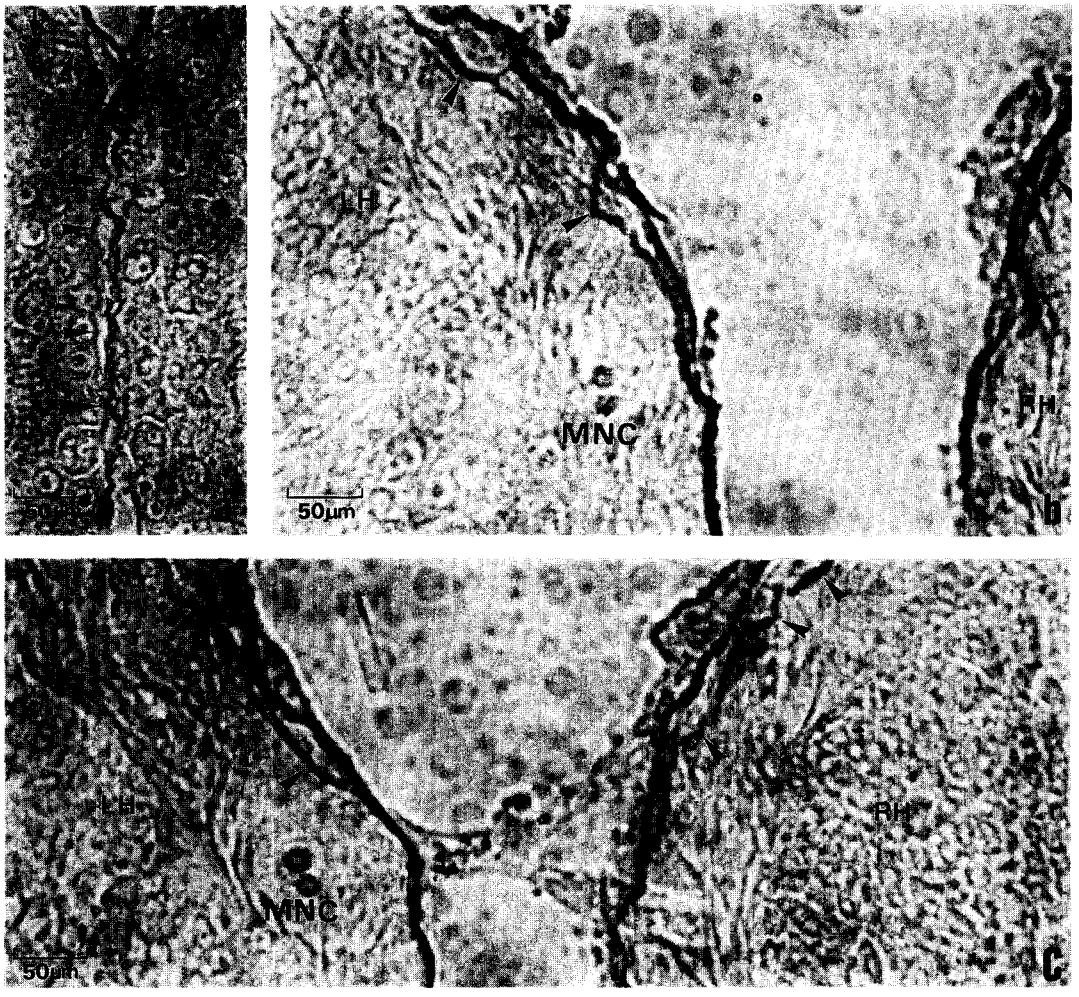


**Fig. 4.** Light micrographs of MNSP and LNSP stained with the paraldehyde fuchsin. (a) The axons (MNSP, small arrowheads) of the MNC (small arrow) located in left cerebral hemispere decussate in a medial cerebral region (large arrowhead), and then run to the right cerebral hemisphere. PI, pars intercerebralis. (b), (c) The axon bundles (LNSP, arrowheads) of the LNC are running down for innervation to the retrocerebral neuroendocrine complex. The two neurosecretory cells (MNC) are also seen. LH, left hemisphere of brain; RH, right hemisphere of brain.

been revealed by immunohistochemical investigations using monoclonal antibodies (Mizoguchi *et al.*, 1991; Ichikawa, 1991).

The cerebral neurosecretory cells in all the insects release their products in neurohaemal organs such as the brain, corpus cardiacum and corpus allatum. In this experiment, the corpus cardiacum and corpus allatum were demonstrated to have functions as principal neurohaemal organs in *Pieris rapae*. The corpus cardiacum of the retrocerebral neuroendocrine complex is connected to the brain by three pairs of the nerves which are formed by cerebral neurosecretory axons. The cerebral neuroendocrine system in the fifth instar larva of *Pieris rapae* was found to be similar to that of *Bombyx mori* which has also three types of neurosecretory cells in the brain. The medial and lateral neurosecretory axons form a large nerve, NCC I + II, immediately after exiting the brain. This nerve projects to the corpus cardiacum. In *Pieris rapae*, some of this nerve terminate in the corpus cardiacum. However, the

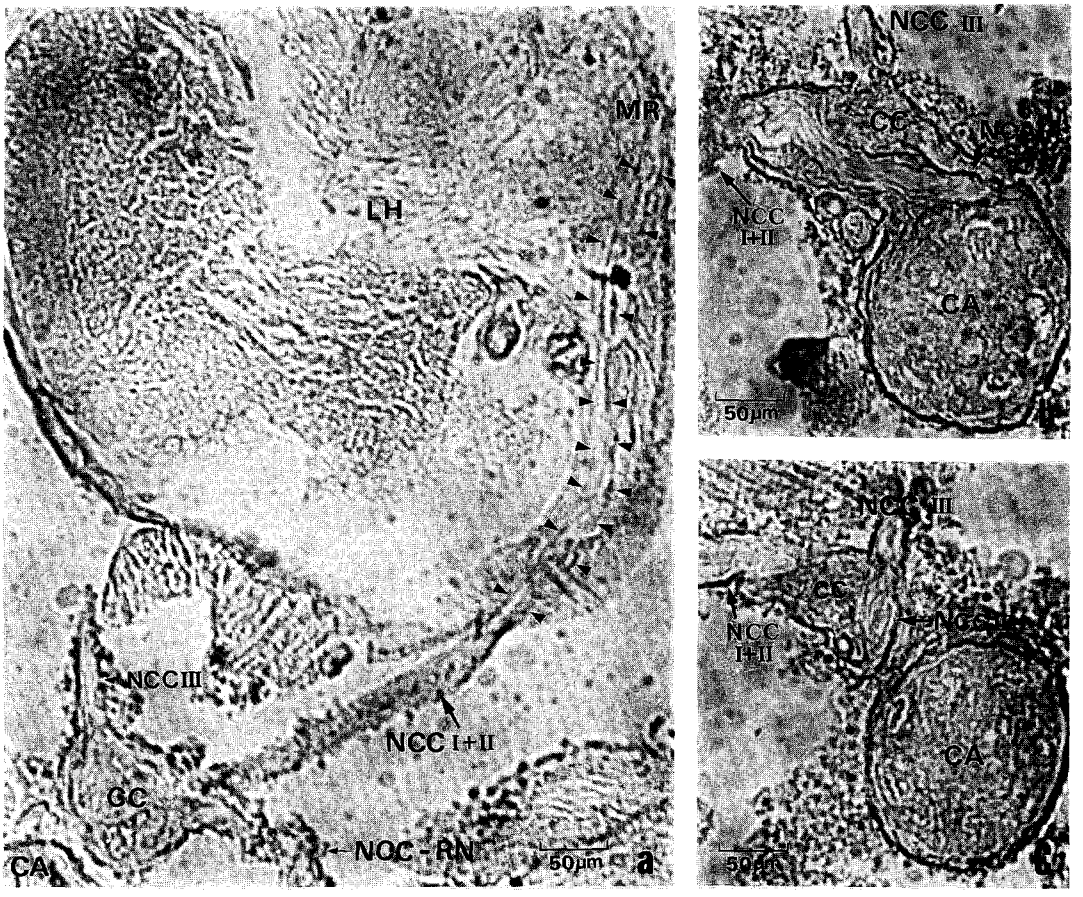


**Fig. 5.** Light micrographs of the NCC I and II (arrowheads) projecting from the left hemisphere of the brain(LH) to the corpus cardiacum(CC) and corpus allatum(CA). (a) The axon bundles (small arrowheads) in medial region(MR) within the brain form the NCC I and NCC II outside the brain, and are then innervated to the retrocerebral neuroendocrine complex. The axon bundle of tritocerebral neurosecretory cells forms a nerve(NCC III) outside the brain, and then projects into the corpus cardiacum. NCC-RN, nervus corporis cardiaci-nervus recurrens. (b) The NCA I runs between the corpus cardiacum(CC) and the corpus allatum(CA). (c) The NCC III passes through the corpus cardiacum(CC), and then projects into another organ.

remaining of the NCC I + II which does not terminate in the corpus cardiacum forms the NCA I. The NCA I was demonstrated to terminate in the corpus allatum in *Pieris rapae*.

The medial neurosecretory axons and lateral neurosecretory axons of colorado potato beetle *Leptinotarsa decemlineata* (Schooneveld, 1974), cockroach *Diploptera punctata* (Duve *et al.*, 1991) and tobacco hornworm *Manduca sexta* (Westbrook *et al*, 1991) have been also reported to project to the corpus cardiacum through a large nerve, NCC I + II, as in *Pieris rapae* and *Bombyx mori* (Ichikawa, 1991). The above three insects include only medial and lateral neurosecretory cells in the brains. However, the medial and lateral neurosecretory cells of earwig *Labedura riparia* (Awasthi, 1975), beetle *Mylabris pustulata* (Sidhra *et al.*, 1983) and *Pericalia ricini* (Singh and Arif, 1983) project to the corpus cardiacum through the NCC I and NCC II respectively.

The tritocerebral neurosecretory cells of *Pieris rapae* project to the corpus cardiacum through a nerve, NCC III, as in *Bomyx mori* (Ichikawa, 1991). Sidhra *et al.* (1983) have described presence of ventral neurosecretory cells in the brain of *Mylabris pustulata*. These neurosecretory

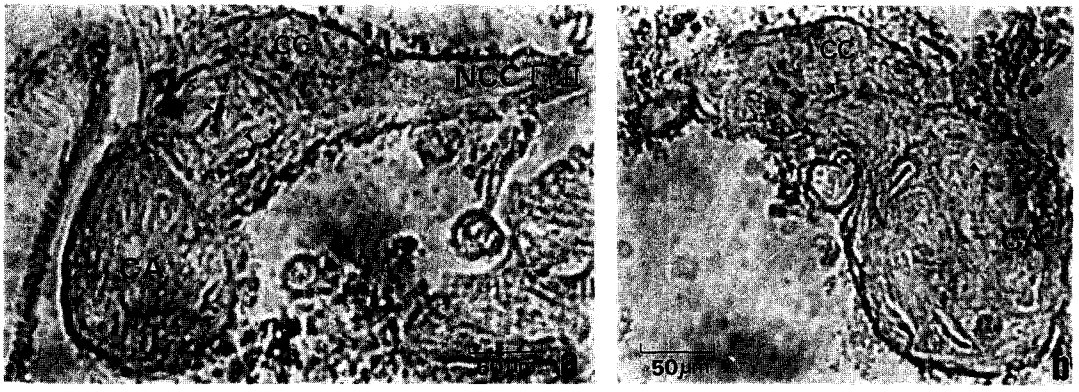


**Fig. 6.** Light micrographs of the corpus cardiacum(CC) and corpus allatum(CA) containing paraldehyde-fuchsin-positive neurosecretory substances. These substanses are transported by the NCC I and II from the neurosecretory cell bodies in the brain. Sometimes the neurosecretory substanses (small arrowheads) are included only in the corpus cardiacum of the retrocerebral neuroendocrine complex (a), but can be also found in both corpus cardiacum and corpus allatum (b).

cells have structural characteristics that their axons join the NCC II, which is separate nerve from the NCC I, immediately prior to existing the brain. In *Schistocerca vaga*, the axons of tritocerebral neurosecretory cells join the NCC I immediately before exiting the brain (Mason, 1973).

The NCC III which projects to the corpus cardiacum in *Pieris rapae* appears to pass through it without branching. It has been also reported in *Bomyx mori* that the axons running down along the NCC III pass through the corpus cardiacum without branching and extend several long terminal branches among the stomodeal dilator muscles (Ichikawa, 1991). In *Schistocerca vaga* (Mason, 1973),however, axons of the NCC III arise from cell group in the tritocerebrum and end in both corpus cardiacum and corpus allatum.

## References

Awasthi, V.B., 1975. Neurosecretory system of the earwig *Labedura riparia*, and the role of the aorta as a neurohaemal organ. *J. Insect Physiol.* **21:** 1713-1719.

Duve, H., A. Thorpe and S.S. Tobe, 1991. Immunocytochemical mapping of neuronal pathways from brain to corpora cardiaca / corpora allata in the cockroach *Diploptera punctata* with antisera against Met-enkephalin-Arg-Gly-Leu. *Cell Tissue Res.* **263:** 285-291.

Gabe, M., 1953. Sur quelques applications de la coloration par la fuchsine-paraldéhyde. *Bull. Microsc. Apl.* [2] **3:** 153-162.

Geldiay, S. and J.S. Edwards, 1973. The protocerebral neurosecretory system and associated cerebral neurohemal area of *Acheta domestica*. *Z. Zellforsch.* **145:** 1-22.

Ichikawa, T., 1991. Architecture of cerebral neurosecretory cell systems in the silkworm *Bombyx mori*. *J. Exp. Biol.* **161:** 217-237.

Lee, B.H., M.Y. Kim, K.S. Kim and W.K. Kim, 1990. Morphologic changes on neurosecretory cells in brains of cabbage butterflies, *Pieris rapae*, during metamorphosis. *Korean J. Entomol.* **20:** 169-177.

Mason, C.A., 1973. New features of the brain-retrocerebral neuroendocrine complex of the locust *Schistocerca vaga* (Scudder). *Z. Zellforsch.* **41:** 19-32.

Mizoguchi, A., M. Hatta, S. Sato, H. Nagasawa, A. Suzuki and H. Ishizaki, 1991. Developmental changes of bombyxin content in the brain of the silkmoth *Bombyx mori*. *J. Insect Physiol.* **36:** 655-664.

Raabe, M., 1983. The neurosecretory-neurohaemal system of insects: anatomical, structural and physiological data. *Adv. Insect physiol.* **17:** 205-303.

Rowell, H.F., 1976. The cells of the insect neurosecretory system: constancy, variability and the concept of the unique identifiable neuron. *Adv. Insect Physiol.* **12:** 63-121.

Schooneveld, H., 1974. Ultrastructure of the neurosecretory system of the colorado potato beetle, *Leptinotarsa decemlineata* (Say). I. Characterization of the protocerebral neurosecretory cells. *Cell Tissue*

*Res.* **154:** 275-288.

Sidhra, D.V., V.K. Tharkare and D.B. Tembhare, 1983. The cephalic neuroendocrine system of the beetle, *Mylabris pustulata* (Thumb) (Meloidae: coleoptera). *J. Hirnforsch.* **24:** 71-78.

Singh, Y.N. and M. Arif, 1983. Metamorphic changes in the cephalic neuroendocrine complex of *Pericalia ricini* Fabricius (Lepidoptera: Arctide). *J. Hirnforsch.* **24:** 189-200.

Westbrook, A.L., M.E. Haire, W.M. Kier. and W.E. Bollenbacher,1991. Three-dimensional architecture of identified cerebral neurosecretory cells in an insect. *J. Morphol.* **208:** 161-174.

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## 배추흰나비 5령유충의 뇌신경내분비계의 구조

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본연구는 배추흰나비 5령유충의 뇌신경내분비계의 구조를 밝히기 위해서 수행되었다. 뇌신경내분비계의 구조를 관찰하기 위하여 뇌와 뇌뒤의 신경내분비복합체(카디아카체와 알라타체가 주 구성원)를 paraldehyde fuchsin으로 염색하였다. 5령유충의 뇌는 medial neurosecretory cell(MNC), lateral neurosecretory cell(LNC), tritocerebral neurosecretory cell (TNC)등 3종류의 신경분비세포를 포함하였다. 세포체가 뇌간부에 존재하는 MNC는 medial neurosecretory pathway(MNSP)를 따라 뇌를 달린다. MNSP는 뇌의 정중부에서 좌우교차하는 특징을 가지고있다. MNC의 축색은 뇌를 빠져나가면 NCC I을 형성한다. 전뇌의 배측부에 세포체가 있는 LNC는 lateral neurosecretory pathway(LNSP)를 따라 뇌를 달리는데 이같은 LNSP는 좌우 교차하지 않고 MNSP와 함께 달린다. LNC의 축색은 뇌밖에서 NCC II를 형성하는데 NCC I과 결합하여 하나의 커다란 신경(NCC I+II)으로 카디아카체로 들어간다. 세포체가 후뇌에 있는 TNC는 tritocerebral neurosecretory pathway(TNSP)를 따라 뇌를 달린다. TNC의 축색은 뇌밖에서 NCC III를 형성하여 역시 카디아카체로 들어간다. 카디아카체로 들어간 NCC I+II는 그곳에서 일부가 종지하고 나머지는 NCA I를 형성하여 알라타체로 들어가 그곳에 종지한다. 역시 카디아카체로 들어간 NCC III는 카디아카체에 거의 종지하지 않고 다른 기관으로 달리는 것으로 관찰되었다.