

microscope; 2. 1 drop was placed on a cleaned glass slide and dried.

Under a 800–1000-times magnification, the laser was focused (1–2  $\mu\text{m}$ ) on digestive vacuoles containing apparently insoluble material. The major diffusion lines were surveyed, then a Raman spectrum could be studied between 100 to 3500  $\text{cm}^{-1}$ ; the more interesting diffusion lines occur between 100 and 2000  $\text{cm}^{-1}$ .

**Results and discussion.** It has been possible to obtain spectra for intravacuolar pesticides with both methods: figures 1 and 2 give reference spectra, and figures 3 and 4 give the spectrum response of digestive vacuoles of ciliates. The table gives the wavelength and characteristics of the lines found in the control and the samples for 4',4'-dichlorodiphenyl and  $\beta$ -endosulfan respectively. In both cases, the most important lines of reference of the sample are the same as those of the pesticide added. No essential modification in chemical structure occurs, because both the reference and sample spectra are the same.

Both wet and dry methods can give good results in analysis but each method has its own advantages. With the wet method, research is quite easy. But currents can occur in the preparation due to the local thermic effect of the laser beam, so that the cell can move and no longer remain in focus. With a very thin preparation, the phenomenon is reduced. With the dry method, many cells are drastically altered morphologically, and collapsed, so it is very difficult to see precisely the structures that are interesting for

analysis. The advantage is that the cells do not move under laser focus.

Theoretically, it is possible to embed the cells in a solid medium, but many solvents can extract the interesting product so that it is lost during preparation. The research into good preparation conditions continues. The medium has to be hydrosoluble, must not be fluorescent and must give a minimal Raman diffusion spectrum.

With the Raman microprobe, it is not possible to identify particles below a size of 1 or 2  $\mu\text{m}$ . The concentration of the substance must be sufficiently high; this last condition is always fulfilled when we study precipitated or crystalline material in the cell. It is necessary to know the reference spectrum of a substance to identify it in the cell.

So we think that Raman spectrometry can be a very interesting method for biological studies which involve looking for chemical changes in studying their crystalline configuration.

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## Neurosecretory system and storage of paraldehyde fuchsin positive neurosecretory material in the dorsal aorta in an earwig, *Anisolabis annulipes* Lucas (Dermaptera: Labiduridae)

Y. N. Singh and R. Narain<sup>1</sup>

Department of Zoology, University of Allahabad, Allahabad-211 002 (India), 22 October 1979

**Summary.** Each of the 2 groups of medial neurosecretory cells has 10–12 A and 2–3 B-cells. Each pars intercerebralis lateralis has 3–4 B-cells only. The 2 nervi corporis cardiaci I (Ncc I) join with the lateral wall of the aorta and Ncc II terminate in corpora cardiaca (Cc). Only 1 corpus allatum is present. The paraldehyde fuchsin positive neurosecretory material is stored in the dorsal aorta and not in the Cc which indicates the neurohaemal nature of the aorta.

In most of the insect orders the paraldehyde fuchsin positive (PF-positive) neurosecretory material (Nsm) produced by the medial group of brain neurosecretory cells (Nsc) is stored in the corpora cardiaca (Cc), but in Dermaptera divergent views have been given regarding the storage of the Nsm<sup>2–8</sup>. Ozeki<sup>2</sup> and Gabe<sup>3</sup> pointed out that Cc stores the medial as well as lateral neurosecretion in all Dermaptera, whereas Awasthi<sup>5,6</sup>, studying 2 species of earwigs, found Nsm in the dorsal aorta only. These variations suggested it might be useful to gather more information on this topic, and the present communication describes the neurosecretory system and storage organ for Nsm in the earwig *A. annulipes*.

**Materials and methods.** Earwigs were reared in the laboratory on sliced bread. The brain and endocrine aortal complex was taken out by dissecting the insects in Bouin's fixative, and fixed for 18–24 h. The fixed materials (both bulk preparations and sections) were stained in PF (Ewen<sup>9</sup>) and sections were cut 6–8  $\mu\text{m}$  thick.

**Observations.** The neurosecretory cells. Each protocerebral hemisphere of *A. annulipes* bears a group of median Nsc. Each group consists of 10–12 compactly arranged A-cells and 3–4 B-cells which are somewhat oval in shape. Nsc are

lodged very close to the periphery of the brain and are dorsal in position. The pars intercerebralis lateralis has 2–3 B-cells which are smaller in size than the median B-cells. Cyclic secretory activities have been observed in A-cells only. In a freshly moulted earwig these cells have a very small amount of Nsm, which gradually increases as the insect becomes older and is maximal during mating and oviposition.

The neurosecretory pathways. The axons of each group of Nsc in the brain converge and form a medial neurosecretory pathway (Mnp) (figure 1) in each brain lobe. The 2 pathways of the 2 brain lobes cross over. Posterior to the crossing point they run separately on the dorsal side of the protocerebrum and emerge as nervi corporis cardiaci I (Ncc I). Each Ncc I enters the dorsal aorta on its lateral side. Similarly 2 short Ncc II arise from the posterior region of the brain and join with the anterior region of the Cc.

The dorsal aorta. The aorta is located above the Cc and the corpus allatum (Ca) and is full of Nsm. The Cc and Ca lack PF-positive Nsm. The wider anterior part of the aorta receives the neurosecretory axons of the Ncc I from the lateral sides. This region has many more neurosecretory granules than the posterior region. The granules are totally

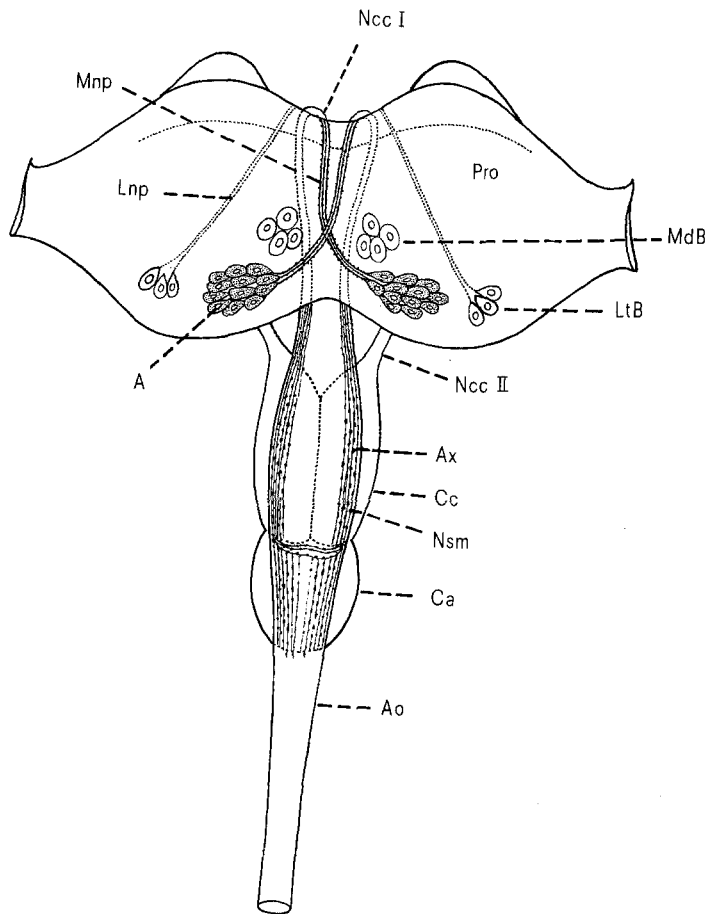


Fig. 1. Diagram of the neurosecretory system and retrocerebral endocrine glands of *A. annulipes* Lucas. A, A type neurosecretory cells; Ao, aorta; Ax, axons; Ca, corpus allatum; Cc, corpora cardiaca; LtB, lateral B-type Nsc; Lnp, lateral neurosecretory pathway; MdB, median B-type Nsc; Mnp, median neurosecretory pathway; Ncc I and II, nervi corporis cardiaci I and II; Nsm, neurosecretory material; Pro, protocerebrum.

lacking in the region posterior to the level of Ca. The amount of Nsm in the aorta of a nymph is less than in the adult.

The corpora cardiaca and corpus allatum. Each Cc is an elongated body lying on the dorsal surface of the oesophagus. The 2 Cc are closely apposed medially but never fuse completely. Each is composed of oval or spherical secretory cells of variable size. Their secretion stains orange green with PF-counter stain. The Ca is an oval to globular body which is attached to the posterior part of the Cc. A thin noncellular membrane encircles the central cellular region. These cells are small, alike and PF-negative.

**Discussion.** The basic plan of the neuroendocrine aortal complex of *A. annulipes* closely resembles that of gymnoceratan Heteroptera<sup>4</sup>. The medial A and B-cells are similar to those of *Anisolabis maritima*<sup>2</sup>, *Labedura riparia*<sup>5</sup> and *Euborellia annulipes*<sup>6</sup>. Ozeki<sup>2</sup> reported the fusion of Ncc I and Ncc II, and the former joining to the storage part and the latter to the secretory part of the Cc. On the other hand Gabe<sup>3</sup> pointed out that both of these nerves join the rostral part of the Cc in Dermaptera. Awasthi<sup>5-7</sup> reports the entrance of Ncc I into the cephalic aorta and Ncc II joining the Cc of the ipsilateral side. The Nsm of the medial Nsc is found in the cephalic aorta whereas the Cc completely lack PF-positive Nsm. We find that in *A. annulipes*, the dorsal aorta is responsible for the storage of PF-positive Nsm of

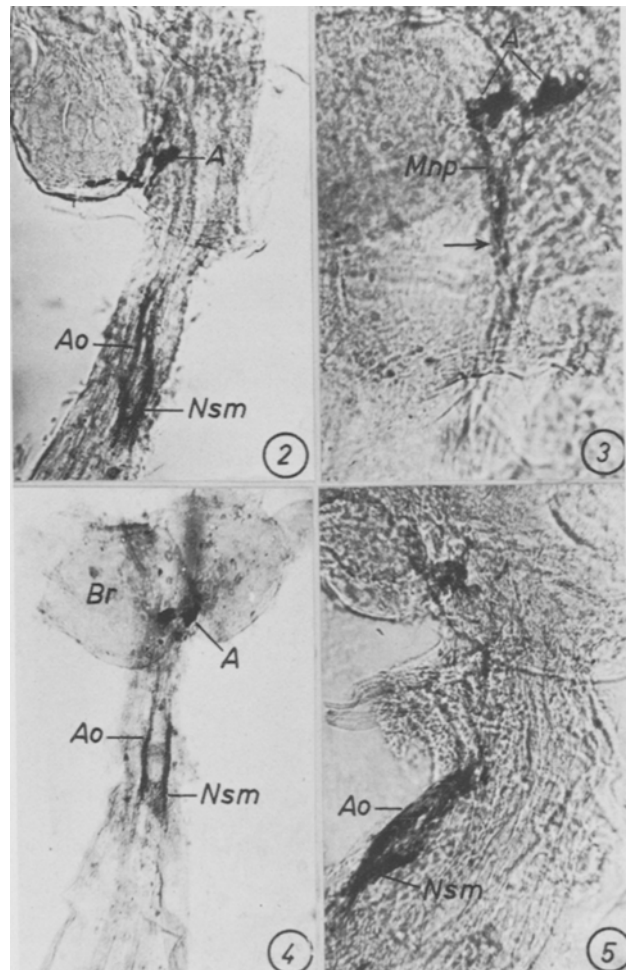


Fig. 2. Whole mount of brain and retrocerebral endocrine aortal complex of adult showing the presence of Nsm in the A-cells (A) and aorta (Ao).  $\times 58$ . Fig. 3. Whole mount of brain of adult female showing 2 groups of A-cells and crossing over of Mnp on the dorsal side of the protocerebrum ( $\rightarrow$ ).  $\times 116$ . Fig. 4. Whole mount of brain and retrocerebral endocrine aortal complex of a male during mating. Note the neurosecretory axons and aorta heavily loaded with Nsm.  $\times 18$ . Fig. 5. Whole mount of brain and retrocerebral endocrine aortal complex of female 1 day after laying. Note the presence of large amount of Nsm in the aorta.  $\times 365$ .

the medial A-type Nsc whereas the PF-negative Nsm of lateral groups of Nsc is stored in the Cc along with their intrinsic secretion. Thus our observations support Awasthi<sup>5-7</sup> and are contrary to those of other authors<sup>2,3,8</sup>. As in *A. annulipes*, Lhoste<sup>8</sup> observed a maximum amount of Nsm in the medial Nsc of *Forficula auricularia* during copulation and oviposition.

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