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Short Communication

A new gland associated with the retrocerebral complex of the adult corn earworm, *Helicoverpa zea*

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ABSTRACT

We report the discovery of a single-celled putative new gland associated with the retrocerebral complex in the adults of *Helicoverpa zea*. The gland was not observed in *Manduca sexta* and few other species of moths. The pair of glands, each 50.6 ± 5.5 μm in diameter, is located on either side of the recurrent nerve. Each gland is connected on one end through a fine nerve to the nervus corporis cardiaci-3 (NCC-3) and at the opposite end to the corpora allata through a thin fiber. The gland is composed of a giant cell with a large nucleus. The cytoplasm has an abundance of mitochondria in addition to dense bodies, electron lucent spheres, concentric whorls of rough endoplasmic reticulum and few vacuoles. At this stage we have no idea as to the function of this new gland.

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1. Introduction

The cerebral neuroendocrine system of insects which consists of the brain and a retrocerebral complex controls most of the aspects of their behavior and physiology (Raabe, 1983). The retrocerebral complex typically consists of a pair of corpora cardiaca (CC) and corpora allata (CA). The CC are primarily neurohemal structures with axonal endings from brain neurosecretory cells as well as their own intrinsic cells (Bowers and Johnson, 1966; Pipa, 1983; Copenhaver and Truman, 1986). In Lepidoptera, several nerves including nervi corporis cardiaci 1 and 2 (or NCC-1 + 2; a combination of the two nerves), NCC-3 and NCC ventralis (NCC-V) enter the CC (Hinks, 1970; Nijhout, 1975; Copenhaver and Truman, 1986). The CA are the sites of juvenile hormone (JH) synthesis.

While working with the corn earworm moths, Helicoverpa zea, it was noticed that a pair of very small perfectly spherical bodies were present in the vicinity of the CC–CA complex. Most of the earlier work on lepidopteran nervous system was carried out in *Manduca sexta* (Eaton and Dickens, 1974; Nijhout, 1975; Copenhaver and Truman, 1986; Eaton, 1988; Davis et al., 1996). None of these authors have described any structure resembling the one we found in *H. zea*. Earlier it was reported that after running anteriorly the NCC-V in *M. sexta* formed a small swelling containing 2–3 intrinsic cell bodies (Eaton and Dickens, 1974; Copenhaver and Truman, 1986). However, this swelling was not connected to the CA. We report the discovery of a new putative gland in *H. zea*, its tentative neural connections and fine structure.

2. Materials and methods

2.1. Insects

H. zea pupae were provided by Dr. Juan Lopez of USDA-ARS, Areawide Pest Management Research Unit, College Station, TX and maintained in an environmental chamber at16L (26 °C):8D (22 °C) and 60–65% RH. Adults were provided 10% sucrose solution for feeding. In addition several species of moths were collected around lights at night in New Orleans, LA or obtained from other researchers. Known or identifiable species among these were *M. sexta* (Sphingidae), *Spodoptera exigua* (Noctuidae) and *Estigmene acrea* (Arctiidae).

2.2. Anatomy and transmission electron microscopy

The brain–retrocerebral complex was dissected in phosphate buffered saline from adults of *H. zea*. Gland dimensions and their neural connections from about 40 moths of both sexes were examined under a dissecting microscope. Five pairs of the new gland together with the attached CC and CA (for the purpose of orientation and handling) were cleared of other tissues and fixed in Karnovsky's fixative for 1 d. The tissues were washed in 3–4 changes of 0.05 M phosphate buffer, postfixed in buffered 2%

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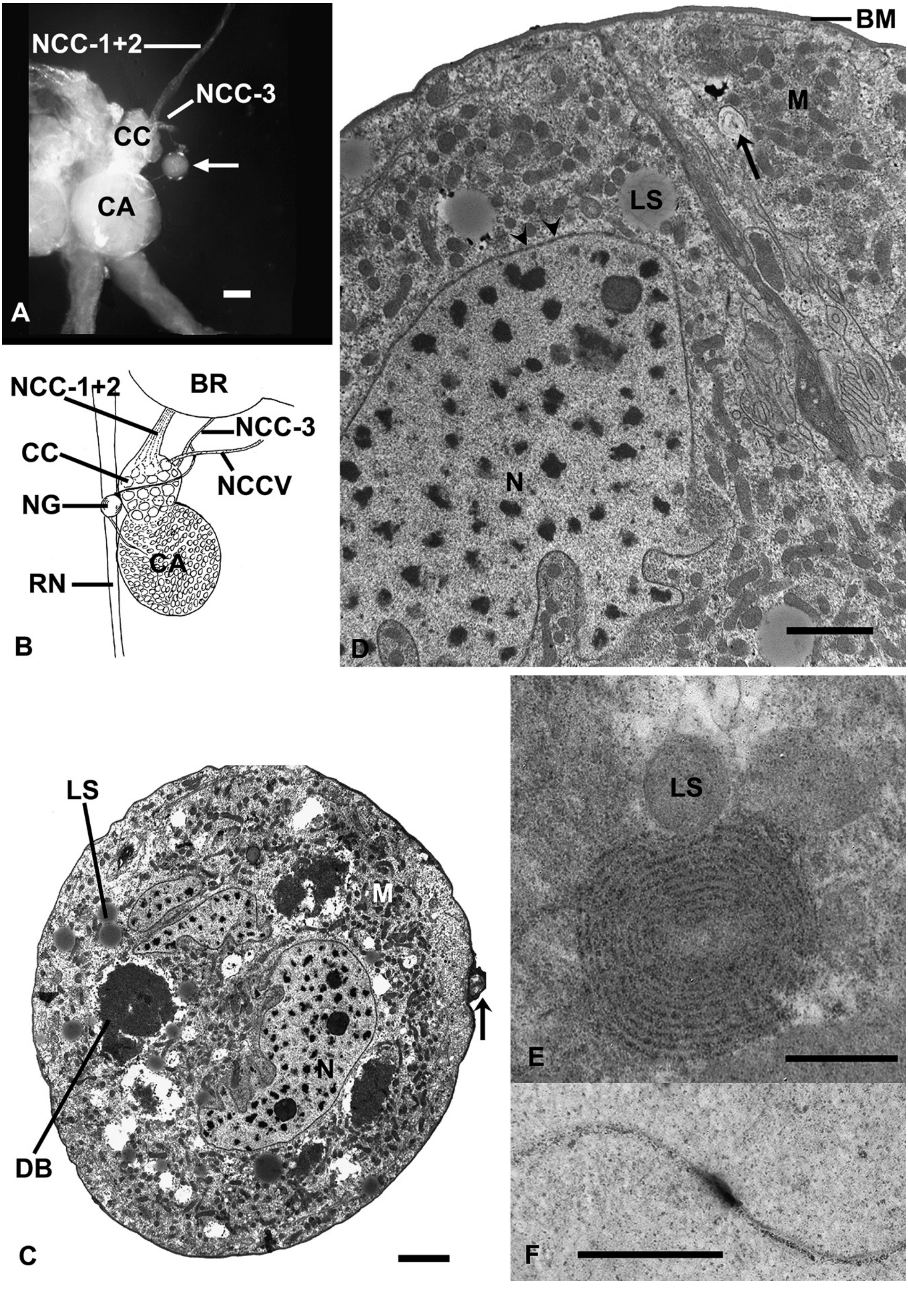
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osmium tetroxide for 2 h, dehydrated in a graded ethanol series, and infilterated with and embedded in Spurr's low-viscosity medium. Ultrathin sections (90 nm) were cut on a Reichert/AO Ultracut (Leica Microsystems, Deerfield, IL) microtome with a Diatome diamond knife. The sections were stained with 2.5% uranyl acetate for 30 min, followed by 3% lead citrate for 5 min. Sections were viewed in a Hitachi H-7000 (Hitachi, Tokyo, Japan) transmission electron microscope. Images were captured with an AMT Advantage 4.0 CCD camera system.

3. Results

During routine dissection of the brain–retrocerebral complex of adult *H. zea*, we often found a pair of very small spherical structures on either side of the recurrent nerve. However, in dissections of other moth species, we did not find these glands in *M. sexta* but they were invariably present in some of the Arctiids such as the tiger moth *E. acrea*. In *H. zea*, the gland has an average diameter of 50.6 ± 5.5 μm (range 42–59 μm, $N = 20$) (Fig. 1A), and it is connected through a fine fiber to the middle region of the CA on its side (Fig. 1A and B). At the opposite end, the gland has a knob-like projection that is connected to the NCC-3 through a fine nerve.

Electron microscopy of the gland revealed that it consists of a single giant cell having a nucleus with lobes or infoldings (Fig. 1C). The gland is enclosed by a 0.11 μm thick basal lamina (Fig. 1D). The nucleus has an extensive convoluted membrane with many pores, several nucleoli and very distinct and dispersed chromatin. The cytoplasm contains several dense bodies, of which one is particularly prominent and visible even under phase contrast (Fig. 1C). The cytoplasm also contains large number of mitochondria, glycogen-like granules, smooth endoplasmic reticulum and many electron lucent spheres with an average diameter of 1.7 μm (Fig. 1C and D). In addition there are vacuoles, some of which appear to contain remnants of membranes. Occasionally, one can see concentric whorls of rough endoplasmic reticulum associated with electron lucent spheres (Fig. 1E). The cytoplasm may also rarely contain a desmosome (Fig. 1F).

The NCC-3, to which the gland is attached by a fine nerve, contains many axons with three types of neurosecretory granules (NSG) (Fig. 2A) and glial cells with prominent nuclei. Of the electron dense granules, the larger and the smaller granules measure 0.17 and 0.07 μm in diameter, respectively. There are also electron lucent granules measuring an average of 0.08 μm in diameter. The connecting fine nerve, approximately 1.5 μm thick, contains small electron dense and electron lucent NSG (Fig. 2B). At the junction of the nerve connecting NCC-3 to the gland, the cell membrane appears to split creating a pocket and perhaps gives the appearance of a knob (Fig. 2A). The connecting nerve internally divides into two chambers. One of these chambers forms a funnel-like depression opening through the membrane into the gland. At the opposite end of this neural connection is a fine fiber (1 μm thick) connecting the gland to the CA. Ultrastructure of this fiber shows that it does not have the characteristics of a typical nerve and that it contains no NSG (Fig. 2C). Actually the fiber looks like a sensillum with a basal

Fig. 1. One side of the retrocerebral complex of adult *Helicoverpa zea*. (A) Dissected specimen. CA – corpus allatum, CC – corpus cardiacum, NCC-1 + 2 – nervi corporis cardiaci-1 + 2, NCC-3 – nervus corporis cardiaci-3, arrow – the new gland. Scale bar = 50 μm. (B) Diagrammatic representation of A. BR – brain, RN – recurrent nerve, NG – new gland. (C) TEM of a cross-section of the gland. DB – dense body, LS – electron lucent sphere, M – mitochondria, N – nucleus, arrow – point at which the connecting nerve from NCC-3 attaches to the gland. Scale bar = 5 μm. (D) Higher magnification showing the basal lamina (BM), abundance of mitochondria, nuclear pores (arrow heads) and vacuole with empty membranes (arrow). Scale bar = 2 μm. (E) Concentric rings of rough endoplasmic reticulum in association with electron lucent spheres. Scale bar = 0.5 μm. (F) A desmosome in the cytoplasm of the gland. Scale bar = 0.5 μm.

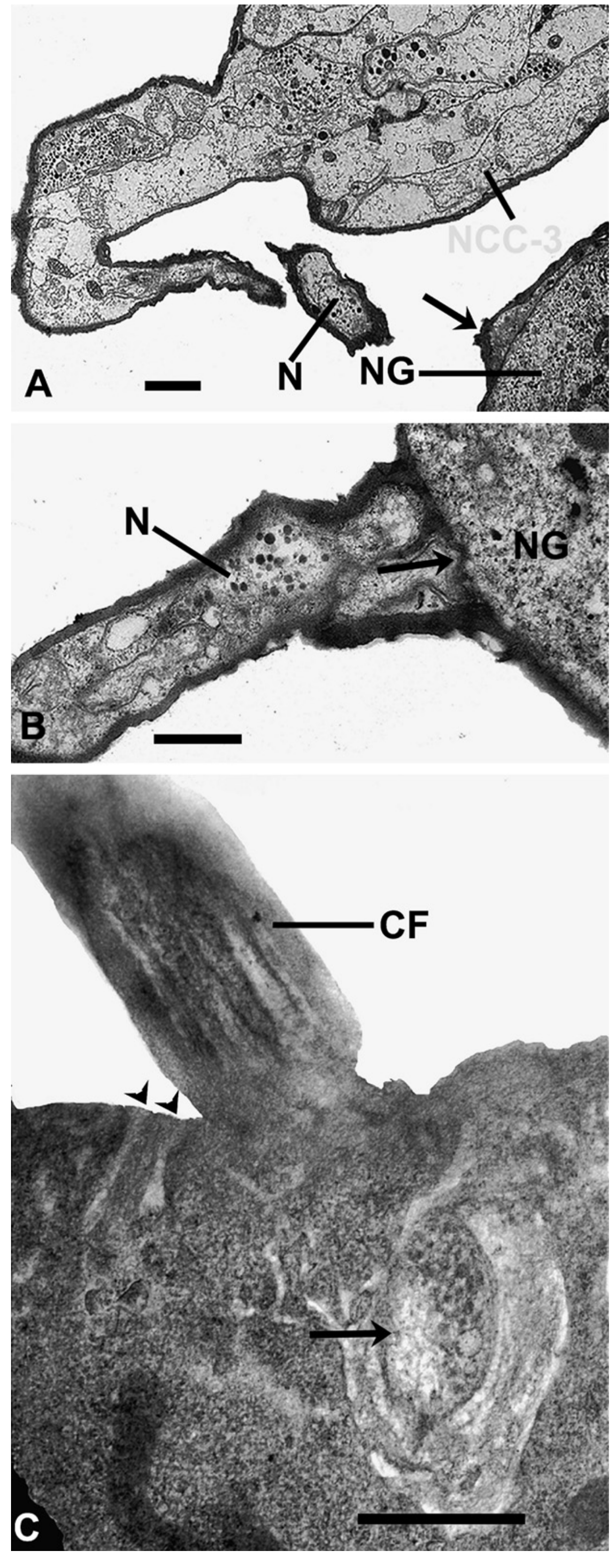

Fig. 2. TEM of the nerve and a fiber connecting the gland to NCC-3 and CA. (A) Connection of NCC-3 to the gland (NG) via a fine nerve (N). The basal lamina at the point of contact of the nerve shows a bulge (arrow) Scale bar = 2 μm. (B) Nerve from NCC-3 connected to the gland. Both electron dense and lucent granules are visible in the nerve. Arrow shows the funnel-like projection of the nerve into the gland. Scale bar = 1 μm. (C) The fiber (CF) connecting the gland to the CA. Within the gland there is a sensillum like structure at the base of the fiber (arrow). There are also invaginations (arrow heads) in the gland surface in proximity to where the fiber is attached. Scale bar = 1 μm.

neuron like structure. Adjacent to the connecting fiber, several invaginations in the surface of the gland are also visible.

4. Discussion

In spite of extensive studies conducted on the structure of brain–retrocerebral complex of insects including lepidopteran species, the structure described here for the first time in adults of the corn earworm, *H. zea*, had not been previously reported. It appears that unlike CC and CA this putative gland is not present in all insect species. Apparently the structure described by Eaton and Dickens (1974) and Copenhaver and Truman (1986) as a swelling containing 2–3 cell bodies in the NCC-V of *M. sexta* is not the gland we report in *H. zea*. Similarly it is different from the cluster of cell bodies that Hinks (1970) described in adult Noctuids as being present at the point of division of NCC-2 dorsalis, a nerve arising from NCC-2. Cazal (1948) in his monograph on the retrocerebral glands in Insecta described an accessory nerve arising from each NCC-2 with no indication of its distal termination.

The giant cell has many characteristics such as the presence of electron lucent spheres, some in association with rings of endoplasmic reticulum, indicating that this may indeed be a glandular structure. Presence of vacuoles containing remnants of membranes suggests that the material from these spheres may possibly be released within the gland itself. Structure of the nucleus is suggestive of very high activity. The fact that neurosecretory material (both electron dense and electron lucent granules) present in the fine nerve connecting the gland to NCC-3 indicates that the CC or the material passing through it may be exerting some influence on the gland's function. However, we did not find the electron dense NSG in the gland itself. At the opposite end, the gland is connected by a fiber to the middle region of CA. This particular connection looks like sensory in nature suggesting the possibility of this structure being a stretch receptor. However, at this time we have no concrete evidence as to the function of this gland.

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