NEUROSECRETORY CELLS OF THIRD-INSTAR LARVAE OF *ANASTREPHA OBLIQUA* MACQUART (DIPTERA, TEPHRITIDAE)

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ABSTRACT. Neurosecretory cells of the nervous system of third-instar larvae of *Anastrepha obliqua* Macquart, 1835 were located and described histomorphologically. Six groups of neurosecretory cells were identified in the brain and one group in the ventral ganglion. The groups differed in position, cell size and staining characteristics. Four of these groups appear to be active throughout the third instar and three, from the prepupal period.
KEY WORDS. *Anastrepha obliqua*, fruit fly, neurosecretory cells

Neurosecretory cells are classically defined as neurons that synthetize peptides (neurohormones), which are released into the bloodstream and act at a distance (SCHARRER 1977; KRIEGER 1983; LAFONT 1991). Today, however, it is also know that the same neuron can produce peptides as well as monoamines, and that a neurosecretion may also act as neurotransmitters and neuromodulators (HÖKFELT *et al.* 1980; ORCHARD 1982; RAABE 1989; ORCHARD *et al.* 1992).

Neurosecretory system, in essence, takes information from outside and inside the animal, screens, processes, and integrates everything, and then directs the appropriate action or activity (GRIER 1984). Specifically during the larval stage, it regulates the initiation of the moulting process and later growth by controlling the activities of endocrine glands (NORMAN 1965; CAZAL *et al.* 1971; BLIGHT & WENHAN 1976; GILBERT *et al.* 1980; GRANGER & BOLLENBACHER 1981; AGUI *et al.* 1980; SEDLAK 1985; HENRICH *et al.* 1987). Futhermore, at the end of the larval stage, it induces differentiation of adult primordia (LOCKE 1981), and metamorphosis events (ZDAREK 1980).

Neurosecretory cells are present in the entire nervous system (RAABE 1989; ORCHARD & LOUGHTON 1985), and analogous groups have been detected in several insects.

A survey of the literature showed the absence of studies on the neurosecretory cells of *A. obliqua* Macquart, 1835. This fruit fly is a pest, whose larval stage causes enormous damage to the fruit-growing industry, and studies of its neurosecretory system are essential for the understanding of its physiology of development. So this paper describes histomorphologically the PF-positive neurosecretory cells of third-instar *A. obliqua* larvae.

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MATERIAL AND METHODS

A. obliqua larvae were obtained in the laboratory from wild adults infesting *Spondias purpurea* Linnaeus (ceriguela) fruits collected and maintained according to the methods used by BRESSAN (1981), POLLONI (1981) and SILVA *et al.* (1985).

Developing larvae were maintained on nutritionally efficient diet (diet-2) (SIMÕES-JORGE 1987), which contained a mixture of brewer's yeast, sucrose, starch, agar, propionic acid, nipagin and distilled water. Larvae were kept in a wood chamber with a thermostat maintaining at temperature of 25°C and with approximately 75% relative humidity. Larval stages were recognized on the basis of morphology and colour of buccal hooks according to TELES DA SILVA (1978).

On each day of the third larval instar, except the third and 11th days, ten larvae were dissected and the complex brain-ventral ganglion-Weismann gland set was fixed in Bouin's for 24 hours. After standard embedding in paraffin, longitudinal and transversal serial sections were cut and stained by the paraldehyde-fucsin technique of EWEN (1962) for the neurosecretory products of insects. All larvae, which were used in the present work, had passed to third instar on the sixth post-hatching day. Furthermore, the larvae of the same age used in the present study were those which presented similar weight and length.

Different groups of neurosecretory cells in the third instar were determined and phases were described on the basis of the cytoplasmic staining of these cells. Neurosecretory cells belonging to the various widest and shortest cellular and nuclear diameters were measured.

RESULTS

Seven groups of neurosecretory cells were identified in the nervous system of third-instar *A. obliqua* larvae. Of these, six were found in each brain hemisphere and one in the ventral ganglion (Fig. 1), as described below:

GROUP 1 - is formed by two cells located in the dorso-anterior region of the brain close to the junction of the two brain hemispheres in the pars intercerebralis (Fig. 1 a-a'). These cells have a maximum cell size of 4x5μm and a maximum nuclear size of 2x3μm (Fig. 2).

GROUP 2. Consists of one cell located in the ventro-anterior region of the brain hemispheres but in the same direction as that of group 1 (Fig. 1 a-a'). The maximum cell and nuclear diameters of this cell are 4x6μm and 2x3μm, respectively. Vacuoles are normally observed in these cells (Fig. 3).

GROUP 3. Has nine to fourteen cells located in the dorso-anterior region of the brain hemisphers (Fig. 1 a-a'). The maximum approximate cell diameters of these cells are 5x7μm, and the nuclear diameters, 2x3μm. These cells may present vacuoles (Fig. 4).

GROUP 4. Has four small cells more or less oval in shape with maximum cellular and nuclear sizes of about 3x4μm and 2x3μm, respectively. This group is detected in the dorso-lateral region of the brain hemispheres (Figs 1 b-b'; 5).

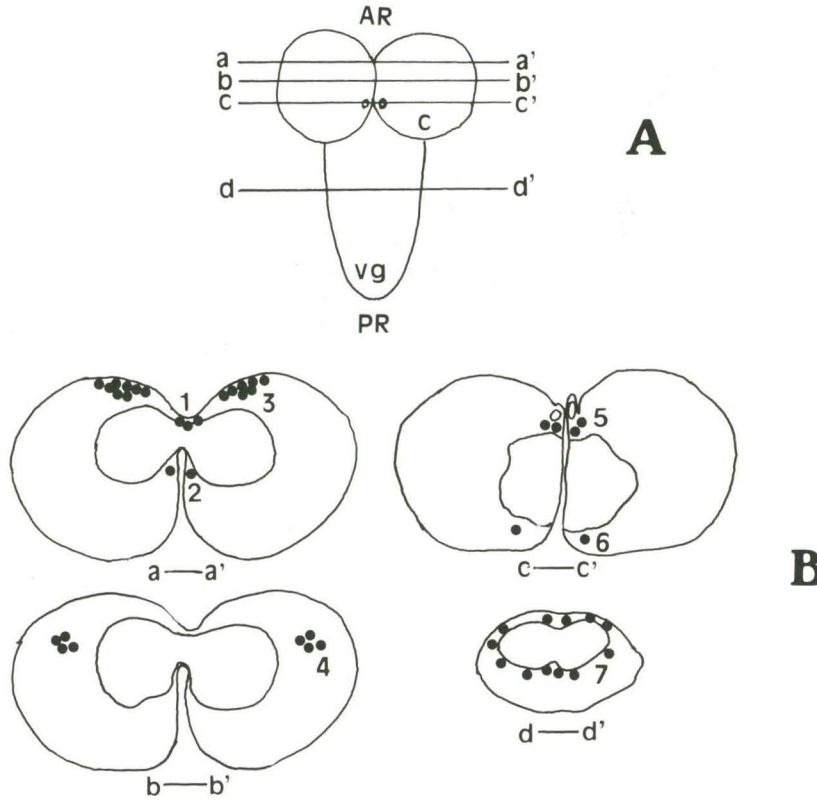


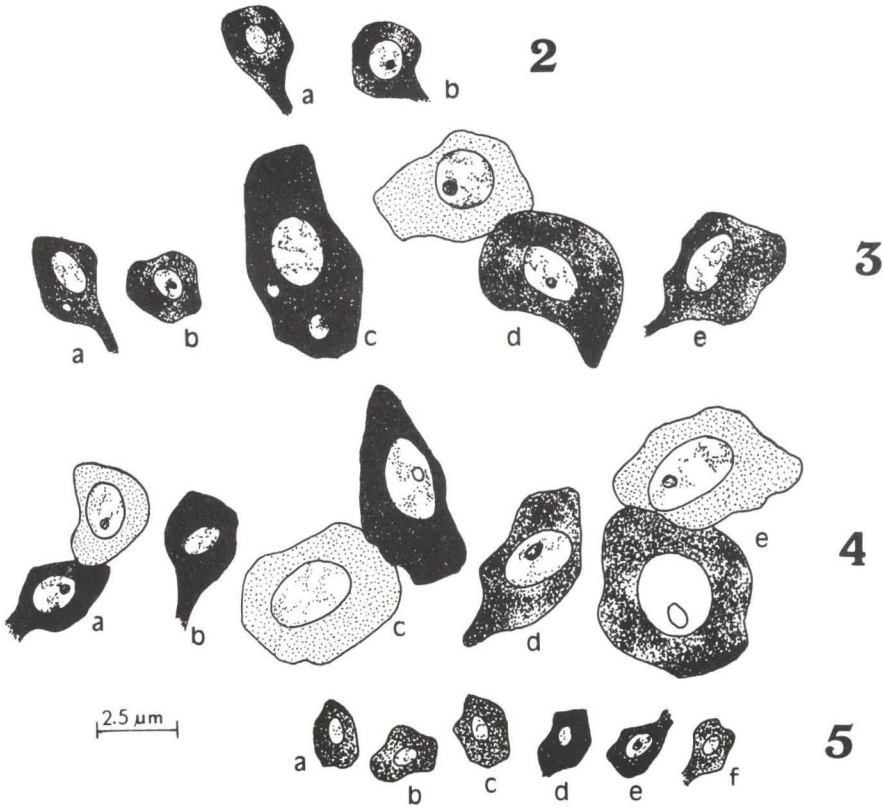
Fig. 1. (A) Schematic dorsal view of the nervous system of third-instar *Anastrepha obliqua* larvae showing the position of the transversal sections a-a', b-b', c-c' and d-d'. (B) Diagrams showing the location of neurosecretory cell groups (black dots) in the nervous system of third-instar *A. obliqua* larvae, according to the sectioning planes. c: brain; vg: ventral ganglion; 1-7: neurosecretory cell groups; AR, PR: anterior and posterior regions.

GROUP 5. Consists of two cells located close to the tracheal opening in the dorso-posterior region of the brain (Fig. 1 c-c'). Their approximate maximum cellular and nuclear sizes are 3x5μm and 2x3μm, respectively (Fig. 6).

GROUP 6. Is formed by one cell located in the ventro-posterior region of the brain (Fig. 1 c-c') which measures a maximum of 3x5μm and has a nucleus of 2x3μm (Fig. 7).

GROUP 7. Has various cells distributed along the ventral ganglion and always around the medullary region of the latter (Fig. 1 d-d'). These cells reach cell diameters of 3x4μm and nuclear diameters of 1x2μm (Fig. 8).

The neurosecretory cells detected in the brain hemispheres (group 1-6) are all unipolar, whereas those located in the ventral ganglion (group 7) are bipolar (Figs 2-8).



Figs 2-5. Schematic light-camera drawing of groups 1, 2, 3, 4 and 5 neurosecretory cells of third-instar *Anastrepha obliqua* larvae, respectively, aged one (a), two (b), seven (c), eleven (d) fourteen (e), seventeen (f) and twenty-one (g) days of life, showing variations in the amount of neurosecretory present in the cytoplasm (black dots) and increasing cell size with larval age.

During the first days of the third larval instar, group 2 and 3 cells underwent a considerably marked increase in volume (Figs 3 and 4). The same was observed for groups 1 and 4 (Figs 2 and 5). Groups 5, 6 and 7 were only identified during the prepupal phase.

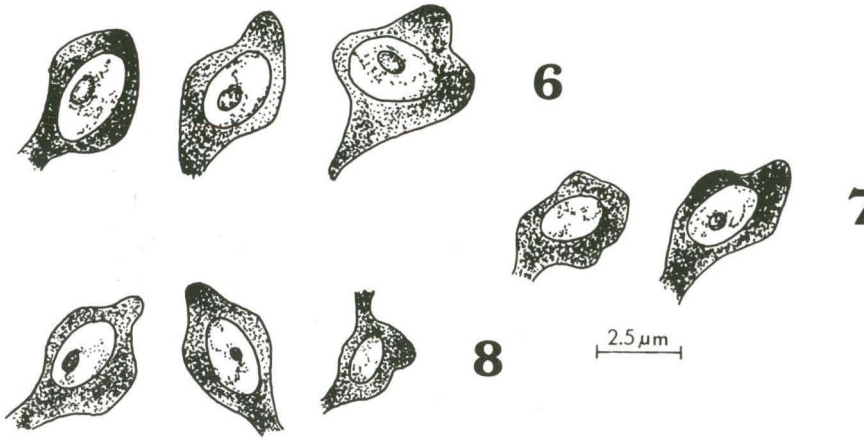
The neurosecretory cells of groups 1-4 showed changing cytoplasm staining during the course of the instar (Tab. I, Figs 2, 3, 4 and 5). These variations were represented by the following symbols:

0 – the cytoplasm stains green.

\+ – the cytoplasm stains light gray.

\+ + – the cytoplasm stains purple but staining is not uniform, *i.e.*, there are regions with deeply stained granules and regions with only a few or no stained granules. At times, stained material can be observed in the axon region.

\+ + + – the cytoplasm is fully stained purple and is completely filled with



Figs 6-8. Schematic light-camera drawing of group 5, 6 and 7 neurosecretory cells of white *Anastrepha obliqua* prepupae.

Table I. Cytoplasm staining in neurosecretory cells of groups 1, 2, 4, 5 , 6 and 7 from third-instar *Anastrepha obliqua* larvae and prepupal phase (PP).

A	1	2	4	5	6	7
01	+ +	+ +	+	0	0	0
02	+ +	+ +	+ +	0	0	0
04	+ + +	+ + +	+ + +	0	0	0
05	+	+	+	0	0	0
06	0	+ +	0	0	0	0
07	+	+ +	+ +	0	0	0
08	0	+ + +	0	0	0	0
09	+ +	+ +	0	0	0	0
10	+	+ + +	+	0	0	0
11	+	+ +	+	0	0	0
12	+ +	+ +	+ +	0	0	0
14	0	+ + +	+ +	0	0	0
16	+	+ + +	+	0	0	0
17	+ +	+ + +	+ +	0	0	0
19	0	+ +	+ +	0	0	0
20	+ +	+ +	+ +	0	0	0
21	0	+ + +	+ + +	0	0	0
PP	+ +	+ + +	+ +	+	+ +	+ +

A: Third-instar days.
0: Green-staining cytoplasm; +: light gray cytoplasm; + +: purple cytoplasm with instained regions; + + +: uniformly purple-stained cytoplasm.

Table II. Number of group 3 neurosecretory cells in each brain hemisphere (B) of the third-instar *Anastrepha obliqua* larvae of identical and different ages (A), and variations in cytoplasm staining in the cells of each hemisphere (C).

A	B	C						
01	09-09		9++		-		9++	
02	10-10	5+	5++		-	5+	3++	
04	10-10	2+	5++	3+++	-	4+	4++	2+++
05	12-10	6+	6++		-	6+	4++	
06	11-10	9+	2++		-	9+	1++	
07	11-10	6+	4++	3+++	-	5+	3++	2+++
08	11-10	7+	3++	1+++	-	6+	4++	
	10-10	6+	4++		-	8+	2++	
09	11-11	6+	5++		-	6+	5++	
10	11-11	8+	3++		-	9+	2++	
	11-10	8+		3+++	-	7+	2++	1+++
11	11-10	6+	5++		-	5+	5++	
	11-10	7+	3++	1+++	-	6+	2++	2+++
12	12-12	7+	2++	3+++	-	6+	2++	4+++
14	11-10	8+		3+++	-	6+		4+++
16	11-10	6+	2++	3+++	-	6+		4+++
	12-11	6+	3++	3+++	-	6+	3++	2+++
17	10-10	6+	3++		-	6+	3++	1+++
	14-11	7+	5++	3+++	-	8+	3++	
18	13-12	8+	4++	1+++	-	8+	3++	1+++
20	14-12	9+	5++		-	5+	6++	1+++
21	11-10	6+	4++	1+++	-	5+	6++	
PP	11-10		9++	2+++	-		10++	

+: light gray cytoplasm; ++: purple cytoplasm with instained regions; +++: uniformly purple-stained cytoplasm.

densely accumulated stained granules. At times the nucleus is poorly visible due to the accumulation of surrounding stained material.

The difference in cytoplasm staining as also detected between group 3 cells in the same hemisphere and in opposite hemispheres of the same individual or of distinct individuals (Tab. II):

The cytoplasm of the neurosecretory cells of groups 5, 6 and 7 was stained light purple (++).

Purple-stained material was detected in the cytoplasm of prothoracic gland cells and in the corpora cardiaca region of white prepupae.

DISCUSSION

The groups of PF-positive neurosecretory cells of the nervous system of *A. obliqua* larvae show correspondence in the location, staining and number of cells with those of *Lucilia caesar* Linnaeus, 1758, *Sarcophaga bullata* Parker, 1916

(Sarcophagidae) and *Calliphora erythrocephala* Meigen, 1830 (Calliphoridae) larvae (all of them Diptera Cyclorrhapha) studied by FRASER (1959 a,b), DOGRA & TANDAN (1965) and VERNIER & VERNIER (1969), respectively.

Groups 1 and 2 of the neurosecretory cells of *A. obliqua* are equivalent to group 1 and 2 of *L. caesar* and *C. erythrocephala*, and group 1 and 3 of *Sarcophaga ruficornis* (Fabricius, 1794). However, it exists in *C. erythrocephala* an additional cell (three rather than two).

Group 3 of *A. obliqua* corresponds to group 2 of *S. ruficornis* and group 3 of *C. erythrocephala*, but these species have a distinct number of cells. This group presents 20-22 cells in *S. ruficornis*, 12 in *C. erythrocephala* and 18-26 in *A. obliqua*. As in *S. ruficornis*, in *A. obliqua* this group does not present correspondence between cell number and variation in cytoplasm staining, between opposite brain hemispheres, or between larvae of the same age. These variations seem to indicate that groups 3 (with 4 cells) and 4 (with 7 cells) of *L. caesar* correspond to group 3 of *A. obliqua*. Thus, the difference in number of group 3 cells among these four species may be due to variations in the amount of neurosecretion.

Group 4 of *A. obliqua* is equivalent to group 5 of *L. caesar*, group 4 of *S. ruficornis* and group 6 of *C. erythrocephala*. However, in *C. erythrocephala* the number of cells in each hemisphere is smaller than in the other species, *i.e.*, there are three rather that four cells.

Group 5 of *A. obliqua* represents group 6 of *L. caesar* and group 5 of *C. erythrocephala*. These species present equal number of cell, i.e., two cells. This group was not observed in *S. ruficornis*.

Group 6 of *A. obliqua* was observed only in *C. erythrocephala*. In this species, group 6 has three cells in each hemisphere, whereas in *A. obliqua* it only has one.

Group 7 of *A. obliqua* was detected only in *L. ceasar*.

The existence of relationship between the PF-positive cells of these species belonging to four different families colaborates the assumption that these neurosecretory cells groups were conserved in the Cyclorrhapha evolutive process.

Although the neurosecretory cells of groups 1, 2, 3 and 4 of *A. obliqua* presented variation in the amount of neurosecretion throughout the stage and even absence of neurosecretion (groups 1 and 4), our data provide no explanation for the origin of these changes, *i.e.*, whether they were due to a process of synthesis or of secretion. However, the continuous presence of neurosecretion in group 2 and 3 during the third instar indicates that these groups may be related to the larval-pupal moult or metamorphosis events.

The presence of stained substances observed inside the cells of the prothoracic gland of *A. obliqua* during the prepupal phase was also observed in *C. erythrocephala* by VERNIER & VERNIER (1969) during the post-feeding period. SLOPER (1957, 1958 *apud* FRASER 1959a) and later FRIEDEL *et al.* (1980) determined that this substance is the amino acid cystine.

Our data show that the NSC of groups 2 and 3 seem to reach their maximum size on the 7th day of third instar. This suggests the possibility that larval system

reach maximum growth during the first days of third instar, a fact that may be advantageous or even necessary for a larva who has the important role of assuring successful pupal development by the accumulation of large amounts of nutritional reserves.

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