

ACTIVITY OF PEPTIDERGIC NEUROSECRETORY NEURONS FROM THE PARS INTERCEREBRALIS IN *MORIMUS FUNEREUS* LARVAE DURING THE INTERMOLT PERIOD. Vera Nenadović, Marija Mrdaković, Jelica Lazarević and Vesna Perić-Mataruga. Siniša Stanković Institute for Biological Research, 11060 Belgrade, Serbia and Montenegro

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The post-embryonic development of insects is under the control of the neuroendocrine system (Sakurai, 1983; Nenadović, 1992; Nijhout, 1999). The main role is played by the prothoracicotrophic hormones (large and small PTTH), ecdysteroids, and juvenile and eclosion hormones (Ishizaki and Suzuki, 1986; Miyoguchi *et al.* 1987; Riddiford, 1981, 1985; Ewer *et al.* 1997; Gammie and Truman, 1999; Gilbert *et al.* 2000; Elkonich and Robinson, 2000).

The larval development time of Cerambycidae is quite long under natural conditions. It lasts for several years and the number of larval instars is variable. For example, a development time of 3-4 years is characteristic of *Morimus funereus* larvae in

the field, while under constant laboratory conditions (temperature of 23°C and artificial diet for *Drosophila*, Roberts, 1989), a development time of 8.5 months and eleven larval instars were recorded (Stanić *et al.* 1989; Nenadović, 1992). Environmental factors (especially food quality and temperature) affect instar number and duration of the intermolt period (Pershing and Lint, 1989; Nenadović, 1992).

The aim of the present work was to investigate the activity of protocerebral medial neurosecretory neurons (A type) during the 6th larval instar in *Morimus funereus* larvae. These neurons were described by Ivanović *et al.* (1975a, 1975b). Depending on morphological and cytological traits, they were divided into four subtypes (Nenadović, 1992). Medial neurosecretory neurons of the 6th instar in *M. funereus* include 56 A1 neurons, 32 A2 neurons, four A1' neurons in the ventromedial region, and six A2' neurons in the dorsomedial region of protocerebrum (Fig 2). It has been found in other insect species that these neurons synthesize allatotropins and allatostatins (Bogus and Scheller, 1994; Veelaert *et al.*, 1995; Gilbert *et al.*, 2000), eclosion hormone (Reynolds, 1983, Gammie and Truman, 1999), small form of prothoracicotrophic hormone (Ishizaki and Suzuki, 1986; Mizoguchi *et al.* 1987; Dai *et al.*, 1994), etc.

Morimus funereus larvae were reared individually under constant laboratory conditions: temperature of 23°C, artificial diet for *Drosophila* (Roberts, 1989), relative humidity of 70%, and absence of light. Under such conditions, the 6th instar lasts for 14 days. The larvae were sacrificed immediately after molting into the 6th instar (0 h) and 6 h and 1, 2, 3, 4, 6, 9, 10, 11, 12, 13, and 14 days after the molting into the 6th instar. After decapitation, heads were fixed in Bouin's solution. The chitinized surface and muscles were removed, and protocerebra were excised. Common histological techniques were employed for embedding in paraffin (Merck, 57-59°C). Serial paraffin sections of 5 µm were stained using Alcian Blue Phloxine and Paraldehyde Thionine Phloxine (Panov, 1980). Analysis of A1, A2, A1', and A2' neurons was performed using a Leitz DMRB light microscope. Three protocerebra were analyzed for each time of the intermolt period.

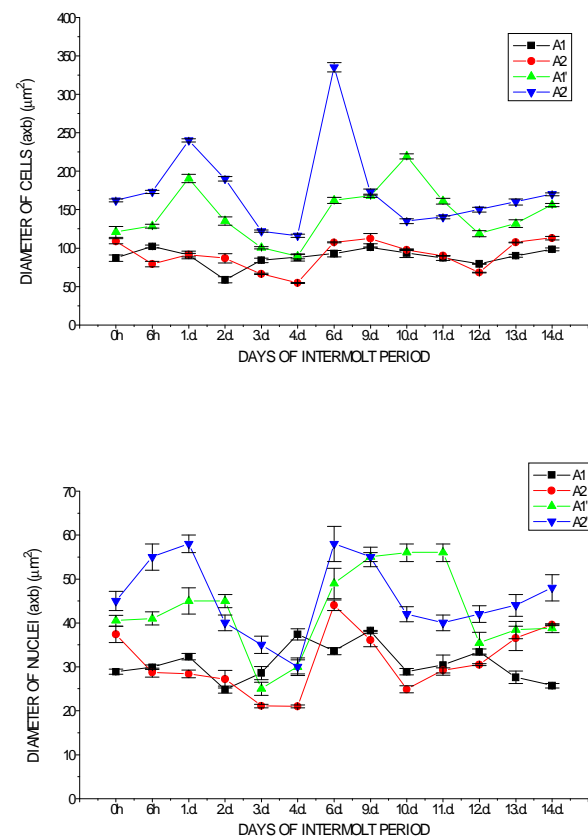


Fig. 1. Diameter of A1, A2, A1' and A2' cells and their nuclei during the intermolt period.

The activity of protocerebral neurosecretory cells was

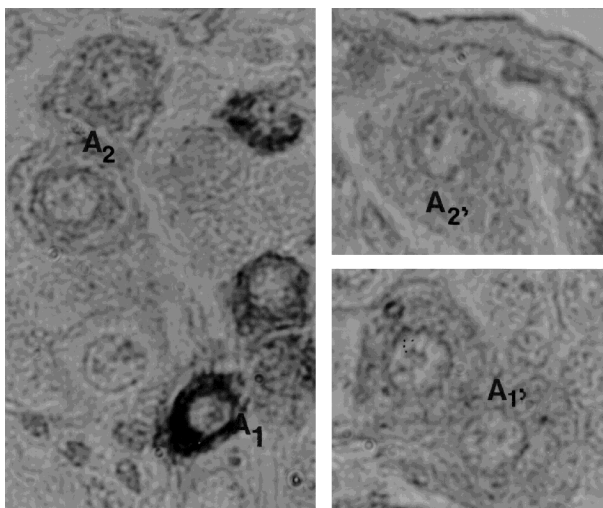


Fig 2. Protocerebral medial neurosecretory neurons (A type) in *Morimus funereus* larvae.

estimated using the following cytological parameters:

- The size of neurosecretory neurons expressed as the mean of the products of the largest and smallest diameters of each neuron (a x b);

- The size of the nucleus, expressed as the mean products of the largest and smallest diameters of each nucleus (a x b).

Increase in the diameter of A1 cells and their nuclei is discernible at the beginning of the intermolt period. The presence of 1-2 large nucleoli, powdery neurosecretory material (NSM) in the perikarya and extended axons points to increased synthesis and fast release of NSM. Already on the second day of the intermolt period, synthesis and release of NSM were observed to be retarded (Figs. 1, 2). Activity increased after that and remained high until the 11th day, when it decreased again. Increased synthesis, but not release was noticed on the next two days. Large agglomerations were formed in both perikarya and extended axons (Fig. 1).

During the first 4 days of the intermolt period, the activity of A2 neurons decreased. On the 4th day of the intermolt period approximately 40% of A2 neurons were without nucleoli in the nuclei and without NSM in the perikarya. Sixty percent of A2 neurons contained a small quantity of powdery NSM and had

one small nucleolus visible in the nucleus. Maximal activity is attained between the 6th and 9th day of the intermolt period. The period between the 10th and 12th day is characterized by moderate synthesis and increased release of NSM. Increased activity of these neurons was recorded before and during molting while a decrease was noticed immediately after molting. On the whole, activity was maximal in the middle of the larval instar and before and during molting (Figs. 1; 2).

The A1' and A2' neurosecretory neurons showed significant oscillations of activity during the intermolt period. Maximal activity of A1' neurons was observed on the 1st and 10th day while activity was minimal on the 4th and 12th day of the intermolt period. High activity of A2' neurons was recorded on the 1st and 6th day while activity was low on the 3rd and 4th day (Figs. 1, 2).

In conclusion, it can be asserted that most neurons attained their highest activity in the middle of the intermolt period. For A2 neurons, activity remained high during molting. The obtained changes in neuron activity point to the synthesis of different neurohormones affecting metabolism, growth, and molting of *M. funereus* larvae.

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