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ACTIVITY OF LATERAL PEPTIDERGIC NEUROSECRETORY NEURONS OF THE *MORIMUS FUNEREUS* PROTOCEREBRUM DURING THE INTERMOLT PERIOD.

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The lateral group of peptidergic neurosecretory neurons in *Morimus funereus* consists of L_1 and L_2 neurons present in the dorsolateral region of the protocerebrum. Their axons inervate contralateral *corpora cardiaca allata* (Ivanović *et al.* 1975a, b; Nenadović, 1992). The L_1 neurons are the smallest peptidergic neurons of the *M. funereus* protocerebrum and are located above the neuropila. Their large nucleus with a large nucleolus is surrounded by a thin level of cytoplasm where large agglomerations of neurosecretory granules are visable. An array of five to seven large L_2 neurons has been noticed above L_1 neurosecretory neurons. In shape, size and basophility, they are similar to medial A_2 neurosecretory neurons (Nenadović, 1992).

By using monoclonal antibodies it has been shown that L_2 neurons synthesize big form of prothoracicotropic hormone (Agui *et al.* 1979; Kawakami *et al.* 1990; Dai *et al.* 1994). Depending on insect species, its molecular weight varies from 11-15kD in *Lymantria dispar* (Kelly *et al.* 1995) to 28-30kD in *Manduca sexta* (Westbrook and Bollenbacher, 1990).

It has been found in some insect species that dorsolateral neurons synthesize allatostatins (Veelaert *et al.* 1995), neurohormones that inhibit biosynthesis of juvenile hormones in *corpora allata* (Bhascaran *et al.* 1990).

The aim of the present work was to investigate activity of dorsolateral neurosecretory neurons of the protocerebrum in *M. funereus* larvae (6th larval instar) during the intermolt period.

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 6h and 1, 2, 3, 4, 6, 9, 10, 11, 12, 13 and 14 days after 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 dorsolateral L_1 and L_2 neurons was performed using a Leitz DMRB light microscope. Three protocerebra were analyzed for each time of the intermolt period.

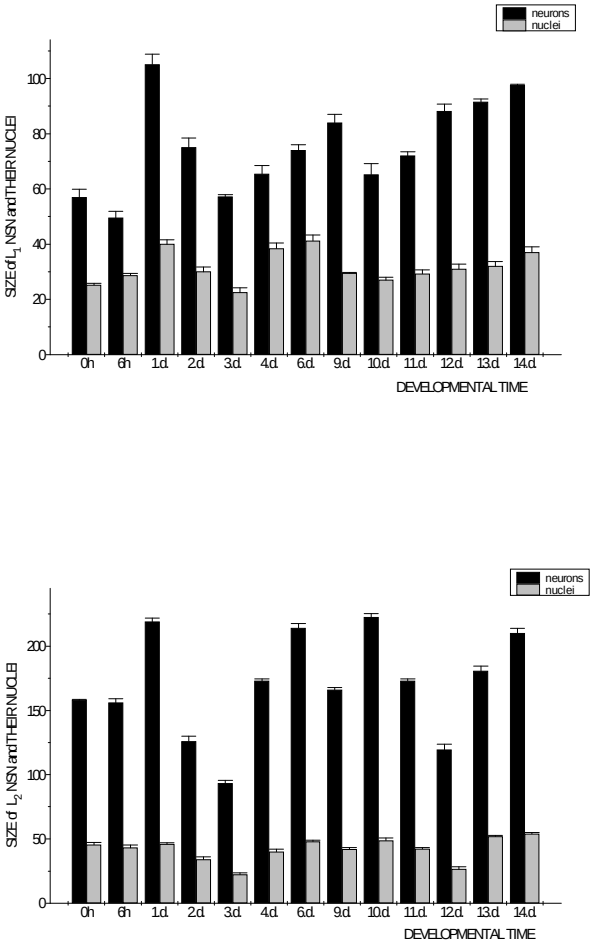

Fig. 1. Size of L_1 (a) and L_2 (b) neurosecretory neurons and their nuclei in the *Morimus funereus* protocerebrum during the intermolt period.

The activity of L_1 neurosecretory neurons was quite low immediately after molting into the 6th larval instar. Maximal activity was recorded on the 1st day of the intermolt period and was succeeded by a decrease in activity on the 2nd and 3rd days.

Activity was moderate from the 3rd to 9th day, when it began to decline lower again until the 12th day. Before the next molting (from the 12th to 14th day), the activity of L_1 neurons rose and showed a maximum on the 14th day of the intermolt period (Fig. 1a). With respect to their morphological and physiological attributes, L_1 neurons of the *M. funereus* protocerebrum most resemble medial A_1 neurons. Periods of high activity of L_1 neurons correspond to periods of low activity of A_1 neurons and *vice versa* (Nenadović, 1992).

It was suggested earlier that L_1 neurons of *M. funereus* possibly synthesized prothoracicotropic hormone (PTTH) (Ivanović *et al.* 1988). The responses of protocerebral neurons to different temperatures (0°C and 23°C) were shown to depend on the season when *M. funereus* larvae were collected (spring or autumn). In addition the titer of ecdysteroid in the hemolymph correlated with the activity of L_1 neurons (Ivanović *et al.* 1980). The same authors pointed to a possible indirect role for A_1 neurons in the synthesis of PTTH.

Fluctuations in activity during the intermolt period were also expressed for L_2 neurons (Fig. 1b). Periods of high activity, i.e., were high synthesis and fast release of neurosecretory material, were noticed on the 1st, 6th, 11th and 14th day. They were followed by periods of low activity on the 2nd and especially on the 3rd and 12th day after molting into the 6th instar.

Along with seasonal changes in the level of activity of L_2 neurons, changes in the quality of neurosecretory material were also noticed. Synthesis of AZ+ instead of PF+ material was detected at the beginning of autumn (Ivanović *et al.* 1980), which could have a role in metabolic changes during the acclimatization to temperature decrease in autmn. Data on some metabolic factors confirmed this suggestion (Ivanović *et al.* 1979, 1980). Synthesis of AZ+ material was also observed during metamorphosis of *M. funereus* whereas PF+ material was synthesized in adults (Nenadović, 1992).

According to some authors, L_2 neurons synthesize neurohormones that regulate the activity of *corpora allata* (Buys and Gibbs, 1981; Janković-Hladni *et al.* 1983; Panov, 1985; Melnikova, 1985). Inervation of *corpora allata* by L_2 neurons was shown using the axonal diffusion technique with horseradish peroxidase (Khan *et al.* 1984).

Results of the present work indicate a difference in dynamics of activities of L_1 and L_2 neurons pointing to the synthesis of different neurohormones.

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References: - Agui, N., Granger, N. A., Bollenbacher, W. E. and Gillbert, L. I. (1979). *Proc. Natl. Acad. Sci.* USA **76**, 5694-5698. - Bhaskaran, G., Dahm, K. H., Berrera, P., Pacheco, J. L., Peck, K. E. and Myzinska-Pytel, M. (1990). *Gen. Comp. Endocrin.* **78**: 123-136. - Buys, C. M. and Gibbs, D. (1981). *Cell. Tissue Res.* **215**, 505-513. - Dai, J. D., Mizoguchi, A., Satake, L., Ishizaki, A. and Gilbert, L. I. (1994). *Dev. Biol.* **171**, 212-223. –Ivanović, J. P., Janković-Hladni,M. I. and Milanović, M. P. (1975). *J. Therm. Biol.* **1**, 53-58. –Ivanović, J., Janković-Hladni, M., Nenadović, V., Frušić, M. and Stanić, V. (1988). In: *Endocrinological Frontiers in Physiological Insect Ecology* (Eds. F. Sehnal, A Zabža and D. Z. Denlinger), **1**, 141-159, Wroclaw Technical University Press, Wroclaw, Poland. - Ivanović, J., Janković-Hladni, M., Stanić, V. and Milanović, M. (1980). *LXXII Acad. Sci. Arts. Cl. Nat. Math. Sci. Nat.* **20**, 91-96. - Ivanović, J., Janković-Hladni, M., Stanić, V., Milanović, M. and Nenadović, V. (1979). *Comp. Biochem. Physiol.* **80A**, 107-112. - Ivanović, J., Janković-Hladni, M. and Milanović, M. (1975). *Comp. Biochem. Physiol.* **50A**, 125-129. - Janković-Hladni, M., Ivanović, J., Nenadović, V. and Stanić, V. (1983). *Comp. Biochem. Physiol.* **77**, 351-357. - Kawakami, A., Kataoka, H., Oka, T., Mizoguchi, A., Kimura-Kawakami, M., Adachi, T., Iwami, M., Nagasawa, H., Suzuki, A. and Ishisaki, H. (1990). *Science*, **247**, 1333-1335. - Kelly, T. J., Masler, E. P., Bell, R. A., Thygaraja, B. S., Davis, R. E., Fescemyer, H. W. and Borkovec, A. B. (1995). In: *Insect Neuropeptides: Chemistry, Biology and Action.* (J. J. Menn, T. J. Kelly and E. P. Masler, eds), American Chemical Society, USA, 27-37. - Melnikova, E. J. (1985). *J. Mikrosk. Anat. Forsch.* Leipzig, **99 (3)**, 503-509. - Nenadović, V. (1992). *Doctoral thesis*, University of Novi Sad. - Panov, A. A. (1980). In: *Neuroanatomical Techniques. The Insect Nervous System.* (Eds. N. J. Stansfeld and T. A. Miller), 25-50. Springer Verlag, New York, Heildelbeg, Berlin. - Panov, A. A. (1985). *Zool. Anz.* Jena, **214(1-2),** 101-110. - Roberts, D. B. (1986). *Drosophila: A Practical Approach.* 15-19, IRL Press, Oxford. - Veelaert, D., Tobe, S. S., Yu, G. G., Schoofs, L. and Loof, A. D. (1995). *Belg. J. Zool.* **125(1)**: 243-249. - Westbrook, A. L. and Bollenbacher, W. E. (1990). *Dev. Biol.* **140**, 291-299.