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EFFECTS OF THERMAL STRESS ON ACTIVITY OF *CORPORA ALLATA* AND DORSOLATERAL NEUROSECRETORY NEURONS IN *MORIMUS FUNEREUS* LARVAE

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Abstract - The effects of different temperatures (23°C and 8°C) on activity of *corpora allata* (CA) and dorsolateral (L_1, L_2) protocerebral neurosecretory neurons were investigated in *Morimus funereus* Mulsant (1863) larvae collected from a natural population during March. Activity of CA was revealed by monitoring of CA volume and cell number. Increase of CA volume after two day exposure to both temperatures was shown to be the result of increase in cell number. Activity of CA was higher at 23°C than 8°C. Activity of L_1 and L_2 neurosecretory neurons was inhibited at both temperatures. Neurosecretory neurons were more sensitive to temperature of 23°C than 8°C. It can be supposed that dorsolateral neurosecretory neurons synthesize neurohormones that affect CA activity, depending on environmental temperature.

Key words: Temperature stress, *corpora allata*, neurosecretory neurons, *Morimus funereus*, Cerambycidae.

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INTRODUCTION

Insects are permanently exposed to the action of abiotic and biotic environmental factors. Among abiotic factors, temperature plays a significant role in survival, development, metamorphosis, reproduction, and population dynamics of insects.

Temperature influences all levels of biological organization in insects. Through changes in the cell membranes, it differentially changes the activity of neurosecretory neurons in the central nervous system, thus disturbing hormonal equilibrium. The neuroendocrine system quickly reacts to environmental changes (Chernysh, 1991). Its activity determines the content and interrelation of hormones in hemolymph, which further leads to changes in insect metabolism and metamorphosis (Borkovec and Gelman, 1986).

The CA synthesize and release juvenile hormones (JHs), which play a prominent role in insect development and reproduction (Nijhout, 1994). Besides their multiple physiological roles, the JHs have a protective role in response to environmental changes. In some species, JHs play a role in regulation of cryoprotectants, ice nucleating agents, and thermal hysteresis proteins (Horwath and Duman, 1983; Baust *et al*. 1985).

Rauschenbach *et al.* (1983) have demonstrated the important role of JHs in resistance of insects to high temperature. Both low and high temperatures cause an increase in the level of JHs and prolongation of their secretion from CA, enabling insects to survive under stressful conditions.

It has been proposed that CA activity may be regulated in two ways (Tobe and Pratt, 1976; Feyereisen, 1985). First, levels of JH biosynthesis may be regulated through modulation of rate-limiting steps in the JH biosynthetic pathway. Second, the capacity of CA to synthesize JH may be regulated by slow developmental changes in the amount of cellular machinery involved in hormone production (Tobe and Stay, 1985). Work on brain-produced allatostatins in cockroaches supports the former mechanism: the neuropeptides exert fast and reversible inhibition of JH synthesis *in vitro* (Stay *et al*. 1994).

Allatostatins are synthesized in the dorsolateral neurosecretory neurons of the insect protocerebrum (Veelaert *et al*. 1995), as in the large form of the prothoracicotropic neurohormone (PTTH) (Dai *et al*. 1994). They are regulators of the metabolic and morphogenetic process in insects (Raabe, 1982; Gilbert *et al.* 1996). The large form of the PTTH exerts a tropic effect on the prothoracic gland by inducing the synthesis of ecdysone (Fescemeyer *et al*. 1995). Allatostatins inhibit CA activity (Okuda and Tanaka, 1997). The absence of allatostatins and/or presence of allatotropins in the median part of the protocerebrum stimulates the CA (Meng-Ping *et al*. 2001). The aim of the present work was to compare changes in the activity of CA and protocerebral dorsolateral neurosecretory neurons in larvae subjected to temperature stress (23°C and 8°C). We expected differences of CA and dorsolateral neuron activity between larvae exposed to different constant temperatures and the control group of larvae.

MATERIAL AND METHODS

The effects of different temperatures (23°C and 8°C) on activities of CA and dorsolateral neurosecretory neurons were investigated in *M. funereus* larvae (500-700 mg) collected from the nature on the mountain Fruška Gora, during March (average daily temperature was 3°-5°C). The control larvae were killed immediately (natural control group - NC). Other larvae were divided into four experimental groups. They were placed in separate test tubes with crumbled oak bark, and exposed to 23°C and 8°C. Larvae were killed after short-term (2 days) and long-term (30 days) exposure to different constant temperatures.

The larvae were killed and head capsules were fixed in Bouin's fixative. The brain complexes were dissected out and after rinsing and dehydration they were embedded in paraffin wax (Merck 59°C). Serial cross sections (5μm thick) were cut and stained by the Alcian Blue Phloxine and Paraldehyde Thionine Phloxine techniques (Panov, 1980). Neurosecretory material in neurons stained dark purple were paraldehyde fuchsin positive (PAF+).

Four pairs of CA per experimental group were analyzed using a light microscope. Activity of CA was estimated by monitoring the volume of CA and the number of their cells. Volumes of the CA were calculated using the formula $V=1/6\times\pi\times a\times b^2$, where "a" represents the larger diameter and "b" the smaller diameter of CA (Huang *et al*. 1991). The total number of cells was determined on the same preparation.

Based on their morphological characteristics, we divided the protocerebral dorsolateral neurosecretory neurons (for ease of monitoring the results) into two groups L_1 and L_2. The activity of the neurons was estimated using the following cytological parameters:

- the number of L_1 and L_2 neurosecretory neurons as noted for each animal (each neuron was observed in all of the serial section in which it appeared);

- the size of the neurosecretory neurons and their nuclei, expressed using the formula $V=1/6\times\pi\times a\times b^2$ ("a" representing the larger diameter and "b" the smaller diameter of each neuron or its nucleus);

- the amount of the paraldehyde stained (PAF+) neurosecretory material in the perikaryon, which was arbitrarily estimated as well-expressed, expressed, poorly expressed, and empty (represented as % of the analyzed neurosecretory neurons);

- the quality of the neurosecretory material, which was described as powdery, fine, medium, or large grained.

The CA volume and size of the neurons and nuclei were determined using the Leica QWIN program, and the results were expressed in μm^3.

Statistical analysis of results was performed using the STATISTICA program, version 5.0. One-way ANOVA and the multiple range test were applied for estimation of significant differences among the five groups for CA volume, the number of CA cells, and the number and size of neurosecretory neurons and their nuclei.

The significance of temperature, exposure duration, and interaction effects was estimated using two-way ANOVA.

RESULTS

The cytological parameters indicate that there were differences in CA activity and activity of the dorsolateral neurosecretory neurons in *M. funereus* larvae under the influence of different constant temperatures.

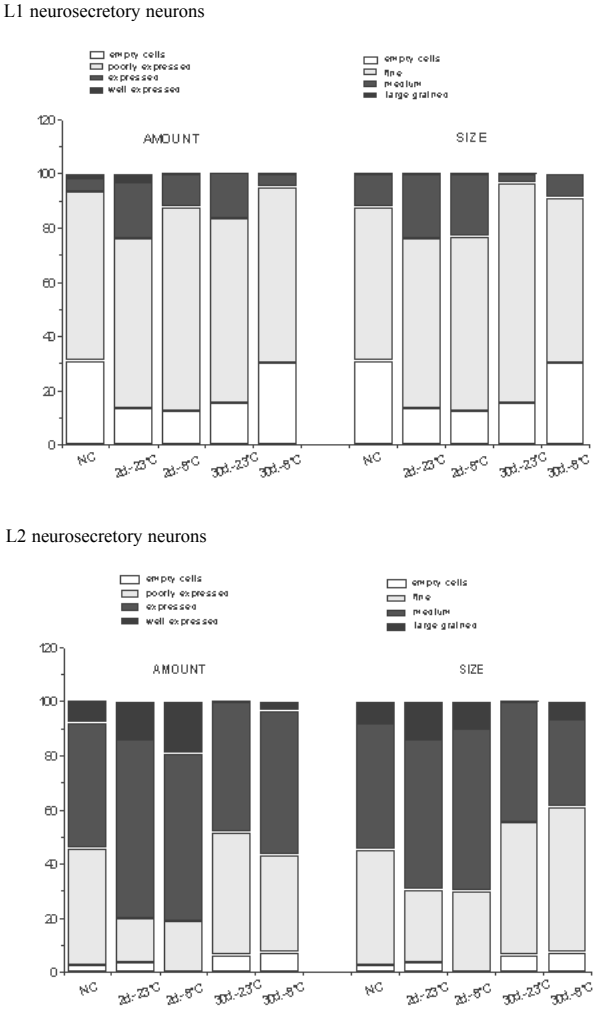

Fig. 1. Quantity and size of PAF+ neurosecretory material in neurosecretory neurons (L_1, L_2) of *Morimus funereus* larvae.

Different constant temperatures provoked increase of CA volume. It was significant after 2 days of exposure to 23°C and 8°C (Table 1; Fig. 3). The number of CA cells was significantly higher regardless of the temperature and exposure time (Table 1). These parameters were higher at 23°C than 8°C. Mitotic divisions were noticeable in the CA during short-term exposure to both temperatures (Fig. 3). Highly significant temperature and exposure duration effects were recorded for CA volume and the number of CA cells (Table 2). The size of L_1 neurons decreased in larvae exposed to constant temperatures of 23°C and 8°C. It was significant in groups exposed to 23°C and 8°C for 30 days (Table 1). The size of the nuclei was also smaller in all groups than in NC, and the difference was significant in the group exposed to 23°C for 2 days, as well as in groups exposed to both temperatures for 30 days (Table 1).

The L_1 neurons of the larvae exposed to different temperatures for 2 days and 30 days contained a poorly expressed amount of powdery PAF+ neurosecretion. Empty cells were recorded in all groups (Figs. 1 and 4).

The number of L1 neurons in larvae exposed to 23°C and 8°C for 2 days and 30 days was lower than in NC (Table 1). The reduction in number was significant in the group exposed to 8°C for 30 days (Table 1). This is indicated by significant "temperature × time" interaction in

Table 1. Means and standard errors of CA volume (CA-V), number of CA cells (CA-N), number of neurosecretory neurons (N), and size of neurosecretory neurons (SN) and their nuclei (Sn) in *M. funereus* larvae. NC-natural conditions.

	NC	23 C-2d.	8 C-2d.	23 C-30d.	8 C-30d.	F	Pr>F
CA-V (m3)	50128.48 ±10321.2a	318164.86 ±53394.7b	181071.95 ±36967.1b	81236.22 ±11896.2a	52048.75 ±12812.5a	25.79	0.0000
CA-N	96.87 ±10.31a	360.89 ±48.99bc	216 ±15.58b	187.75 ±15.26c	180 ±13.24c	20.40	0.0000
L1-N	7.50 ±0.45a	5.80 ±0.22ab	6.25 ±0.29ab	7.00 ±0.47ab	5.80 ±0.65b	3.82	0.0193
L1-SN (m3)	280.78 ±27.35a	172.17 ±11.62ab	174.97 ±12.62ab	156.95 ±13.34b	163.53 ±15.20b	5.41	0.0004
L1-Sn (m3)	73.83 ±8.82a	34.89 ±3.69b	37.6 ±4.38ab	36.85 ±4.34b	39.98 ±5.83b	5.18	0.0006
L2-N	12.83 ±0.2a	11.20 ±0.2b	11.75 ±0.9ab	11.50 ±0.8ab	11.20 ±0.2b	4.26	0.0125
L2-SN (m3)	1137.99 ±65.63ac	912.54 ±39.60abc	855.27 ±54.40bd	768.78 ±45.54b	972.07 ±44.74cd	9.08	0.0000
L2-Sn (m3)	199.96 ±11.97ab	160.45 ±11.01bc	141.04 ±8.07c	143.34 ±10.53c	174.60 ±9.77bc	5.52	0.0003

Table 2. Two-way ANOVA of cytological parameters of *M. funereus larvae*. Temperatures (T) and times (t) are fixed factors. The mean squares (MS) were multiplied by 1000.

		Source of variation			
Trait		**T**	**t**	**T X t**	**Error**
CA-V	df	1	1	1	24
	MS	32.85	235.42	0.13	4.04
	F	8.13**	58.26***	0.03	
L1-N	df	1	1	1	14
	MS	3.32	1.89	15.95	3.44
	F	0.97	0.55	4.64*	
L1-VN	df	1	1	1	107
	MS	1.24	11.90	0.19	4.53
	F	0.27	2.63	0.042	
L1-Vn	df	1	1	1	107
	MS	0.68	0.32	0.06	7.35
	F	0.09	0.04	0.007	
L2-N	df	1	1	1	14
	MS	0.12	012	1.09	0.52
	F	0.22	0.22	2.10	
L2-VN	df	1	1	1	201
	MS	7.55	0.59	26.37	2.04
	F	3.69	0.29	12.90***	
L2-Vn	df	1	1	1	201
	MS	6.32	4.49	23.22	4.22
	F	1.50	1.06	5.51*	

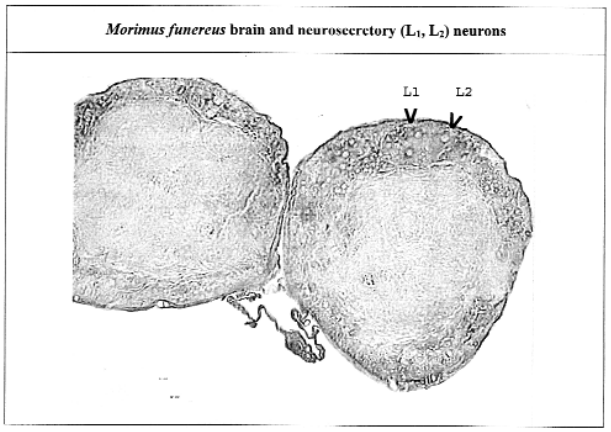

Fig. 2. Frontal cross-section of the brain at the level of the protocerebrum in *Morimus funereus* larvae. The arrows indicate L_1 and L_2 neurons.

two-way ANOVA (Table 2).

In comparison with the NC group, the size of L_2 neurons decreased under the influence of both temperatures. The differences were significant in the group exposed to 8°C for 2 days and the group exposed to 23°C for 30 days. Significant differences were also observed between the groups exposed to 23°C and 8°C for 30 days (Table 1). The size of the nucleus was smaller in all groups than in NC. The decrease of nucleus size was significant in larvae exposed to 8°C for 2 days and larvae exposed to 23°C for 30 days (Table 1). A significant "temperature × time" interaction was recorded for both L_2 neurosecretory neurons and their nuclei (Table 2).

The cytoplasm of L_2 neurons in larvae exposed to 23°C and 8°C for 2 days mostly contained fine-grained PAF+ neurosecretion. The L_2 cells of larvae exposed to different temperatures for 30 days contained powdery neurosecretion, and empty cells were present in both groups (Figs. 1 and 4).

The number of L_2 neurons was significantly lower in the groups exposed to 23°C for 2 days and 8°C for 30 days (Table 1).

DISCUSSION

The cerambycid beetle *M. funereus* inhabits deciduous and coniferous trees in the forests of Southeastern Europe. Temperature is a limiting factor for their spreading to the north (Staniæ *et al*. 1989). In the course of evolution, *M. funereus* larvae have become specialized for seasonal changes of temperature. Their development is long, and the number of larval instars is unknown and dependent upon environmental conditions. Changes in the duration of larval development under stress conditions are in correlation with neuroendocrine activity (Ivanoviæ *et al*. 1991) and with changes in protein and carbohydrate metabolism of *M. funereus* larvae (Ivanoviæ *et al*. 1982, 1992).

Previous studies have demonstrated that there are seasonal differences in the response of *M. funereus* larvae to the effect of different temperatures. The temperature of 8°C was stressful to larvae collected in June (inhibition of neurosecretory neurons), but not to those collected in November. In contrast, the temperature of 23°C provoked stress in November, but not in June (Ivanoviæ *et al*. 1975, 1980).

The *M. funereus* larvae used in our experiment live in their natural habitat under the influence of low temperatures. Constant temperatures of 8°C and especially 23°C were higher than the temperature in the larval environment (3°-5°C). Our results confirm the expected differences in CA activity and the activity of dorsolateral neurons in larvae exposed to both temperatures and the control group.

Volume of the CA increased after short-term exposure to different temperatures and was higher at 23°C than 8°C (Table 1; Fig. 3). The increase of CA volume seems to result from increase of cell number, since mitotic divisions were observed (Fig. 3). Investigations on various species suggest that there is a correlation between secretory activity of the CA and their volume. It has also been shown that changes in CA volume result from mitotic activity within the gland, before increase of gland volume and JH synthesis (Pszczolkowski and Chiang, 2000; Pszczolkowski and Brown, 2003). Since the biosynthesis and release of JHs are highly correlated, and there are no data suggesting the possibility for JH accumulation in the CA, our results suggest that the level of JHs in hemolymph and other tissues might be increased after short-term exposure to both temperatures.

The effects of both temperatures resulted in inhibition of L_1 neurons (Tables 1 and 2; Figs. 1 and 4), such inhibition being markedly expressed at 23°C. A similar result was obtained in *Lymantria dispar* larvae (CA were active, but L_1 neurons inhibited) under nutritional stress (Periæ-Mataruga *et al*. 2001). Concomitant increase in CA volume and inhibition of L_1 neurons indi-

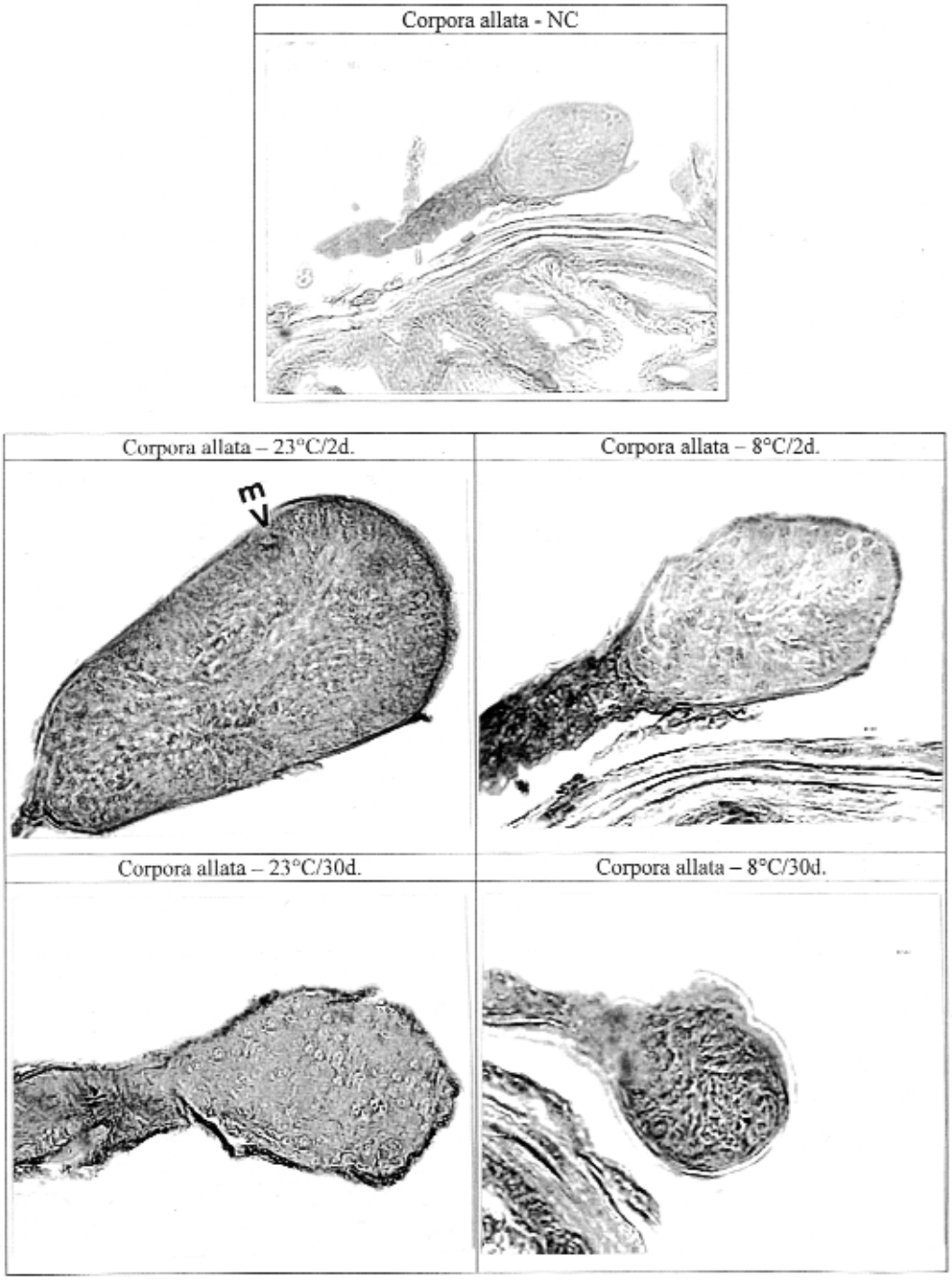

Fig. 3. The CA of *Morimus funereus* larvae exposed to different temperatures (23°C and 8°C) and larvae of the control group (NC); m - mitotic division of CA cells.

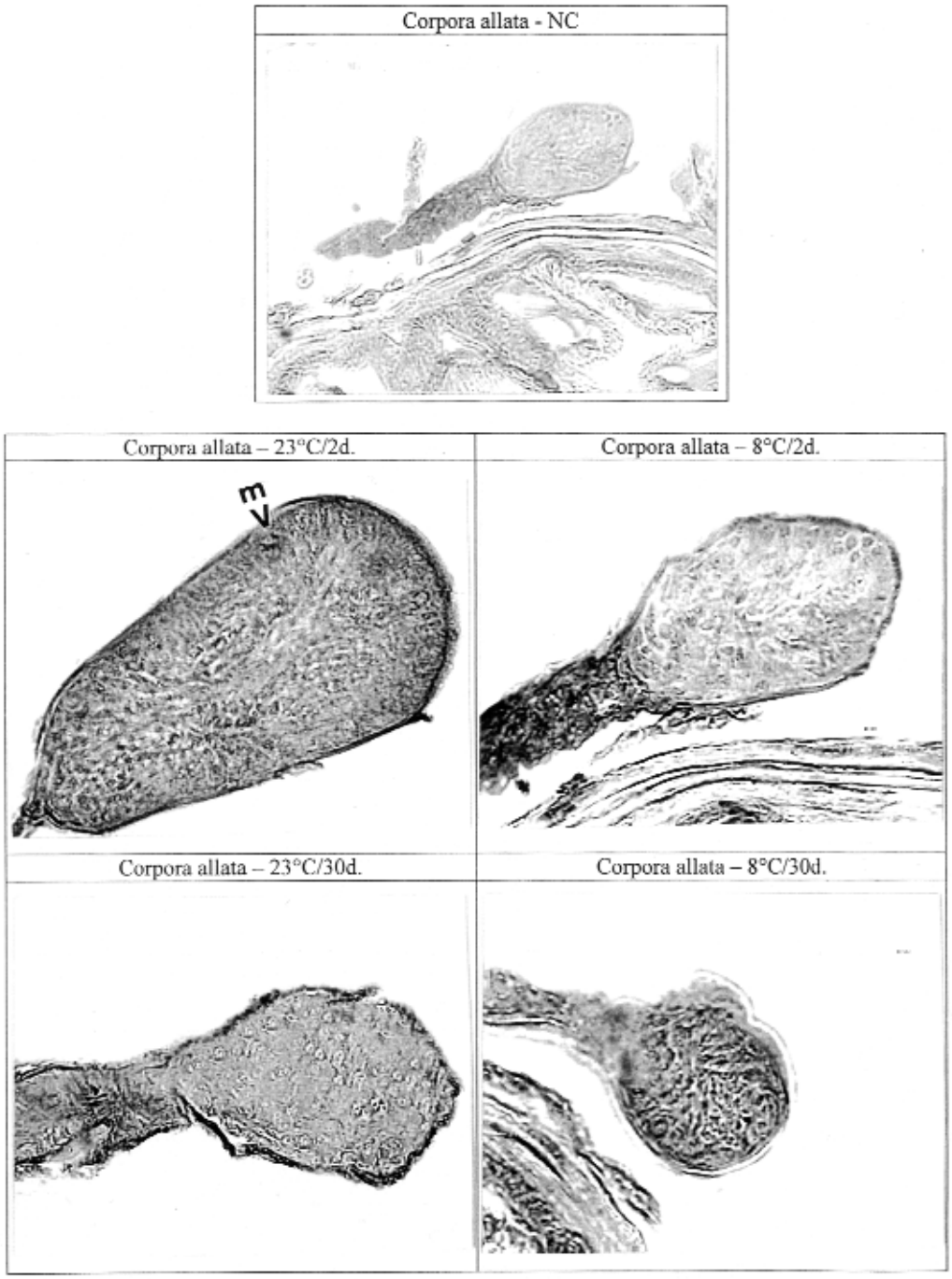

Fig. 4. Protocerebral L_1 and L_2 neurosecretory neurons of *Morimus funereus* larvae exposed to different temperatures (23°C and 8°C) and larvae of the control group (NC).

cates that L_1 neurosecretory neurons may synthesize allatostatins. This has been found in other insects by immunocytochemical methods (Veelaert *et al*. 1995). Increase in CA activity and JH titer is common in insects under stressful conditions (Chernysh, 1991; Gruntenko *et al*. 2000).

Compared to the NC group, the activity of L_2 neurosecretory neurons was depressed regardless of the temperature and exposure time (Tables 1 and 2; Figs. 1 and 4). It is probable that these cells synthesize neurohormones involved in morphogenesis. By using monoclonal antibodies, it has been shown that the large neurosecretory neurons of the dorsolateral region of the brain synthesize the large form of the PTTH (Kawakami *et al*. 1990; Gray *et al.* 1994), which stimulates synthesis and release of ecdysone. It has also been shown that PTTH secretion is usually inhibited by the presence of high JH concentration in the hemolymph (Chernysh, 1991) and occurs only after decrease in JH titer (Nijhout and Williams, 1974; Rountree and Bollenbacher, 1986). We speculate that increased CA activity after short-term exposure to 23°C and 8°C inhibited PTTH secretion in large L_2 neurons in *M. funereus* larvae. The response to temperature changes depends not only on temperatures and time of exposure, but also on the developmental stage (Ivanoviæ *et al.* 1975), phase of the annual cycle (Ivanoviæ *et al.* 1982), and nutrition (Ivanoviæ *et al.* 1989).

Exogenous and endogenous factors that change the activity and number of neurosecretory neurons also change the neurohormonal balance (Ivanoviæ and Jankoviæ-Hladni, 1991). Stress-induced changes in the activity of protocerebral neurosecretory neurons exert a considerable influence on different regulatory and effector systems directly involved in the process of adaptation (Chernysh, 1991).

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REFERENCES

Baust, J. G., Rojas, R. R., Hamilton, M D. (1985). Life at low temperatures: representative insect adaptations. *Cryo-Letters* **6,** 1-199.

Borkovec, A. B., Gelman, D. B. (*eds*) (1986). Insect Neurochemistry and Neurophysiology. Humana Press, Clifton, New York, USA, 1-523.

Chernysh, S. I. (1991). Neuroendocrine system in insect stress. *In*: Hormones and Metabolism in Insect Stress, (*Eds*. J. Ivanoviæ and M. Jankoviæ-Hladni), 69-97. CRC Press, Boca Raton, USA.

Dai, D., Mizoguchi, A., Satake, L., Ishizaki, H., Gilbert, L. I. (1994). Developmental changes in the PTTH content of the *Bombyx mori* brain-retrocerebral complex and hemolymph: analysis by immunogold electron microscopy qualitative image analysis and tissue resolved fluoroimmunoasay. *Dev. Biol.* **171,** 212-223.

Fescemeyer, H. W., Masler, E. P., Kelly, T. J., Lusby, W. R. (1995). Influence of development and prothoracicotropic hormone on the ecdysteroids produced *in vitro* by prothoracic gland of female gypsy moth (*Lymantria dispar*) pupae and pharate adults. *J. Insect Physiol.* **41,** 489-500.

Feyereisen, R. (1985). Regulation of juvenile hormone titer synthesis. *In*: Comprehensive Insect Physiology, Biochemistry and Pharmacology, 7 (*Eds.* J. Kerkut and L. I. Gilbert), 391-429. Pergamon Press, New York, USA.

Gilbert, L. I., Rybcynski, R., Tobe, S. S. (1996). Endocrine cascade in insect metamorphosis. *In*: Metamorphosis: Postembryonic Reprogramming of Gene Expression in Amphibian and Insect Cells (*Eds*. L. I. Gilbert, J. R. Tata and B. G. Atkinson), 60-98. Academic Press, New York, USA.

Gray, R. S., Muehleisen, D. P., Bollenbacher, W. E. (1994). Multiple peptide expression by the L-NSC in the tobacco hornworm *Manduca sexta*. *In*: Insect Neurochemistry and Neurophysiology (*Eds*. A. B. Borkovec and M. J. Loeb), 251-254. CRC Boca Raton, USA.

Gruntenko, N. E., Khlebodarova, T. M., Vasenkova, I. A., Sukhanova, M Jh., Wilson, T. G., Rauschenbach, I. Yu. (2000). Stress-reactivity of a *Drosophila melanogaster* strain with impaired juvenile hormone action. *J. Insect Physiol.* **46,** 451-456.

Horwath, K. L., Duman, J. G. (1983). Induction of antifreeze protein production by juvenile hormone in larvae of the beetle *Dendroides canadensis. J. Comp. Physiol.* **151,** 223.

Huang, Z.-Y., Robinson, G. E., Tobe, S. S., Yagi, K. J., Strambi, A., Stay, B. (1991). Hormonal regulation of behavioral development in the honey bee is based on changes in the rate of juvenile hormone biosynthesis. *J. Insect Physiol.* **37,** 733-741.

Ivanoviæ, J., Jankoviæ-Hladni, M., Milanoviæ, M. (1975). Effect of constant temperature on survival rate, neurosecretion and endocrine cells and digestive enzymes in *Morimus funereus* larvae (Cerambycidae: Coleoptera). *Comp. Biochem. Physiol.* **50,** 1-125.

Ivanoviæ, J., Jankoviæ-Hladni, M., Staniæ, V., Milanoviæ, M. (1980). The role of the cerebral neurosecretory system of *Morimus funereus* larvae (Insecta) in thermal stress. *Bull T. LXXII Acad Serbe Sci. Arts, Classe Sci. Nat. Math. Sci. Nat.* **20,** 91-97.

Ivanoviæ J., Jankoviæ-Hladni, M., Milanoviæ, M., Staniæ V., Bo idarac, M. (1982). Possible role of neurohormones in the process of acclimatization and acclimation in *Morimus funereus* (Insecta) - II Changes in the hemolymph free amino acid content and digestive enzyme activity. *Comp. Biochem. Physiol. 80A* **1,** 107-113.

Ivanoviæ, J., Jankoviæ-Hladni, M., Staniæ, V., Nenadoviæ, V., Fruši æ, M. (1989). The role of neurosecretion and metabolism in development of an oligophagous feeding habit in *Morimus funereus* lar-

vae (Col. Cerambycidae). *Comp. Biochem. Physiol. A94,* **1,** 1-167.

Ivanoviæ, J., Jankoviæ-Hladni, M. (*eds*.) (1991). Hormones and Metabolism in Insect Stress, 178. CRC Press, Boca Raton, USA.

Ivanoviæ, J., Jankoviæ-Hladni, M., Djordjeviæ, S., Stamenoviæ, S., Lazareviæ, J. (1992). Metabolic strategies of *Morimus funereus* larvae during thermal stress. *Comp. Biochem. Physiol. 103A,* **4,** 679-686.

Kawakami, A., Kataoka, H., Oka, T., Mizoguchy, A., Kimura-Kawakami, , M., Adachi T., Iwami M., Nagasawa H., Suzuki A., Ishizaki H. (1990). Molecular cloning of the *Bombyx mori* pro thoracicotropic hormone. *Science*, **247,** 1333-1335.

Meng-Ping, T., Rong, K., Zong-Shun, W., John, G. S. J., Chih-Ming, Y. (2001). Immunolocalization and possible effect of a moth allatotropin-like substance in a fly, *Phormia regina* (Diptera: Calliphoridae). *J. Insect Physiol.* **47,** 233-244.

Nijhout, H. F., Williams, C. M. (1974). Control of moulting and metamorphosis in the tobacco hornworm, *Manduca sexta* (L.): cessation of juvenile hormone secretion as a trigger for pupation. *J. Exp. Biol.* **61,** 1-493.

Nijhout H. F. (*ed.*) (1994). Insect Hormones. Princeton University Press, Princeton.

Okuda, T., Tanaka, S. (1997). An allatostatic factor and juvenile hormone synthesis by corpora allata in *Locusta migratoria. J. Insect Physiol.* **43,** 635-541.

Panov, A. A. (1980). Demonstration of neurosecretory cells in the insect central nervous system. *In*: Neuroanatomical techniques. Insect nervous system. Springer-Verlag, New York, Heidelberg, Berlin.

Periæ-Mataruga, V., Lazareviæ, J., Nenadoviæ, V. (2001). A possible role for the dorsolateral protocerebral neurosecretory neurons in the trophic adaptations of *Lymantria dispar* (Lepidoptera: Lymantridae). *Eur. J. Entomol.* **98,** 257-264.

Pszczolkowski, M. A., Chiang, A. S. (2000). Effects of chilling stress on allatal growth and juvenile hormone synthesis in the cockroach *Diploptera punctata. J. Insect Physiol.* **46,** 923-931.

Pszczolkowski, M. A., Brown, J. J. (2003). Chilling affects allatal cell proliferation via antennae and protocerebral neurons in the cockroach *Diploptera punctata*. *J. Insect Physiol.* **49,** 123-129.

Raabe, M. (*ed*.) (1982). Insect Neurohormones, 1-352. Plenum Press, New York, London.

Rauschenbach, I. Yu., Lukashina, N. S., Korochkin, L. I. (1983). Genetics of esterases in *Drosophila*. VII. The genetic control of the activity level of the JH-esterase and heat resistance in *Drosophila virilis* under high temperature. *Biochem. Genet*. **21,** 1-253.

Rountree, D. B., Bollenbacher, W. E. (1986). The release of the prothoracicotropic hormone in the tobacco hornworm *Manduca sexta* is controlled intrinsically by juvenile hormone. *J. Exp. Biol.* **120,** 1-41.

Staniæ, V., Jankoviæ-Hladni, M., Ivanoviæ, J., Nenadoviæ, V. (1989). Joint effects of temperature, food quality and season on development of cerambycid *Morimus funereus* under laboratory conditions. *Entomologia experimentalis et applicata*, **51,** 261-267.

Stay, B., Tobe, S. S., Bendena, W. G. (1994). Allatostatins: identification, primary structures, functions and distribution. *Adv. Insect Physiol.* **25,** 267-337.

Tobe, S. S., Pratt, G. E. (1976). Farnesenic acid stimulation of juvenile hormone biosynthesis as an experimental probe in corpus allatum physiology. *In*: The Juvenile Hormones (*Ed*. L. I. Gilbert), 147-163. Plenum Press, New York, USA.

Tobe, S. S., Stay, B. (1985). Structure and regulation of the corpus allatum. *Adv. Insect Physiol.* **18,** 305-432.

Veelaert, D., Tobe, S. S., Yu, G. G., Schoofs, L., Loof, A. D. (1995). Allatostatic and allatotropic factors in the brain of the desert locust *Schistocerca gregaria*. *Belg. J. Zool.* **125,** 243-249.

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MARIJA MRDAKOVI], LARISA ILIJIN, MILENA JANKOVI]-TOMANI], MILENA VLAHOVI], Z. PROLI], VESNA PERI]-MATARUGA, JELICA LAZAREVI] iVERA NENADOVI]

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Ispitivan je uticaj razli~itih temperatura (23°C i 8°C) na aktivnost *corpora allata* (CA) i dorzolateralnih (L_1 i L_2) protocerebralnih neurosekretnih neurona kod larvi *Morimus funereus* Muls. (1863), sakupqenih iz prirode tokom meseca marta.

Aktivnost CA je procewivana pra}ewem promena veli~ine CA i broja }elija CA. Pokazano je da je pove}awe veli~ine CA, nakon 2 dana izlagawa larvi obema temperaturama, rezultuje u pove}awu broja }elija. Aktivnost CA je ve}a na temperaturi od 23^{0}C, nego na 8^{0}C. Aktivnost L_1 i L_2 neurosekretnih neurona je bila inhibirana delovawem obeju temperatura. Neuro-sekretni neuroni su osetqiviji na delovawe temperature od 23^{0}C, nego na 8^{0}C.

Moglo bi se pretpostaviti da dorzolateralni neurosekretni neuroni sinteti{u neurohormone koji uti~u na aktivnost CA, u zavisnosti od temperature spoqa{we sredine.