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TEMPERATURE AND MAGNETIC FIELD EFFECTS ON THE ACTIVITY OF PROTOCEREBRAL NEUROSECRETORY NEURONS AND *CORPORA ALLATA* IN *CERAMBYX CERDO* L. LARVAE

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Abstract - The effects of constant temperature and an extremely low-frequency magnetic field (ELFMF, 50 Hz and average induction of 20 mT) on the activity of medial protocerebral neurosecretory neurons (A1 and A2) and *corpora allata* were investigated in *Cerambyx cerdo* L. larvae after 30 days of exposure. Both constant temperature of 23°C and the ELFMF led to decrease in activity of A1 and A2 neurosecretory neurons and increase in activity of *corpora allata* compared to the control group (larvae from natural conditions). The changes are more pronounced in A2 than A1 neurons.

Key words: Medial neurosecretory neurons, *corpora allata*, temperature, magnetic field, *Cerambycidae*

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INTRODUCTION

Neurosecretory neurons in the insect protocerebrum synthesize and release neurohormones in response to various exogenous and endogenous stimuli. Among peptide neurohormones, the most important role belongs to prothoracicotropic neurohormones (Ishizaki and Suzuki, 1986; Vaenstra *et al.* 1985), allatotropins and allatostatins (Bogus and Scheller, 1994; Veelaert *et al.* 1995; Gilbert *et al.* 2000), hypotrechalosemic and hypoglycemic neurohormones.

Our previous investigations on the role of the neuroendocrine system in response and adaptation to stressful conditions (physical and chemical stressors) in xylophagous insects showed that the response at the level of peptidergic neurons preceded the response at the organism level (Ivanović *et al.* 1975a, 1975b; Ivanović *et al.* 1979; Ivanović *et al.* 1980; Nenadović *et al.* 1982; Janković-Hladni *et al.* 1983; Ivanović *et al.* 1991; Leković *et al.* 2001; Mrdaković *et al.* 2004).

It is well known that a magnetic field affects insect orientation (Gould, 1980; Vacha, 1997a), development (Prolić and Jovanović, 1986; Prolić and Nenadović, 1995), behavior (Prolić *et al.* 2003), extracardial pulsation (Vacha, 1997b), mutation rate (Giorgi *et al.* 1992).

However, the changes provoked by magnetic fields at the level of the neuroendocrine system, especially peptidergic neurons, have been scarcely studied. Such investigations are rare even in vertebrates, where it was shown that a magnetic field influenced the neurosecretory function of the hypothalamus and circadial biorhythms in mice (Zagorskaya, 1981).

The aim of the present work was to examine the effects of constant temperature of 23°C and an extremely low-frequency magnetic field on the activity of peptidergic neurosecretory neurons in the medial region of the protocerebrum and activity of *corpora allata* in larvae of the large oak beetle (*Cerambyx cerdo*) after 30 days of exposure.

MATERIALS AND METHODS

Thirty larvae of the large oak beetle *C. cerdo* (weight range from 700 to 900 mg) were collected at the Progar locality near Belgrade in October. They were randomly assigned to three experimental groups. The first group,

natural control (NC), was sacrificed immediately. The second group (T23°C) was reared in the dark under constant laboratory conditions for 30 days (constant temperature of 23°C, relative humidity of 70%, and subcortical region of oak as feeding substrate). The third group (ELFMF) was exposed to an extremely low-frequency magnetic field of 50 Hz and average induction of 20 mT during the same period of rearing under constant laboratory conditions. After 30 days, larvae of the T23°C and ELFMF group were sacrificed.

Decapitated head regions were fixed in Bouin's fixative and embedded in paraffin (Merck, 57-59°C) using standard histological procedure. Serial 5 µ-thick sections were dyed by the Alcian blue - Phloxine method (P a n o v, 1980). The activity of protocerebral peptidergic neurosecretory neurons (NSN) of the medial group was analyzed according to J a n k o v i ć - H l a d n i *et al.* (1983) using a light microscope from Leitz DMRB. The numbers of A1 and A2 were determined. Size of NSN and their nuclei was calculated using the formula for an ellipsoid (H u a n g Z h i Y o n g *et al.* 1991). The same formula was applied to obtain the size of *corpora allata.* The quantity of neurosecretory material (NSM) was arbitrarily estimated and expressed as the percentage of NSN with low, medium, and high quantity. The quality of

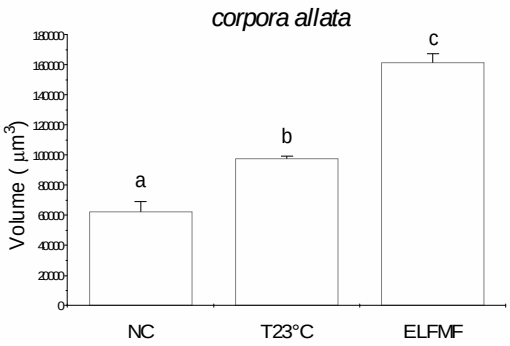

Fig. 1. Volume of corpora allata in *Cerambyx cerdo* larvae from natural conditions (NC) exposed to constant temerature of 23°C (T23°C) and a magnetic field (ELFMF). One-way ANOVA revealed significant differences among the groups (F=98.39; P<0.001). Bars indicated by different letters (a, b, c) differ significantly (LSD test).

NSM was described as powdery, fine-grained, and coarse-grained and expressed as the percentage of NSN with each type of NSM. Data analysis was carried out by one-way ANOVA and the multiple range test (LSD).

RESULTS

Medial peptidergic NSN in the protocerebrum of larvae from the NC group were shown to have large cytoplasm and nucleus with a large nucleolus in the center of nuclei. A medium to high quantity of NSM tending to form large agglomerations was found in A1 NSN (Table 1). At the same time, there was a small quantity of NSM in the neurochemal organs *corpora cardiaca allata.*

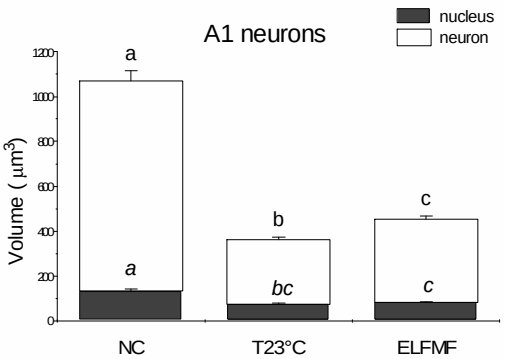

Fig. 2. Volumes of A1 neurosecretory neurons and their nuclei in *Cerambyx cerdo* larvae from different experimental groups. One-way ANOVA revealed significant differences among the groups with respect to size of the neurons (F=240.83; P<0.001) and their nuclei (F=34.50; P<0.001). Bars indicated by different letters differ significantly (LSD test).

Cytological parameters (i.e., small volume) indicated low activity of *corpora allata* in NC larvae (Fig. 1).

Thirty days of exposure to constant temperature (T23°C) and a magnetic field (ELFMF) led to changes in medial NSN. The size of NSN and their nuclei decreased compared to the NC group. One-way ANOVA and the LSD test confirmed statistically significant differences in the size of A1 and A2 NSN among the groups. The

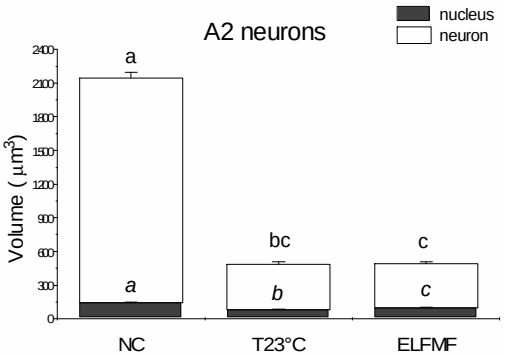

Fig. 3. Volumes of A2 neurosecretory neurons and their nuclei in *Cerambyx cerdo* larvae from different experimental groups. One-way ANOVA revealed significant differences among the groups with respect to size of the neurons (F=351.01; P<0.001) and their nuclei (F=35.22; P<0.001). Bars indicated by different letters differ significantly (LSD test).

decrease in size of NSN and their nuclei was more pronounced in the T23°C group than in the ELFMF group (Figs. 2 and 3).

Table 1. Quantity of neurosecretory material in A1 and A2 neurosecretory neurons in Cerambyx cerdo larvae from different experimental groups presented as the percentage of cells with small /medium /large quantity of NSM. Quality of NSM is presented as the percentage of cells with powdery /fine-grained /coarse-grained NSM.

	Trait	NC	T=23°C	ELFMF
A1	Quantity of nsm (%)	0 / 61.77 / 38.23	0 / 23.81 / 76.19	91.07 / 8.93 / 0
	Size of nsm (%)	0 / 76.47 / 23.53	0 / 28.57 / 71.43	5.36 / 91.07 / 3.57
A2	Quantity of nsm (%)	26.67 / 73.33 / 0	0 / 41.46 / 58.54	100 / 0 / 0
	Size of nsm (%)	40.00 / 60.00 / 0	10.00 / 52.50 / 37.50	78.57 / 21.43 / 0

The distribution of NSN with a certain NSM quantity (small/medium/large) and (powdery/fine-grained/coarse - grained) is presented in Table 1. It can be seen that large agglomerations of NSM were formed in A1 NSN of the T23°C group, while a small quantity of fine-grained NSM was characteristic for A1 NSN of the ELFMF group. The quantity of NSM increased in A2 NSN of the T23°C group compared to the NC group. A small quantity of powdery NSM was discernible in A2 NSN after exposure to a magnetic field.

The number of NSN increased in the T23°C (A1=21.0±1.4, A2=18.5±0.7) and ELFMF (A1=28.0±2.8, A2=21.0±1.4) grups compared to the NC group (A1=17.5±2.1, A2=15.5±0.7).

Corpora allata in the T23°C group and especially in the ELFMF group were more active than in the NC group (Fig. 1). Exposure to constant temperature and a magnetic field also resulted in significant accumulation of NSM by the *corpora cardiaca allata.*

DISCUSSION

It is well known that distribution and activity of living organisms depend on external temperature. Because temperature, as a measure of thermal energy, affects the velocity of molecular movement and thereby influences the velocity of chemical reactions, it also determines the development and metabolism of living organisms.

Our previous investigations on xylophagous cerambycid larvae have shown that constant temperature leads to changes in neuroendocrine regulation of development and metabolism of carbohydrates, proteins, and amino acids. These changes and their reversibility (or irreversibility) depend on the quantity of thermal energy, duration of organism exposure, season, developmental stage, and physiological state of the individual (Ivanović *et al.* 1975a, 1975b; Ivanović *et al.* 1980; Janković - Hladni *et al.* 1983; Ivanović *et al.* 1988; Leković *et al.* 2001; Mrdaković *et al.* 2004). Temperature changes affect various parameters of individual performance and population dynamics of a species (Lindroth *et al.* 1997).

Data on effects of magnetic fields on the insect neuroendocrine system are almost nonexistent. Ours is the first paper showing changes in NSN activity in response to a magnetic field. Exposure of *C. cerdo* larvae to an ELFMF at constant temperature of 23°C led to reduced activity of A1 and A2 NSN compared to the control group (Table 1, Figs. 2 and 3). The presence of a small quantity of powdery NSM and extended axons of A2 NSN pointed to stimulated release of NSM under the influence of a magnetic field compared to the T23°C group. The nuclei of A2 NSN were larger in the ELFMF group than in the T23°C group. They usually had two nucleoli, indicating the beginning of synthetic processes. Goodman (1987) reported increased RNA synthesis in the interband region of salivary gland chromosomes of the dipteran *Sciara coprofila* in a magnetic field of approximately 0,3 mT.

The presence of a large quantity of NSM in *corpora cardiaca allata* showed that increased synthetic and releasing activity of protocerebral NSN preceded the physiological state provoked by a magnetic field, in which the need for neurohormones was obviously increased. Previous investigations on *C. cerdo* larvae collected from natural conditions during November and acclimated to 10°C have shown activation of medial NSN and suppressed activity of *corpora allata* after 14 days of exposure to constant temperature of 23°C (Janković-Hladni *et al.* 1983; Nenadović *et al.* 1982).

In the present work, increased activity of *corpora allata* in the T23°C group and especially in the ELFMF group (Fig. 1) indicated increased synthesis and release of juvenile hormone (JH). One of the numerous functions of JH is to coordinate the concentrations of hemolymph steroids. It is also known to activate ecdysteroid receptors (Diehl-Jones *et al.* 1996). The stress-protective role of 20-hydroxyecdysone at low concentrations is similar to the stress-protective role of glucocorticoids in vertebrates (Kobayachi and Kimura, 1967). Numerous papers have described changes in JH and ecdysteroid hemolymph concentrations in response to stressful conditions (Kelly *et al.* 1986; Rauschenbach, 1991; Chernysh, 1991). Increased levels of hemolymph ecdysteroids were found in *Morimus funereus* larvae collected during winter and exposed to constant temperature of 23°C (Ivanović *et al.* 1980).

Intensive synthesis of metabolic and morphogenetic neurohormones can also be achieved through an increased number of NSN, which was observed in *C. cerdo* larvae of the T23°C and ELFMF groups in the present work (see Results). Increased numbers of medial NSN have been found as a response to an unsuitable feeding substrate in larvae of *M. funereus* (Nenadović *et al.* 1989; Ivanović *et al.* 1991) and *Lymantria dispar* (Perić-Mataruga and Lazarević, 2004).

Some papers have demonstrated the influence of magnetic fields on processes of differentiation. For example, a magnetic field accelerated metamorphosis in *Tenebrio molitor* pupae (Prolić and Nenadović, 1995), prolonged development time in *Rhyzoperta dominica* (Starick *et al.* 2005), and led to an increased number of hypodermal wing cells in *Drosophila melanogaster* (Giorgi *et al.* 1992). We speculate that differentiation of NSN was accelerated in *C.cerdo* larvae of the ELFMF group.

Exposure of *C. cerdo* larvae to a magnetic field changed the response obtained at constant temperature of 23°C. Additional experiments are needed to understand mechanisms underlying the modulatory effects of magnetic fields.

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УТИЦАЈ ТЕМПЕРАТУРЕ И ЕКСТРЕМНО НИСКОФРЕКВЕНТНОГ МАГНЕТНОГ ПОЉА НА АКТИВНОСТ ПРОТОЦЕРЕБРАЛНИХ НЕУРОСЕКРЕТНИХ НЕУРОНА И *CORPORA ALLATA* ЛАРВИ *CERAMBYX CERDO* L.

ВЕРА НЕНАДОВИЋ, МАРИЈА МРДАКОВИЋ, ЈЕЛИЦА ЛАЗАРЕВИЋ, Д. МИРЧИЋ, ДАЈАНА ТОДОРОВИЋ и З. ПРОЛИЋ

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Испитиван је утицај константне температуре и екстремно нискофреквентног магнетног поља (ELFMF, 50 Hz и просечна индукција 20mT) на активност медијаних протоцеребралних неуросекретних неурона (A1 i A2) и *corpora allata* код ларви *Cerambyx cerdo* после 30 дана излагања и константна температура од 23°C и ELFMF доводе до смањења активностиi A1 и A2 неуросекретних неурона и повећања активности *corpora allata* у поређењу са контролном групом (ларве из природних услова). Промене су више изражене код A2 него код A1неурона.