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Thermal acclimatization does not affect the resting activity of type T_1 trichobothrium in the firebug (*Pyrrhocoris apterus*; Heteroptera)

Temperaturna aklimatizacija ne vpliva na mirovno aktivnost trihobotrija tipa T_1 pri rdečem škratcu (*Pyrrhocoris apterus*; Heteroptera)

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Abstract. Firebugs (*Pyrrhocoris apterus)* pass through the winter in adult state. They undergo a series of physiological changes in order to increase their capacity to survive at low ambient temperatures. Nevertheless, even during winter their body temperature can rise up to 28 °C for a few hours on a sunny day, which is comparable to summer conditions. To establish the impact of cold acclimatization on the function of mechanoreceptors, the resting activity of T_1 type trichobothria warmed up to 20 °C is compared in cold and warm acclimatized animals, as well in animals acclimated to laboratory conditions. In cold acclimatized animals the mean resting activity is 3300 imp/min (SE 90, n=13), in warm acclimatized 3400 imp/min (SE 60, n=15), and in animals acclimated to laboratory conditions 3700 imp/min (SE 130, n=17). The similar trend is observed in the variability of inter-impulse time intervals. The mean coefficient of the interval variation is in both cold and warm acclimatized animals 0.28 (cold SE 0.013, n=13; warm SE 0.010, n=15), and in animals acclimated to laboratory conditions 0.33 (SE 0.012, n=17). These data show negligible differences between the three groups. We can conclude that the resting activity of type T_1 trichobothrium remains limited to a narrow range, regardless of the phase of the acclimatization process.

Keywords: *Pyrrhocoris apterus*, mechanoreception, trichobothrium, resting activity, thermal acclimatization.

Izvleček. Stenica, rdeči škratec *(Pyrrhocoris apterus)* preživlja hladno zimsko obdobje v stanju imaga. Preživetje v obdobju nizkih okoljskih temperatur ji omogoča niz fizioloških prilagoditev. Kljub temu se lahko v tem obdobju temperatura telesa ob sončnih dneh za kratek čas povzpne do 28 °C, kar približno ustreza poletnim pogojem. Vpliv aklimatizacije na delovanje čutilnih celic smo želeli ugotoviti s pomočjo mirovne aktivnosti trihobotrija tipa $T_{1.}$ Poskus je bil zasnovan tako, da smo telesno temperaturo, hladno, toplo in na temperaturo laboratorijskega okolja aklimatiziranih živali nastavili na 20°C in posneli spontano mirovno aktivnost. Izmerjeni povprečni vrednosti mirovne aktivnosti sta bili pri živalih aklimatiziranih na hladno 3300 imp/min (SE 90, n=13) in pri živalih aklimatiziranih na toplo 3400 imp/min (SE 60, n=15). Pri živalih aklimatiziranih na laboratorijske pogoje pa je bila povprečna vrednost 3700 imp/min (SE 130, n=17). Podobno razmerje med skupinami se je pokazalo tudi pri analizi variabilnosti intervalov med živčnimi signali. Tako je bil pri hladno in toplo aklimatiziranih živalih povprečni koeficient variacije intervalov 0.28 (hladno SE 0.013, n=13; toplo SE 0.010, n=15), pri živali aklimatiziranih na laboratorijsko okolje pa 0.33 (SE 0.012, n=17). Navedeni podatki kažejo, da so razlike med različno aklimatiziranimi živalmi neznatne. Torej je mirovna oziroma spontana aktivnost trihobotrija T_1 proces, ki ne glede na stopnjo termične aklimatizacije, ostaja znotraj ozkega območja.

Ključne besede: *Pyrrhocoris apterus*, mehanorecepcija, trihobotrij, mirovna aktivnost, temperaturna aklimatizacija.

Introduction

Mechanosensitive filiform sensillae in Heteroptera are commonly characterized by the name trichobothria (Tullgren 1918). As it is typical for filiform sensillae, they have a simple structure with a single receptor neuron (reviewed by Keil 1997). In the firebug (*Pyrrhocoris apterus*) they are classified into three types. The registration of a single unit activity is very simple, therefore their functional properties have been well defined (Drašlar 1973, 1980). For type T_1 trichobothrium a constant resting activity in the level of 3000 imp/min is characteristic. Experiments in other insects have shown that the origin of the resting activity in filiform sensilla is predominantly endogenous (Buno et al. 1981; Dagan & Volman 1982; Hamon & Guillet 1994; Landolfa & Miller 1995). It has been suggested that the main source of the activity are depolarizing ion currents through the mechanosensitive transduction channels (Buno et al. 1981; Thurm 2001). The involvement of the transduction site in the resting activity of type T_1 trichobothrium has been confirmed also by our current investigations (Drašlar & Škorjanc 1997; Škorjanc & Drašlar 2001; paper in preparation).

A typical feature of the firebug is its resistance to low temperatures. It can be seen active not only during the summer but also on the sunny winter days. Its cold hardiness depends on the ability to increase the supercooling capacity and therefore avoid freezing at temperatures below 0°C. The process of cold acclimatization has two phases. The first is induced by the onset of the reproductive diapause in the late summer and the second by the lowering of the ambient temperatures in the late autumn. The acquired cold hardiness persists throughout the winter months, and is terminated by the rising temperatures in the early spring (Socha 1993; Hodkova & Hodek 1994; Koštal & Šimek 2000; reviewed by Hodkova & Hodek 2004). During the acclimatization the concentration of cryoprotective polyols in haemolymph is increased (Koštal & Šimek 2000; Koštal et al. 2001, 2004; Šlachta et al. 2002) and the phospholipid membranes are remodelled (Hodkova et al. 1999, 2002; Tomčala et al. 2006). Recent molecular studies have shown that the lipid composition of the cell membrane could affect the gating of mechanosensitive channels (Kung 2005). The resting activity of type T_1 trichobothria could be therefore used as a probe for detecting seasonal changes in functional properties of the receptor due to the remodelling of the membrane lipids. On the basis of these presumptions we proposed a simple hypothesis. If the cell membranes are significantly remodelled, the resting activity of preparations suddenly exposed to 20 °C should significantly differ between cold and warm acclimatized animals.

Material and Methods

Experiments were performed on the adult male firebugs (*Pyrrhocoris apterus* L., Hemipteroidea, Heteroptera). Animals were collected near Ljubljana. The animals, regarded as a cold acclimatized, were collected in January and February 2007, and the animals regarded as warm acclimatized in May and June 2007. Experiments were performed within a few weeks after the animals were collected. During this period they were maintained in glass containers, supplied with lime seeds and water ad libitum. To imitate natural conditions containers were kept outside. They were placed in a covered box, which was partly buried into the ground. The air temperature and the humidity were monitored by wireless weather station BAR 628 HG (Oregon Scientific USA). In the cold period the mean temperature was 5°C (T_{min} = 3.3 °C; T_{max} = 8.1 °C) and in the warm period 17°C (T_{min} = 13.5 °C; T_{max} = 20.5 °C). The relative humidity was 90 ± 5 %. As a reference we used the data obtained between the years 2003 and

2007 in bugs that were kept in the laboratory at 20 ± 2 °C. These animals were collected on different locations and in different periods. We believe that this reference gave us a fair insight into the general level of the resting activity and therefore enabled a valid comparison of the resting activity in closely synchronized cold and warm acclimatized animals.

To avoid the thermal stress during preparation, cyanoacrylate adhesive (Loctite Super Attack, gel) was chosen instead of a warm wax-resin. Measurements showed that the glue has no toxic effects. Bugs were glued with their dorsal side down to a preparation holder. After the fixation, their legs were removed and the wounds sealed with Kwik-Sil (WPI, Sarasota, USA).

We recorded the resting activity of type T_1 trichobothrium from the fifth abdominal segment. For the nerve impulse registration tungsten electrodes were used (Goodfellow, Cambridge, UK). The registration electrode was inserted a few millimeters away from the trichobothrium, while the reference electrode was placed in the abdomen through the last abdominal segment (Drašlar 1973, 1980). Signal was processed by an AI 402 preamplifier and CyberAmp 320 signal conditioner (Molecular Devices, USA), and then digitalized by CED 1401 plus or CED 1401power interface (Cambridge Electronic Design, UK). The recording, spike sorting and analysis of the data were performed on a PC computer by Spike2 v.6.02 (Cambridge Electronic Design, UK) software. For statistical analysis Prism v.4.02 (GraphPad Software Inc.) was used.

The measurements were performed at the temperature of 20 ± 0.3°C. For this purpose the temperature of the preparation holder was regulated by a homemade controller based on a miniature Peltier element (RO3.3–3.9 Conrad).

The frequency of the nerve impulses was a measure of the resting activity level. The relative variability of inter-impulse time intervals (IPI) was estimated with the coefficient of variation (CV). The coefficient of variation was determined as a ratio between IPI standard deviation and mean value. To compare the IPI distributions between the three groups of animals average IPI histograms were calculated. Only data obtained in trichobothria with long-term stability of the resting activity and normal response to a deflection of the hair were considered as acceptable. In this paper data from 13 cold and 15 warm acclimatized and 17 animals from the reference group are presented.

In the field experiments measurements of temperature were performed with K-type thermocouples (HH501DK, Omega). The body temperature was measured with the thermocouple inserted into the abdomen whereas the ambient temperature was determined with thermocouple placed next to the animal. The reference air temperature was measured in the shadow, 2 meters above the ground.

Results

The resting activity of type T_1 trichobothrium was used as a probe for detecting the effect of the thermal acclimatization on the function of sensory cell. For the test temperature, at which the resting activity of cold and warm acclimatized animals was compared, we chose 20 °C. The data obtained throughout a period of four years in animals kept at the laboratory ambient temperature were used as reference.

In the reference group the resting activity ranged from 2800 to 4600 imp/min (SD 540, n = 17), which was in a good agreement with published data (Drašlar 1973, 1980). In the group of animals acclimatized to cold (winter) conditions the activity ranged from 2600 to 3700 imp/min (SD 300, n = 13). Similarly, in the group of animals acclimatized to warm (summer) conditions the range was between 2900 and 3800 imp/min (SD 230, n = 15) (Fig. 1a). The variability between individual preparations was higher in the reference group. However, this is not surprising, considering the greater diversity in this group. The animals were of different origin, collected in different seasons over a longer period. The corresponding mean values of the resting activity rate were 3700 imp/min (SE 130) in the reference group, 3300 imp/min (SE 90) in the cold acclimatized and 3400 imp/min (SE 60) in the warm acclimatized group. No significant difference was established in the rate between the cold and warm acclimatized animals. This conclusion was confirmed by the Student's t-test (α= 0.05). The test also showed that the difference between the mean rate attained in the reference and the one attained in the cold acclimatized animals was statistically significant (α = 0.05). However, the mean rates could not be distinguished between the reference and warm acclimatized animals.

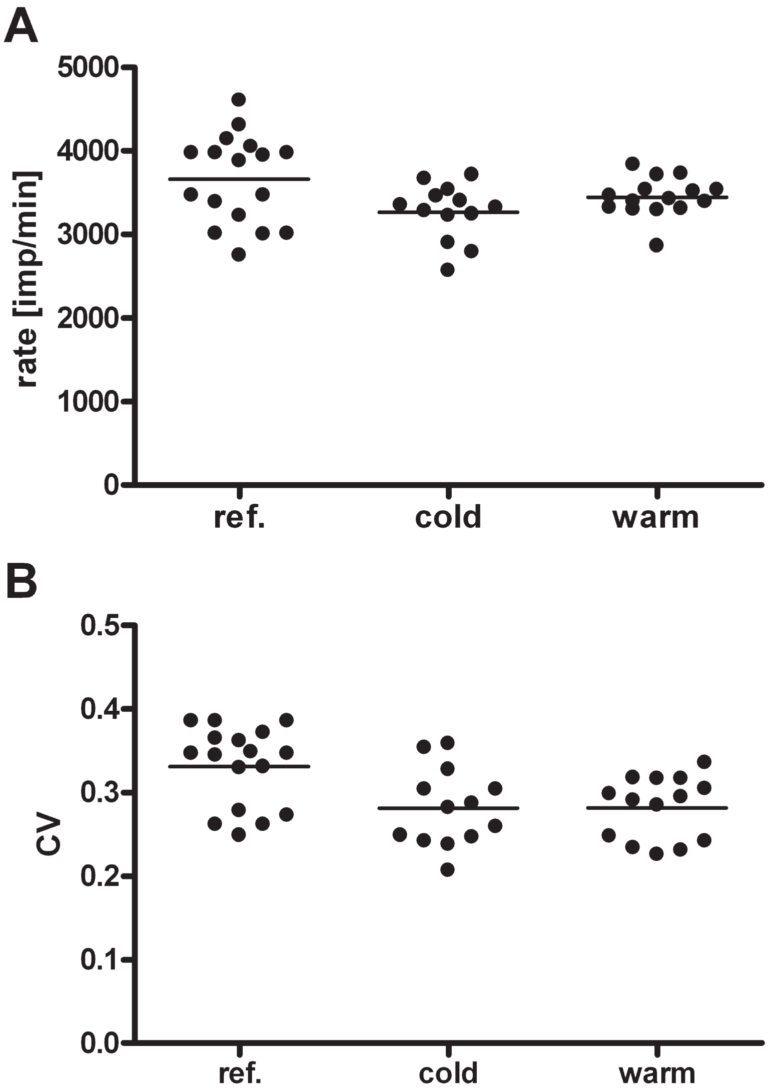

Figure 1: Resting activity in cold and warm acclimatized, and to laboratory conditions acclimated firebugs. **A:** Resting activity rate. **B:** Coefficient of variation of inter-impulse time intervals. Points represent individual experiments and lines the corresponding mean values.

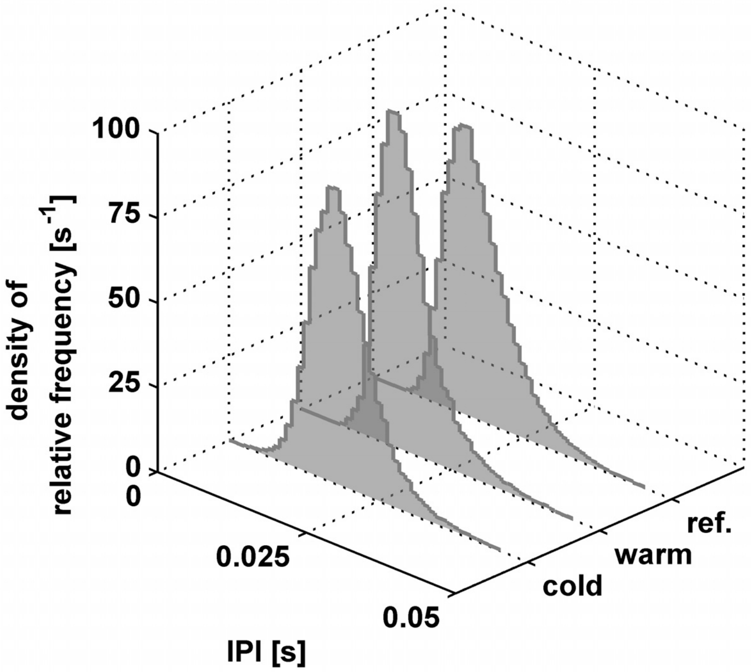

Figure 2: Average inter-impulse time interval (IPI) histograms obtained in cold and warm acclimatized, and to laboratory conditions acclimated firebugs.

Another parameter that describes the resting activity is the coefficient of variation (CV) of inter-impulse intervals (IPI), which quantifies the relative IPI variability and describes the regularity of impulse discharge. In the reference group the mean CV value was 0.33 (SE 0.01). In the cold as well as in the warm acclimatized group the CV was 0.28 (SE 0.01) (Fig. 1b). Again, no difference could be established between the cold and warm acclimatized animals and the CV was statistically higher in the reference group (α=0.05). However, even if these differences are considered, the resting activity rate and CV are limited to a very narrow range. This is well reflected by the similarity of the average IPI histograms in the three compared groups of firebugs (Fig. 2). The process of thermal adaptation therefore has no obvious influence on the resting activity of T_1 type trichobothrium.

To get an approximate estimation of the thermal conditions that allow the bug to become active, the temperature was measured in the natural habitat on a sunny winter day (19. 12. 2003). In the morning the bugs moved out from the shelter and assembled in the grooves of the tree bark. Despite the air temperature being close to 0°C, the temperature inside the grooves reached 25°C. However, the layer of the warm air extended only a few millimetres above the bark. The body temperature of the bug that was placed in the groove, exposed to the sunlight, increased from 5 °C to 28 °C. In comparison, in June (13. 6. 2003), at the air temperature of 31.5 °C, it increased to 37 °C (Fig. 3). Despite the difference of more than 30°C in the air temperature, the final body temperature in December was only 10°C lower than in June. Although the firebugs spent most of the time during the winter at low temperatures, in the sun they were warmed up to summer-like temperatures for a few hours (Fig. 4).

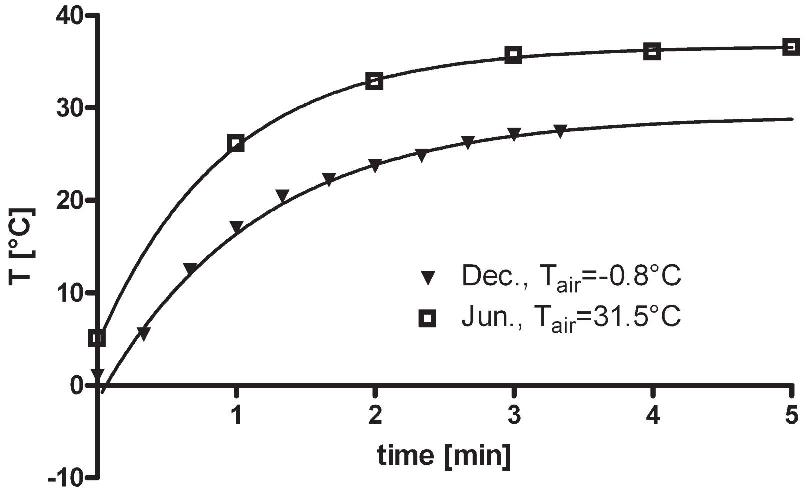

Figure 3: Warming of the firebug body. The temperature of the abdomen was measured. The animal with the body temperature of 5°C was placed into a bark groove exposed to the sun. The temperature of the air was measured in the shadow, 2 meters above the ground.

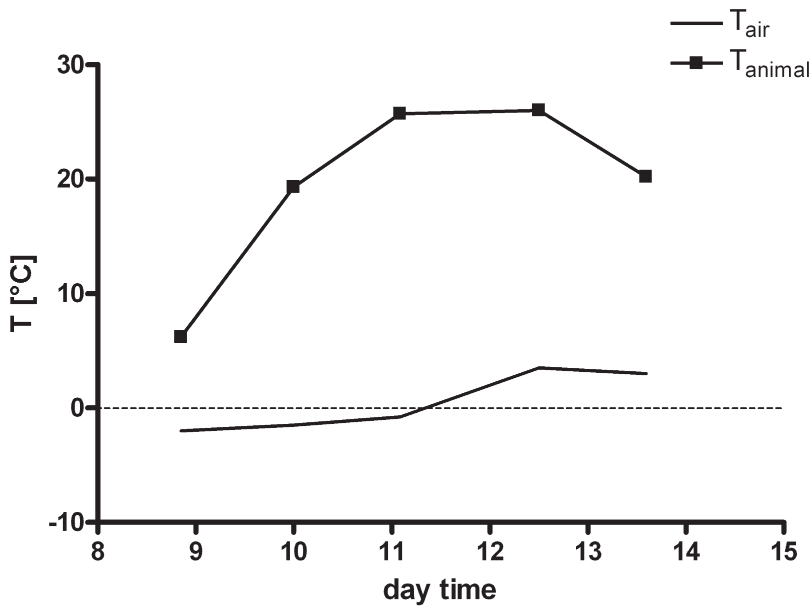

Figure 4: Daytime related change of the firebug body temperature. The position of the animal was in the bark groove on the sunny side of the tree. The measurement was done on 19. 12. 2003.

Discussion

In the summer a new generation of adult firebugs appears. They enter a diapause, and the animals that make it through the winter start breeding in the spring. To survive low temperatures during the winter they undergo a series of processes, including a modification of membrane phospholipids (Hodkova et al. 1999, 2002; Tomčala et al. 2006). Most of the data regarding the cold hardiness were obtained in the Bohemian population. The adaptation to low temperature can vary in geographically separated populations. The Bulgarian population, which is exposed to higher temperatures, for example, shows lower cold hardiness (Kalushkov & Nedved 2000). Because the annual temperatures in Slovenia are similar to the Bohemian, similar adaptation can be expected.

The composition of the cell membrane could affect the gating of the mechanosensitive ion channels (Kung 2005), which are the most likely candidate for the source of the resting activity in filiform sensilla (Buno et al. 1981, Thurm 2001). The resting activity of type T_1 trichobothrium should therefore reflect the effects of the acclimatization on the sensory cell function.

Our results show no significant difference neither in the rate nor the regularity of impulse discharge between the cold and warm acclimatized animals, when suddenly warmed to 20 °C. When interpreting these findings, tissue specificity has to be taken into consideration. Data regarding the membrane remodelling were obtained in the muscle and fat body (Hodkova et al. 1999, 2002; Tomčala et al. 2006). Extending these findings to the receptor cell seems appealing, however, it should be done with reservations. Perhaps the lipid composition of the sensory cell membranes is regulated in a way that ensures a constant resting activity throughout the year. Namely, the resting activity is important for the sensory receptor function, as it sets the reference level necessary for the stimulus encoding (Drašlar 1973, 1980). It is important that the sensory receptor remains calibrated when the animal gets active. In the sun the body temperature of the bug increases to summer-like temperature and remains high for a couple of hours even during the winter. Thus the body temperature, at which the firebugs are active, remains in the same range throughout the year. We suppose that the firebugs could have adapted in a way that enables them to survive low temperatures during the winter and also to preserve the sensitivity and calibration of the receptor when they get warmed and become active.

Conclusion

The level of resting activity in trichobothria of type T_1 in the firebug (*Pyrrhocoris apterus)* remains within narrow limits regardless of the season and thermal adaptation.

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