

## Electrophysiological characterization of the cold receptors in the ground beetle *Pterostichus oblongopunctatus*

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**Abstract.** Several insects possess thermoreceptors, which aid them in feeding and survival. Campaniform sensilla at the tip of antennae of ground beetle *Pterostichus oblongopunctatus* (Fab., 1787) (Coleoptera, Carabidae) show action potentials of the three sensory cells, A-, B- and C-cells, different in their spike amplitudes. Only the A-cell, with the largest spike amplitude, is highly sensitive to temperature fluctuations, showing remarkable changes in its firing rate induced already by changes in temperature of 0.1°C. A-cells respond to a rapid temperature drop with a strong phasic-tonic reaction; larger decreases in temperature evoke higher peak frequency values. Maximum peak frequencies in A-cells, varying from 344–588 Hz in different specimens, are induced by temperature drop of 7.9–15.7°C, whereas temperature rise strongly inhibits impulse activity of the A-cell. Number of action potentials per first second of response of eight beetles' was very various, from 7 to 210 impulses, induced by temperature drop from 0.1 to 14.6°C, higher temperature decreased the number of impulses. This knowledge serves as prerequisite information for future electrophysiological studies related to different habitat selection of ground beetles'.

**Key words:** campaniform sensilla, cooling, impulse activity, single-sensillum recording, thermoreceptor

### INTRODUCTION

The environment is to a large extent determined by the average climate and its seasonal variations. Superimposed is the capricious weather with frequent shifts in air temperature, precipitation rain or snow, and solar radiation. Insects have evolved mechanisms to survive, therefore, rapid detection of environment and body temperature is important for their survival (Tichy & Gingl, 2001). The control of insect body temperature by physiological or behavioural means implies the presence of thermoreceptors packaged in many different external cuticular structures (Altner & Prillinger, 1980; Altner & Loftus, 1985; Zacharuk, 1985). Certain butterflies possess thermoreceptors on their wings and antennae that protect them from heat damage during basking (Schmitz & Wasserthal, 1993; Schmitz, 1994). In beetles, thermoreceptors of the cave beetle *Speophyes lucidulus* have been examined both physiologically and morphologically (Loftus & Corbière-Tichanè, 1981; Corbière-Tichanè & Loftus, 1983). By electrophysiological recording, of campaniform sensilla on the antennae of the ground beetle *Pterostichus aethiops* Pz. were classified as cold receptors (Merivee et al., 2003). Small *sensilla campaniformia* in ground beetles *Bembidion lampros* Hbst. and *B. properans* Steph., and *sensilla campaniformia* type 1

in *Platynus dorsalis* Pont., usually occur in pairs close to each other on the flagellomeres. Their number reaches maximum of 5–9 on the terminal flagellomere, whereas 3–5 are located at the very tip of the flagellomere (Merivee et al., 2000, 2001, 2002).

The purpose of this electrophysiological study was to ascertain the function of the campaniform sensilla located at the tip of the terminal flagellomere of the ground beetle *Pterostichus oblongopunctatus* (Fab., 1787).

## MATERIALS AND METHODS

### **Insect**

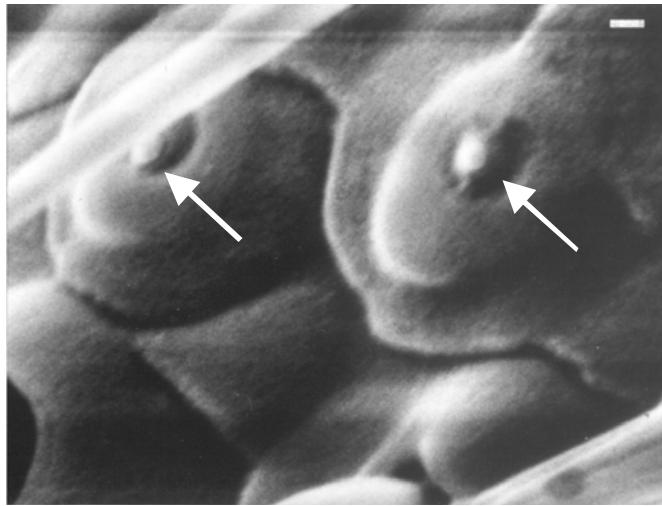
Adult ground beetles *P. oblongopunctatus* used in this study were collected from their hibernating aggregation at forest margin in southern Estonia in May 2003 and preserved in plastic boxes with moistened sand at +2°C. Before the experiments, the beetles were kept at room temperature (23–25°C) for a week. The beetles provided with pure water and were fed with moistened cat food (Kitekat, Master Foods, Poland).

### **Preparation and stimulation**

Single-sensillum recordings were made with microelectrodes comprised a 0.1 mm tungsten wire, that were electrolytically sharpened with a highly concentrated solution of potassium hydroxide. The electrode was inserted into the base of a campaniform sensillum. For electrophysiological recordings, the beetles were fastened to an aluminium stand in a suitable position with special clamps and beeswax, so that campaniform sensilla at the tip of the terminal flagellomere were visible and recognizable under the light microscope at a magnification of 600x, well exposed to cooling and warming airstreams, and easily accessible for microelectrode manipulations. Recordings were made on a total of 8 campaniform sensilla of eight beetles, of successive cooling series, were obtained. For deliver thermostimuli were used two air streams emerging at 2m/s from two copper tubes, hot and cold tube, with an inner diameter of 2.5 mm. The orifices of the tubes were placed 10 mm from the tip of the antenna. The temperature of the cold tube was equal to the room temperature (approximately 25°C). Rapid temperature changes were produced by presetting the temperature of the airstreams and switching rapidly between them. During these rapid cooling test series, the initial temperature of the heated air was gradually increased and held constant for 3 min or more before each cooling to guarantee adaptation of the sensory cells. About electrodes and their insertion, recordings of action potentials, temperature stimulation and measurement has been described elaborately and previously (Merivee et al., 2003).

### **Data management and analysis**

The nerve impulses from several sensory cells of a campaniform sensillum could be distinguished by differences in spike amplitudes. The frequency (Hz) of impulses was calculated as the reciprocal of the period between two successive impulses using TestPoint and Microsoft Excel (Microsoft Corp., Redmond, U.S.A.) software. Linear multiple regression analysis was used to investigate the relationship between  $\Delta t$  °C and the response (impulses per second) of campaniform sensilla. To normalize the data, a  $\log_{10}$  and square-root transformation was applied.



**Fig. 1.** Antennal campaniform sensilla in a ground beetle. Arrows show the central cap in the middle of the small dome surrounded by a larger cuticular collar. Scale bar = 1  $\mu\text{m}$ .

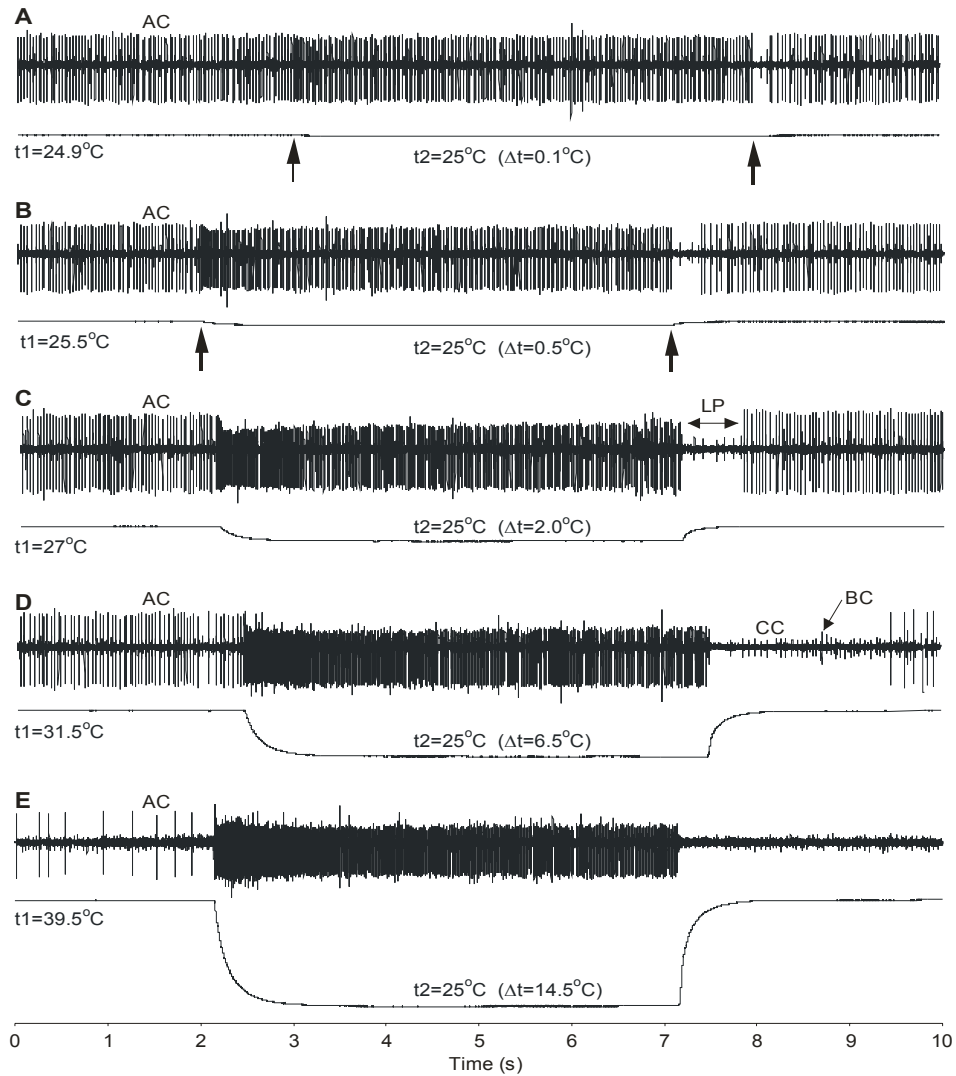
## RESULTS

The tested campaniform sensilla were in a row at the very tip of the terminal flagellomere of the ground beetle *P. oblongopunctatus*. *Sensilla campaniformia* are small domes with radial ribs surrounded by a raised cuticular collar. A tiny cap lie in the centre of the dome (Fig. 1). A recording microelectrode inserted into the dome base of these sensilla picks up action potentials of three sensory cells, A-, B-, and C-cells, distinguished by differences in their spike amplitudes (Fig. 2). Only the A-cell, with the largest spike amplitude, is highly sensitive to temperature fluctuations. The single sensillum recordings (Fig. 2) illustrate the responses of a typical terminal campaniform sensillum to rapid cooling and rewarming elicited by different  $\Delta t$ .

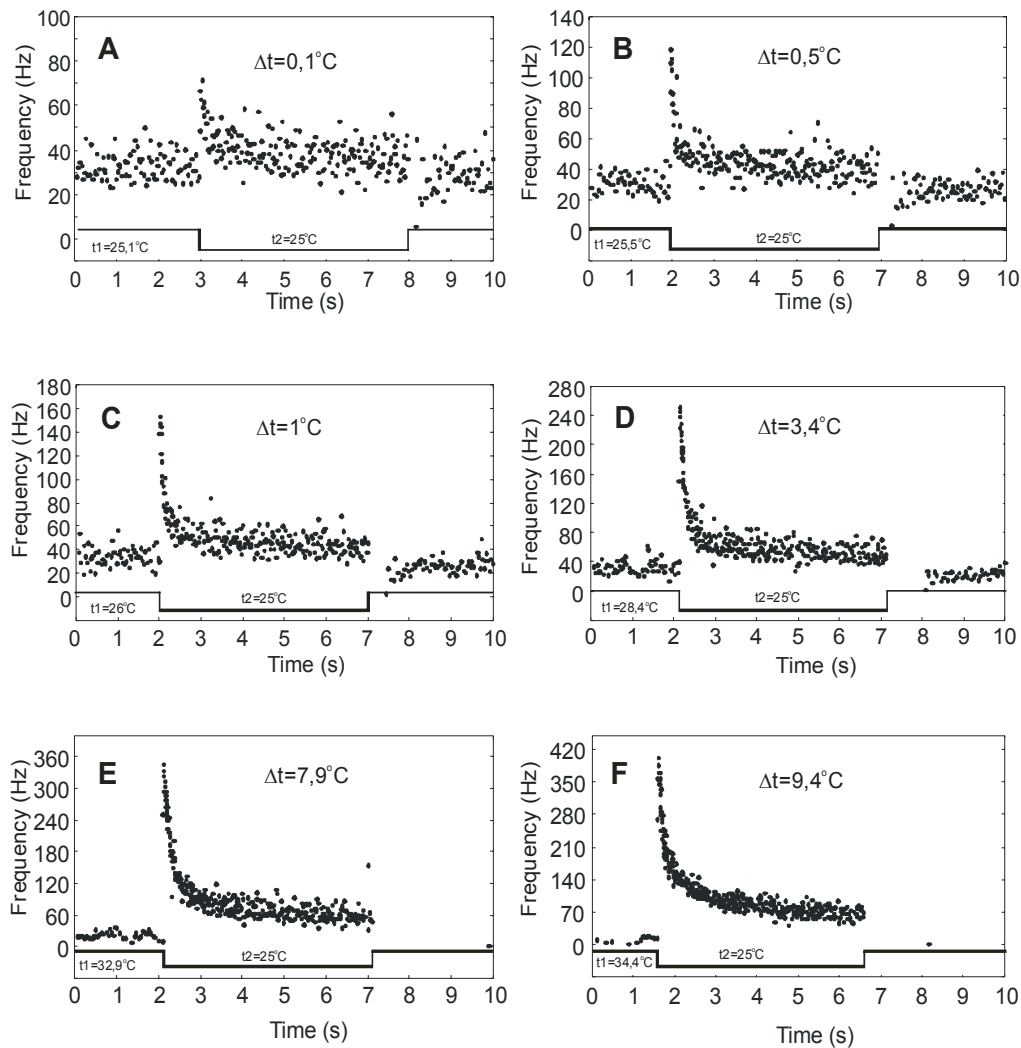
A-cells from campaniform sensilla of different beetles are various in their firing rate. Their activity, calculated from the 2-s precooling period of the recordings, was influenced strongly by the temperature. The higher the temperature of the air stream, the lower the impulse activity of A-cells (Fig. 2, precooling part of the recordings; and 3, precooling part of the recordings). A-cells responded to rapid drop in temperature with a strong phasic-tonic rise in their firing rate, even at  $\Delta t$  of 0.1–0.5°C (Fig. 2). The larger the difference between initial and cooling temperature, the higher were peak frequencies in the phasic response (Fig. 3), and the higher was the mean impulse frequency in the tonic part of the response. Maximum peak frequencies in A-cells, varying from 344 to 588 Hz in different specimens were observed at  $\Delta t$  of 7.9–15.7°C. Increasing  $\Delta t$  further, by increasing the initial temperature, led to a drop in values of peak frequencies (Fig. 4). The number of A-cell impulses in eight beetles' campaniform sensilla per first second stimulus was different, varying from 7 to 210, are caused by temperature drop from 0.1 to 14.6°C, higher temperature decreased

impulse activity (Fig. 4). After that adaptation period, the A-cell tended to return to the initial firing rate (Fig. 2, see the precooling parts of the recordings).

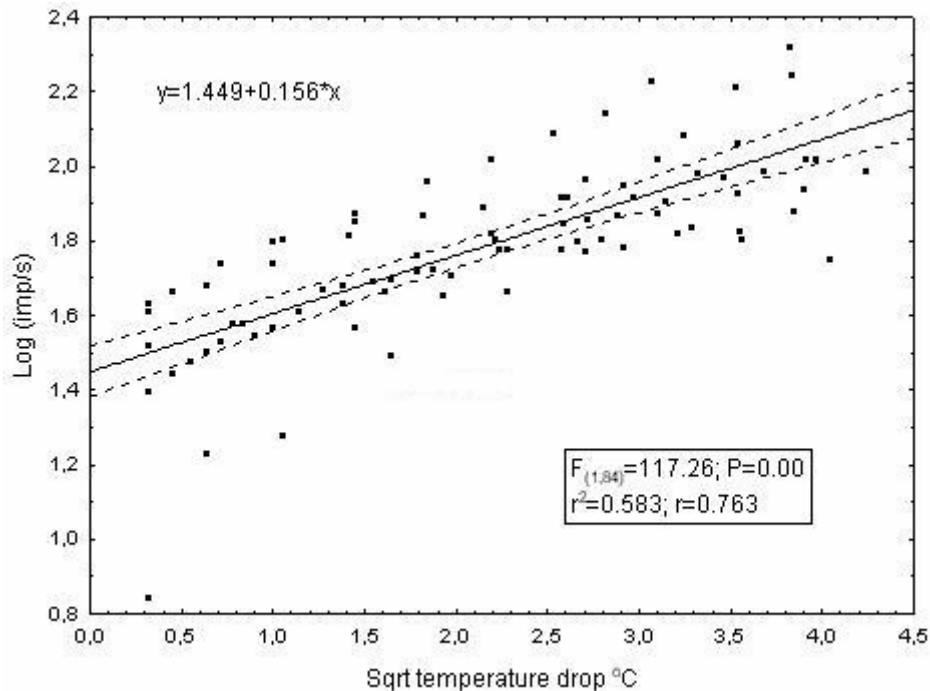
The B- and C-cell did not show a phasic component in their responses to rapid cooling and rewarming. As a response to rewarming, an interspike period occurred, followed by a return of the firing rate to the previous precooling rate (Fig. 2).



**Fig. 2.** Responses of a *P. oblongopunctatus* campaniform sensillum to different degrees of rapid cooling and rewarming. The continuous line under recording indicates the change in temperature, measured by a thermocouple circuit. AC, BC and CC show action potential of the A-, B- and C-cells, respectively. Vertical arrows indicate the start and the end of thermal stimulus (A, B).  $t_1$ , initial temperature;  $t_2$ , cooling temperature;  $\Delta t$ , change in temperature; LP, long interspike period. Recordings are based on the same set of sensory cells.



**Fig. 3.** Changes in the firing rate of an A-cell as a response to different degrees of rapid cooling and re-warming. Instantaneous frequency values (Hz) were calculated as a reciprocal of the period between two successive impulses beginning from the second impulse in a sequence. The start and end of cooling stimulus are schematically shown with continuous lines.  $t_1$ , initial temperature;  $t_2$ , cooling temperature;  $\Delta t$ , the difference between initial and cooling temperature. Before each cooling, initial temperature was gradually increased and held constant for 3 min or more in order to guarantee adaptation of the cell. Note that steady firing rate decreases when the value of steady temperature increases (the first two periods of the recordings A–D). A phasic-tonic reaction pattern of the A-cell to rapid cooling can be observed. The peak frequencies of the phasic component increase when  $\Delta t$  increases.



**Fig. 4.** Linear multiple regression analysis was used to investigate the relationship between  $\Delta t$  °C and the response (impulses per second) of campaniform sensilla. To normalize the data, a  $\log_{10}$  and square-root transformation was applied.

## DISCUSSION

Our recordings show that the campaniform sensilla at the tip of the ground beetle *P. oblongopunctatus* is a thermoreceptor (i.e. cold receptor). Action potentials of three sensory cells, A-, B- and C-cells, were distinguished by differences in their spike amplitudes. The A-cell show activity to temperature fluctuations, peak frequencies increased up to 588 Hz. Any decrease in temperature results in a phasic-tonic increase in spike frequency. On the other hand, warming causes suppression of the discharge rate. This response behaviour of a cold receptor has frequently been found in the coeloconic and short hair-like sensilla on antennae of other insects (Lacher, 1964; Loftus, 1968; Waldow, 1970; Loftus & Corbière-Tichané, 1981). These sensilla generally increase their firing rate to decreases in temperature and decrease their firing rate to increases in temperature. Cold receptors are located also on the antennae of previously tested campaniform sensilla of the ground beetle *Pterostichus aethiops*. These sensilla had similar responses to temperature fluctuations (Merivee et al., 2003).

The impulse activity of A-cells in eight *P. oblongopunctatus* beetles' campaniform sensilla per first second stimulus was very different, varying from 7 to 210 impulses, are induced by temperature decrease of 0.1 to 14.6°C. Temperature rise decreased the number of impulses. This kind of information is necessary for future studies on different habitat selection by means of insects' thermoreceptors.

By contrast to the A-cell, in the response to temperature changes by B- and C-cells, the phasic component is always missing. This kind of nonspecific temperature dependence has been observed on insects' olfactory-, carbon dioxide- and hygrometers (Lacher, 1964; Loftus, 1968; Waldow, 1970; Altner & Loftus, 1985). Thermoreceptive cells are usually in combination with hygrometers (Tichy & Gingl, 2001). The function of the B- and C-cells in terminal campaniform sensilla of *P. oblongopunctatus* is not clear.

To compare the pattern of response of the *P. oblongopunctatus* and previously tested *P. aethiops* (Merivee et al., 2003) receptors, the differences absent, probably because these species are living in the same habitat. In all probability thermoreceptors are involving with habitat selection in carabid beetles and they enable to avoid a hyperthermia of insects.

## CONCLUSIONS

Insects evidently detect variations in temperature, yet the function and location of receptors is poorly known. This study shows that terminal campaniform sensilla at the tip of antennae of the ground beetle *P. oblongopunctatus* contains three sensory cells. One cell, with the largest spike amplitude was distinguished as cold receptor. The thermosensitive cells of *P. oblongopunctatus* are probably powerful tools for detecting and measuring environmental temperatures. For a better interpretation of thermosensitivity of insects, more research is required.

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