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Responses of the antennal bimodal hygroreceptor neurons to innocuous and noxious high temperatures in the carabid beetle, *Pterostichus oblongopunctatus*

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ABSTRACT

Electrophysiological responses of thermo- and hygroreceptor neurons from antennal dome-shaped sensilla of the carabid beetle *Pterostichus oblongopunctatus* to different levels of steady temperature ranging from 20 to 35 °C and rapid step-changes in it were measured and analysed at both constant relative and absolute ambient air humidity conditions. It appeared that both hygroreceptor neurons respond to temperature which means that they are bimodal. For the first time in arthropods, the ability of antennal dry and moist neurons to produce high temperature induced spike bursts is documented. Burstiness of the spike trains is temperature dependent and increases with temperature increase. Threshold temperatures at which the two neurons switch from regular spiking to spike bursting are lower compared to that of the cold neuron, differ and approximately coincide with the upper limit of preferred temperatures of the species. We emphasise that, in contrast to various sensory systems studied, the hygroreceptor neurons of *P. oblongopunctatus* have stable and continuous burst trains, no temporal information is encoded in the timing of the bursts. We hypothesise that temperature dependent spike bursts produced by the antennal thermo- and hygroreceptor neurons may be responsible for detection of noxious high temperatures important in behavioural thermoregulation of carabid beetles.

1. Introduction

Temperature and humidity conditions are undoubtedly the most important environmental factors influencing geographical distribution and habitat selection in insects (Thiele, 1977; Lövei and Sunderland, 1996; Holland, 2002). Ground dwelling carabid beetles are very vulnerable to desiccation and high temperature injury (Thiele, 1977; Denlinger and Yocum, 1998; Hochachka and Somero, 2002; Robertson, 2004; Klose and Robertson, 2004; Chown and Terblanche, 2007). On entering direct sunlight, due to solar IR radiation, a 10-mg insect can heat up by 10 °C in only 10 s (Heinrich, 1993). The temperature at which total heat paralysis begins in various carabids lies in a narrow range between 47.4 and 51.7 °C (Thiele, 1977). Steep temperature gradients are common both above and below the ground surface, and the microclimatic conditions can be greatly modified, especially in areas with vegetation. In Estonia, on sunny summer days with air temperatures above 22 °C, maximum soil surface temperatures in sunlit areas may reach lethal high values above 50 °C while minimum soil surface temperatures in shaded areas below the foliage remain 30–40 °C lower (Must et al., 2006a). Brief forays into sunlit high temperature zones are readily tolerated, however, as long as the animal has the option of retreating frequently to a more moderate environment to prevent overheating and desiccation (Thiele, 1977; Denlinger and Yocum, 1998; Chown and Nicolson, 2004). Behavioural thermoregulation is a complex process that includes sensing of temporal and spatial variation in the thermal environment, and subsequent processing of environmental information (Cooper, 2002; Seebacher and Shine, 2004; Seebacher and Franklin, 2005). In *Pterostichus oblongopunctatus* (Fabricius, 1787) (Coleoptera, Carabidae), the subject of the current experiments, temperature preference depends on ambient air humidity and lies between 10 and 25 °C (Thiele, 1977). As a eurytopic forest species with a palaearctic distribution, the adults are more or less euryhygric to dry-preferring (Lindroth, 1986).

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In carabid beetles, the sensory neurons responsible for detection of ambient temperature are located on the surface of the antennae within dome-shaped or campaniform sensilla. These sensilla occur pairwise on the ventral surface of all nine flagellomeres, and in addition, 5–6 of them lie at the distal margin of the terminal flagellomere (Merivee et al., 2000, 2001, 2002). Both outer and inner structure of the dome-shaped sensilla has been described in the myrmecophilous carabid beetle *Paussus favieri* (Di Giulio et al., 2012). These sensilla are innervated by three neurons, one thermoreceptor (TN) and two hygroreceptor neurons. Electrophysiological experiments confirm the existence of two physiologically different types of hygroneurons: the dry neuron (DN) and the moist neuron (MN) (Merivee et al., 2010), and one thermoreceptor (cold) neuron (CN) (Merivee et al., 2003) in the antennal dome-shaped sensilla in various carabids. A similar triad of thermo- and hygroneurons has been found in various insects and spiders (Waldow, 1970; Tichy, 1979; Altner and Prillinger, 1980; Altner and Loftus, 1985; Tichy and Loftus, 1996; Tichy and Gingl, 2001).

Our knowledge on insect thermoreception is incomplete. Though, the firing rate of the CN increases with rapid temperature decrease and vice versa (Loftus, 1968; Merivee et al., 2003), it does not measure ambient temperature correctly. In the honeybee *Apis mellifera* L. and some carabid beetles, the firing rate of the CN decreases with temperature increase and, at high temperatures above 30–40 °C, spike production may fall to zero, especially when stimulated with a rapid temperature increase (Lacher, 1964; Merivee et al., 2003; Must et al., 2006a,b), making precise detection of dangerously high temperatures impossible. Second, in the American cockroach *Periplaneta americana* and some carabids, the stationary firing rate of the CN does not depend on temperature ranging from 20 to 35 (40) °C at all (Loftus, 1968; Must et al., 2006a,b). Third, the CN fires a high frequency phasic spike train (peak frequency burst) in response to a rapid temperature drop and adapts after several seconds to a frequency determined by the new steady temperature (Loftus, 1968; Merivee et al., 2003; Must et al., 2006a). The peak frequency of the initial spike burst depends primarily upon the initial temperature when the antenna is exposed to 25–32 °C, but above or below this range the extent of temperature change becomes prevalent. Thus, a particular peak frequency can be achieved by several different combinations of initial temperature and magnitude of temperature drop. This ambiguity and significant fluctuations in frequency at steady temperature make it unlikely that the CN is a useful thermometer (Loftus, 1968). These results suggest that additional noxious high temperature encoding mechanisms should be hidden in the spike trains of the neuron triad. In this context, it is of interest that at high temperatures the antennal CNs of the carabid beetle *Platynus assimilis* switch from regular spiking to a bursting manner of firing (Must et al., 2010). The threshold temperature of spike bursting varies in different neurons from 25 to 47 °C. Interspike interval (ISI) analysis showed that the burstiness of the spike trains are temperature dependent and may precisely encode noxious high temperatures in a graded manner.

Hygroneurons may also be involved in thermoreception of insects. In *P. americana* and *Locusta migratoria*, the DN responds to both temperature and humidity resulting in such excessive ambiguity that the unit has been termed bimodal (Waldow, 1970; Loftus, 1976; Altner and Loftus, 1985). The MN of *Carausius*, *Apis* and *Locusta* is another bimodal unit whose firing rate is affected by both humidity and temperature (Lacher, 1964; Waldow, 1970; Altner and Loftus, 1985; Tichy, 1987). By contrast, relative humidity alone has been shown to be an adequate stimulus for the antennal MN of *P. americana* (Yokohari and Tateda, 1976; Yokohari, 1978). No research has been conducted on the temperature sensitivity of the hygroneurons in carabids. The biological significance of bimodality of the hygroneurons remains unclear.

The aim of this study was to test the sensitivity of the antennal DN and MN to temperature in *P. oblongopunctatus*. The electrophysiological experiments were carried out in the range of air temperatures from 20 to 35 °C which approximately falls within the range of preferred temperatures of the species (Thiele, 1977). The responses of the hygroneurons to temperature were measured in two ambient humidity situations: at constant relative (RH) and absolute humidity (AH) conditions, respectively. Results of the experiments are presented in this paper.

2. Materials and methods

2.1. Test beetles

Adult beetles were collected from a local population in southern Estonia before they left their preferred overwintering sites in brown-rot decayed wood in spring 2014. The beetles were kept in $20 \times 30 \times 10$ cm plastic boxes filled with moist pieces of brown-rotted wood and moss at 5 °C for a couple of weeks until they were used in tests. Three to four days prior to experiments, the beetles were exposed to room temperature (20 °C), fed with larvae of *Tenebrio molitor* and given tap water to drink every day.

For electrophysiological experiments, each test beetle was immobilised by placing it firmly into a conical tube made of thin sheet-aluminium of a size that allowed its head and antennae to protrude from the narrower end of the tube. The wider rear end of the tube was blocked with a piece of plasticine to prevent the beetle from retreating out of the tube. The antennae of the beetle were fastened horizontally onto the edge of a special aluminium stand with tiny amounts of beeswax so that dome-shaped sensilla at the tip of the terminal flagellomere were visible from above under a light microscope, were well exposed to stimulating airstreams, and were readily accessible for microelectrode manipulation from the side.

2.2. Electrophysiology

2.2.1. Single sensillum recordings

Tungsten wire (0.08 mm diameter) microelectrodes were sharpened electrolytically in a concentrated KOH solution. The indifferent electrode was inserted into the antennal lumen at the base of the antennal flagellum. The recording electrode was thrust into the base of the dome-shaped sensillum at the distal margin of the terminal flagellomere using the micromanipulator DC-3KS with push-button control (Stoelting CO., USA) under visual control with the upright electrophysiological light microscope Eclipse FN1 (Nikon, Japan) at a magnification 500–1000×. The microscope was equipped with the ITS-FN1 Physiostage (Nikon, Japan) consisting of the X-Y Translator and stainless steel Stage mounted on the Passive Anti-Vibration ScienceDesk (ThorLabs, UK). All experiments were performed in the $100 \times 120 \times 100$ cm Faraday Cage FAR01 (ThorLabs, UK).

Spikes picked up by the recording electrode and initially amplified (input impedance 10 GΩ) by a custom-made Preamplifier Board (Interspectrum, Estonia) were led to input of the main amplifier ISO-DAM 8A (World Precision Instruments, USA), filtered with a bandwidth set at 10–3000 Hz, monitored on an oscilloscope screen, and relayed to a computer via an A/D input board DAS-1401 (Keithley, Taunton, Massachusetts) for data acquisition and storage using testpoint software (Capital Equipment Corp., Billerica, Massachusetts) at a sampling rate of 10 kHz (channel 1). The recordings were made from one sensillum of each test beetle. The number of tested beetles (N) is shown directly everywhere