



Bursty spike trains of antennal thermo- and bimodal hygro-thermoreceptor neurons encode noxious heat in elaterid beetles



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ABSTRACT

The main purpose of this study was to explain the internal fine structure of potential antennal thermo- and hygroreceptive sensilla, their innervation specifics, and responses of the sensory neurons to thermal and humidity stimuli in an elaterid beetle using focused ion beam scanning electron microscopy and electrophysiology, respectively. Several essential, high temperature induced turning points in the locomotion were determined using automated video tracking. Our results showed that the sensilla under study, morphologically, are identical to the dome-shaped sensilla (DSS) of carabids. A cold-hot neuron and two bimodal hygro-thermoreceptor neurons, the moist-hot and dry-hot neuron, innervate them. Above 25–30 °C, all the three neurons, at different threshold temperatures, switch from regular spiking to temperature dependent spike bursting. The percentage of bursty DSS neurons on the antenna increases with temperature increase suggesting that this parameter of the neurons may encode noxious heat in a graded manner. Thus, we show that besides carabid beetles, elaterids are another large group of insects with this ability. The threshold temperature of the beetles for onset of elevated locomotor activity (OELA) was lower by 11.9 °C compared to that of critical thermal maximum (39.4 °C). Total paralysis occurred at 41.8 °C. The threshold temperatures for spike bursting of the sensory neurons in DSS and OELA of the beetles coincide suggesting that probably the spike bursts are responsible for encoding noxious heat when confronted. In behavioural thermoregulation, spike bursting DSS neurons serve as a fast and firm three-fold early warning system for the beetles to avoid overheating and death.

1. Introduction

Environmental temperature is of great significance for geographical distribution, habitat selection, ecological performance and survival of small poikilothermic animals such as insects. They have no way other than behavioural thermoregulation to control their body temperature. To detect circumambient temperature insects are equipped with special thermosensory sensilla on their antennae. Although cuticular parts of these sensilla are highly variable in shape, typically they are innervated by a triad of sensory neurons composed of one temperature sensitive (cold) neuron (CN) and two antagonistically responding hygroreceptor neurons, the dry (DN) and the moist air neuron (MN), respectively (Altner and Prillinger, 1980; Altner and Loftus, 1985; Chapman, 1998;

Merivee et al., 2003, 2010; Piersanti et al., 2011; Must et al., 2017). Studies on ultrastructure of the classical (coeloconic) hygro- and thermoreceptive sensilla (HTSs) have explained their innervation specifics (Altner and Loftus, 1985; Steinbrecht, 1989; Chapman, 1998; Tichy and Kallina, 2010). The unbranched outer dendritic segments (ODSs) of the two hygroreceptor neurons tightly fill the peg lumen and reach the tip of the cuticular peg. The dendrite of the CN, larger in diameter than that of the MN and DN and irregular in cross-section due to branching and lamellation, terminates below the peg base. Below the cuticular peg, all the dendrites are concentrically enveloped by the dendritic sheath and three auxiliary cells, the thecogen, trichogen and tormogen cells, respectively.

The existing data suggest, however, that firing rate of the CN does

Abbreviations: AH, absolute humidity; CHN, cold-hot neuron; CN, cold neuron; CTMax, critical thermal maximum; CV of ISI, the coefficient of variance of interspike intervals; DAN, dendrite of the A-neuron; DBN, dendrite of the B-neuron; DCN, dendrite of the C-neuron; DHN, dry-hot neuron; DN, dry neuron; DSS, dome shaped sensilla; FIB/SEM, focused ion beam/scanning electron microscope; HTN, hygro- and thermoreceptor neurons; HTS, hygro- and thermoreceptive sensilla; ISI, inter-spike interval; ISIH, inter-spike interval histogram; LA, locomotor activity; MHN, moist-hot neuron; MN, moist neuron; ODS, outer dendritic segments; OELA, onset of elevated locomotor activity; PCA, principal component analysis; RH, relative humidity; SE, standard error; SFR, stationary firing rate; TP, total paralysis

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not encode ambient temperatures, especially noxious heat above 30–35 °C, correctly. The CN is extremely sensitive to rapid changes in temperature. Its firing rate phasically or phasic-tonically increases with temperature step-decrease and vice versa, the spike production of the neuron decreases or stops for a certain period of time in response to rapid warming (Loftus, 1968; Waldow, 1970; Merivee et al., 2003; Piersanti et al., 2011). These responses of the CN depend on both initial temperature and the extent of temperature increase. At the high end of physiologically relevant temperatures (30–35 °C), rapid warmings by several degrees may cause silent periods with no spikes lasting for several seconds or more (Merivee et al., 2003; Nurme et al., 2015), making detection of further warmings impossible. Due to ambiguity derived from the fact that particular rates of spike firing can be achieved by many combinations of initial temperature and the extent of change in temperature, it has been considered unlikely that firing rate of the CN can precisely encode ambient temperatures (Loftus, 1968). In many insects, such as the American cockroach *Periplaneta americana*, the dragonfly *Libellula depressa* and some carabid beetles, the stationary firing rate of the CN does not depend on temperature at all (Loftus, 1968; Must et al., 2006a, 2006b; Piersanti et al., 2011).

Encoding noxious high temperatures by insects' thermoreceptor neurons has been a mystery for a long time; however, recently it was discovered that, in carabid beetles, at temperatures above 25–30 °C, the pattern of the spike trains produced by the CN and two bimodal hygroreceptor neurons, housed in antennal dome-shaped sensilla (DSSs) changes, and the neurons switch from regular spiking to spike bursting (Must et al., 2010; Nurme et al., 2015). Several parameters of the bursty spike trains, the percentage of bursty neurons on the antenna, the coefficient of variance of interspike intervals (CV of ISIs) and the percentage of bursty spikes in a spike train, the number of spikes and the ISIs in a burst unambiguously depend on temperature and thus may precisely encode both noxious heat up to 45 °C as well as rapid step-changes in it. It has been hypothesised that thermal information encoded by the bursty spike trains may be used by the beetles in their behavioural thermoregulation (Must et al., 2010; Nurme et al., 2015).

Surprisingly, neurons capable of temperature dependent spike bursting have not been found in insect HTSs other than the DSSs of carabid beetles. Inner fine structure of cuticular parts of the DSSs differs fundamentally from all known types of insect sensilla (Nurme et al., 2015; Must et al., 2017). In the carabid *Pterostichus oblongopunctatus*, they are composed of a round cuticular dome, approximately 6.5 µm in diameter, and of a non-perforated conical peg, about 3 µm in length, tightly inserted into the sensillum socket so that the tiny tip of the peg (diameter 0.5 µm; height 0.3 µm) is directly exposed to ambient air. As in the classical coeloconic HTSs, DSSs of *P. oblongopunctatus* are innervated by the triad of hygro- and thermoreceptor neurons (HTNs) (Must et al., 2017). However, some fundamental structural differences exist in ODSs of the sensory neurons between the two types of sensilla. In contrast to the classical HTSs, in DSSs, the strongly branched and lamellated large ODS of the CN enters deeply into the peg lumen, up to a half of its length and ODSs of both hygroreceptor neurons have numerous, small apical branches. These sensilla, although small in number, are common on the antennal flagellum of carabid beetles (Merivee et al., 2000, 2001, 2002; Di Giulio et al., 2012).

In this study we hypothesise that spike bursting is a fundamental property of insect thermoreceptor neurons responsible for detecting noxious high temperatures to avoid overheating and death, and should be widely distributed among insects, in addition to carabid beetles. Elaterid beetles (Coleoptera, Elateridae) seem to be suitable insects to test this hypothesis and the species *Agriotes obscurus* seems to be a good representative of the family. First, this species prefers to live in open agricultural grasslands and crop fields (Traugott et al., 2015) exposed to direct sunlight where, on sunny days, soil surface maximum temperatures may reach lethal high values of up to 50 °C (Must et al., 2006a). In these thermal conditions, proper sensing and avoidance of noxious heat is crucial for the beetles to survive. No data is available on the upper

thermotolerance limits in *A. obscurus*. In the carabids, living in open habitats together with *A. obscurus*, threshold temperatures for total heat paralysis lie in a narrow range between 47.4 and 51.7 °C (Thiele, 1977). However, first indications of partial paralysis (of the hind legs) begin at 44.4 °C (Must et al., 2010). Threshold temperatures of both partial and total paralysis are related to critical thermal maximum of a species (CTMax) (Ernst et al., 1984; Ribeiro et al., 2012) which is widely used to assess thermal limits in ectothermic animals (Lutterschmidt and Hutchison, 1997). It is defined as the temperature above which individuals of a given species respond with disturbances to normal locomotion, subjecting the animal to likely death. Second, antennal cuticular structures, externally similar to dome-shaped sensilla of carabid beetles (Nurme et al., 2015; Must et al., 2017), have been found in a number of elaterid beetles including *A. obscurus* (Merivee, 1992; Merivee et al., 1997, 1998, 1999; Zauli et al., 2016). Because of lack of data on the inner structure of elaterid beetles, different studies and authors have incorrectly and/or inconsistently classified the sensilla campaniformia (Merivee et al., 1998, 1999; Zauli et al., 2016), DSSs (Merivee et al., 1997), and DSSs proposed as a subtype of s. coeloconica (Merivee, 1992). In elaterid beetles, these sensilla are few in number varying between 20 and 40 per antenna (Merivee, 1992; Merivee et al., 1997, 1998, 1999; Zauli et al., 2016), and they are almost evenly distributed on the flagellomeres varying between 2 and 7 depending on the species, except for the terminal flagellomere where their number is higher reaching up to 15 in *Elater ferrugineus*. We also hypothesise that these sensilla on the antennae of elaterid beetles by their morphological type and innervation are identical to the DSSs of *P. oblongopunctatus* but fine morphological studies on their inner structure and electrophysiological experiments are needed to confirm this.

The aims of this study are:

- to clarify morphological type of the possible HTSs on the antennae of *A. obscurus* on the basis of their inner fine structure using a FIB/SEM combined technique;
- through electrophysiological experiments, to test responsiveness and reaction type of the sensory neurons innervating the possible HTSs to ambient air temperature and humidity;
- through electrophysiological experiments, to test the expected ability of the sensory neurons for high temperature induced spike bursting in the range of 25–45 °C;
- through behavioural experiments, to determine CTmax and threshold temperature for total paralysis (TP) for the beetles.

The results of the study are reported in this paper.

2. Material and methods

2.1. Test beetles

Reproductively-immature adults of *A. obscurus* overwintering in soil were collected in South Estonia in September and October of 2014 and 2015. The beetles were kept in plastic boxes (20 × 20 × 10 cm) filled with moistened sand and moss in a refrigerator at 2–3 °C. Four to five days prior to the experiments, they were placed singly in 50-mm Petri dishes with moistened filter paper (Whatman International, UK) and kept at 20 °C in a Versatile Environmental Test Chamber MLR-35 1H (SANYO Electric, Japan) at 16 h light and 8 h dark (16L:8D) photoperiod. Conditioning of the test insects at room temperature was imperative to achieve good electrical contact with the sensory neurons inside the antennal DSS and an acceptable signal-to-noise ratio. The beetles were not fed but were provided with clean tap water every day. Electrophysiological recordings and behavioural experiments were carried out from March to May i.e. after the cold reactivation of the beetles.

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