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Carbon dioxide detection in adult Odonata

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ABSTRACT

The present paper shows, by means of single-cell recordings, responses of antennal sensory neurons of the damselfly *Ischnura elegans* when stimulated by air streams at different CO_2 concentrations. Unlike most insects, but similarly to termites, centipedes and ticks, Odonata possess sensory neurons strongly inhibited by CO_2, with the magnitude of the off-response depending upon the CO_2 concentration. The Odonata antennal sensory neurons responding to CO_2 are also sensitive to airborne odors; in particular, the impulse frequency is increased by isoamylamine and decreased by heptanoic and pentanoic acid. Further behavioral investigations are necessary to assign a biological role to carbon dioxide detection in Odonata.

1. Introduction

Carbon dioxide (CO_2) is a sensory cue that plays multiple roles in insect behavior, such as foraging behavior by hematophagous insects (e.g., mosquitoes), foraging and oviposition behavior of phytophagous insects and behavior of social species (Guerenstein and Hildebrand, 2008). CO_2 is clearly an olfactory stimulus, but its properties are quite distinctive because it is ubiquitous, it is a very volatile and relatively inert and small molecule compared to other olfactory cues, and the sensory neurons detecting CO_2 have a higher threshold than those tuned to other olfactory stimuli (Stange and Stowe, 1999). In addition, temporal and spatial CO_2 gradients are created by nearly all living matter, and for this reason it is difficult to understand where and when CO_2 signals are sources of ecologically relevant information for different species (Stange and Stowe, 1999). CO_2 sensory cells on the antennae of bees (Lacher, 1964) and ants (Dumpert, 1972) were identified electrophysiologically long ago. Sensory structures able to detect atmospheric carbon dioxide, usually composed of clusters of wall-pore type sensilla, have been described on the palps and the antennae of different insect orders, such as Lepidoptera, Diptera, Hymenoptera and Isoptera, on the head capsule of Chilopoda and on the forelegs of Ixodidae (Stange and Stowe, 1999). CO_2 sensory neurons and CO_2 receptors have been deeply investigated in *Drosophila* and in different species of mosquitoes and moths (see review in Jones, 2013). In many insect orders CO_2 sensory neurons have never been reported, even though numerous behavioral studies suggest that perception of carbon dioxide is a widespread capacity in insects and other animals (Nicolas and Sillans, 1989; Scott, 2011). On this account, researchers speculate that in many insects CO_2-sensitive sensilla are still undetected and that research in the field of carbon dioxide perception in terrestrial arthropods needs much more attention (Stange and Stowe, 1999; Guerenstein and Hildebrand, 2008).

Odonata adults are considered to be primarily visually oriented and so far there have been no data on the detection of carbon dioxide in dragonflies and damselflies, neither on the antennae nor on any other sensory organ. First evidence of the use of olfaction in Odonata behavior has been provided by recent investigations (Piersanti et al., 2014a) demonstrating that adults of the blue-tailed damselfly *Ischnura elegans* Vander Linden (Odonata: Coenagrionidae) are attracted in the laboratory by olfactory cues emitted by prey. These behaviorally relevant odorants were found to be perceived by antennal sensilla (Piersanti et al., 2014a). Ultrastructural investigations of the antennae of this and other Anisoptera and Zygoptera species revealed the presence of sensilla located in pits on the latero-ventral side of the flagellum. Some of them show the morphology of peculiar coeloconic single-walled olfactory sensilla while two other types (deeply sunken sensilla), sharing some features of thermo-hygroreceptor sensilla, are located at the bottom of cavities which reside completely within the antennal

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cuticle and open under the pits (Rebora et al., 2008, 2009; Piersanti et al., 2010, 2014b). Electrophysiological recordings from Odonata antennae confirmed the presence of functional olfactory sensory neurons (OSNs) (Rebora et al., 2012; Piersanti et al., 2014b) and thermo-hygroreceptive neurons in this insect group (Piersanti et al., 2011). Neuroanatomical investigations on the brain of dragonflies described an aglomerular antennal lobe showing spherical knots that could allow for the perception of odor (Rebora et al., 2013).

In order to test the hypothesis that Odonata antennae can perceive atmospheric carbon dioxide, the present paper investigates, by means of single-cell recordings (SCR), the responses of OSNs of *I. elegans* when stimulated by air streams at different CO_2 concentration. Some insects, such as termites (Ziesmann, 1996), Lepidoptera (Bogner et al., 1986), mosquitoes (Tauxe et al., 2013), and flies (Ai et al., 2010), have CO_2-sensory neurons showing additional responses to particular odors. On this account, we also included carboxylic acids and amine, which constitute the strongest ligands for Odonata OSNs (Piersanti et al., 2014b), as stimuli in the current study.

2. Materials and methods

2.1. Study insects

The damselfly *I. elegans* was chosen as model species because it is one of the few odonatan species that can be easily reared in the laboratory (Piersanti et al., 2015) and because previous SCR were performed on antennal OSNs in the same species (Piersanti et al., 2014b).

I. elegans was maintained at conditions of 25 ± 2 °C, a photoperiod (L:D) of 16:8 h and 60–80% relative humidity (RH). The larval stages were reared in aquaria and fed ad libitum with *Artemia salina* nauplii and freshwater plankton (*Daphnia* sp., *Cyclops* sp.). After emergence, males and females were held separately and reared in small insectaries (50 cm × 50 cm × 50 cm wooden cages covered with bee netting). Adults were fed ad libitum with *Drosophila melanogaster* flies. Insectaries and aquaria were provided with light via artificial solar illumination (TL-D 36W/94; Philips, Eindhoven, The Netherlands).

2.2. Single-cell recordings

2.2.1. Insect preparation

SCR were carried out on antennae of male and female adults 2–14 days after emergence. Before testing, each insect was placed inside a channel (diameter 4 mm) drilled through a Plexiglas cube and immobilized with Patafix (UHU Bostik, Milano, Italy) and adhesive tape. The antennae, exposed at the top of the holder, were fastened to the Patafix with tungsten hooks. The pits containing the sensilla, on the latero-ventral side of the antennae, were well exposed to the airstreams used for stimulation and easily accessible for microelectrode manipulation. A total of 50 insects (23 males and 27 females) were used for the recordings.

2.2.2. Recording technique

Nerve impulses from single sensory neurons were recorded extracellularly using tungsten microelectrodes sharpened in a highly concentrated solution of KNO_2. The recording electrode was inserted so as to penetrate the cuticle inside one of the pits located on the latero-ventral side of the flagellum, using a micromanipulator (MMO-203; Narishige, Tokyo, Japan) under visual control with a light stereomicroscope (Wild M420 connected to a Wild zoom 1:5; Wild, Heerbrugg, Switzerland) at 200x. During the insertion of the recording electrode into the pit it was not possible to distinguish the different sensilla under the stereomicroscope because they are located inside cavities. The indifferent electrode was inserted into the pedicellum of the same antenna, making contact with the hemolymph. A conventional electrophysiological set-up for extracellular single-cell recording was used. The ring was mounted on an anti-vibration table and shielded with a Faraday cage. For data acquisition a 10x gain probe (Universal Single Ended Probe, Type PRS-1; Syntech, Kirchzarten, Germany) was used. The amplified analog signal, which was monitored on an audio monitor, was captured and processed with a data acquisition controller (IDAC-4; Syntech). Spike activity was recorded by the computer software Autospike (Syntech). All electrophysiological recordings were carried out at a room temperature of 22 ± 0.5 °C and a relative humidity of 30 ± 5%, according to standard methods.

2.2.3. Stimulus delivery

Stimuli were provided as 4 s puffs of purified air into a continuous charcoal-filtered, humidified (up to 60% RH obtained by bubbling air through a washing glass bottle) main air stream at 1200 ml min^{-1}, which was flowing over the antennal preparation at a velocity of 50 cm/s generated by an air stimulus controller (CS-55; Syntech). The continuous air stream was at room temperature (≈ 22 °C). To make it CO_2-free, it was passed through a soda lime cartridge (Sigma-Aldrich, St. Louis, MO, USA) before entering the charcoal filter. This flow was delivered over the specimen through a plastic pipette (50 mm long, 6 mm inner diameter and 2 mm inner diameter in the tip) oriented towards the antenna (~5 mm away from the preparation). To allow stimuli to be added in the continuous air stream, the tip of a glass Pasteur pipette (150 mm in length) was inserted into a small hole (diameter 1.5 mm) drilled into the plastic pipette.

Gas stimuli were represented by CO_2 at 0.001%, 0.01%, 0.1%, and 1% (the remainder N_2), CO_2 100%, and N_2 100% used as control. All stimuli were delivered by a 100 ml manually activated plastic syringe connected to the glass pipette by a tygon tube 30 cm long. The gas stimuli flow, at about 10 ml/s (we manually delivered 40 ml during the 4 s of stimulation) and at room humidity (RH ≈30%) and temperature (≈22 °C), was inserted into the continuous air stream (CO_2-free and up to 60% RH) to replace half of it. Owing to this, gas stimuli reaching the insect sensilla resulted in a dry (RH ≈45%) nitrogen-enriched air flow containing 0.0005%, 0.005%, 0.05%, 0.5% and 50% of CO_2. The control stimulus consisted of a dry nitrogen-enriched CO_2-free air flow (N_2).

To exclude cells that responded to humidity and temperature changes from the experiments, humidity stimulations (≈30–60% RH, obtained by turning on or off the passage of the continuous main air stream through the washing glass bottle) and temperature stimulations (≈1 °C change, obtained by turning on or off the microscopy lights over the insect preparation) were performed. Gas stimuli were delivered in the following sequence: 50% CO_2, to test if the sensory neurons responded to CO_2 stimulations, and after some minutes, in order to allow the recovering of the spontaneous activity, 0.0005%, 0.005%, 0.05%, 0.5%, 50% CO_2, with N_2 as control.

Odor stimuli included isoamylamine, pentanoic acid and heptanoic acid. The odor stimuli were selected on the basis of previous recordings on antennal OSNs of the same species (Piersanti et al., 2014b). The odor stimuli were provided as puffs of purified, charcoal-filtered CO_2-free (soda lime cartridge; Sigma–Aldrich) air generated by an air stimulus controller (CS-55, Syntech) and inserted in the continuous air stream as described above. They were delivered as 20 μl samples placed on a filter paper (15 mm × 15 mm) inserted into the glass pipette to obtain an odor cartridge. The control stimulus consisted of a similar pipette containing a filter paper impregnated with a 20 μl aliquot of the corresponding solvent. Fresh stimulus pipettes were prepared every day. A pipette with only filter paper was also used to check any contamination of the filter paper and to exclude responses