Perception of Carbon Dioxide and Other "Air-Condition" Parameters in the Leaf Cutting ant *Atta cephalotes*

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Social insects often build subterranean nests to protect themselves and their brood from predators and environmental influences. These nests can reach remarkable sizes, covering an area of up to $40 m^2$ and reaching 6 m below the surface [1]. The fungus gardening leaf cutting ants (genus *Atta*) have special demands for their nests, including stable microclimatic conditions required by the fungus. The climatic parameters, such as humidity, temperature, and the concentration of CO_2, have direct impact on the development of the brood and on the fitness of the whole colony [2]. The ants appear to be able to regulate these conditions by constructing and, when needed, altering ventilation channels, which are an important part of the nest [3].

Various types of sensilla, often hair-shaped sensory organs with up to 30 receptor neurons densely cover the antennae and perceive chemical and mechanical stimuli. In *A. cephalotes* there are about 1400 sensilla on each antenna, and thus an enormous number of sensory cells relay the information to the brain [4]. In addition to pheromones, food odors, and mechanical stimuli, changes in environmental parameters are detected by antennal sensilla.

Much work has been carried out to classify the different morphological types of antennal sensilla, mainly for phylogenetic studies [5]. In ants, however, little is known about the physiology of most types of sensilla [6, 7, 8]. Furthermore, in insects the morphological characteristics of sensilla do not necessarily reveal their specific stimulus modality, for example, hygroreceptors have been identified in sensilla basiconica of the moth [9] and in sensilla coelocapitula of the honey bee [10].

In order to understand the biological role of the antennal receptors we first identified the adequate stimulus modalities of different types of sensilla in *A. cephalotes* by extracellulary recording the activity of their receptor cells. The technique was similar to that described by Masson and Friggi [7], except that the indifferent electrode was placed in a different position, to avoid recording of muscle activity, which otherwise could easily be mistaken for sensory activity. The recording electrode was either an electrolytically sharpened tungsten wire or, for tip-recording [11], a glass electrode was used. Signal amplitudes were in the range of $0.1-0.6 mV$ (bandpass filtered between 60 Hz and 2 kHz). None of the hair-shaped sensilla responded to changes in temperature and humidity or to CO_2 stimuli. Sensory cells of the sensilla trichodea curvata responded to the alarm pheromon 4-methyl-3-heptanon. The sensilla basiconica carry contact chemoreceptors reacting to various salt solutions while the sensilla chaetica, which we always found associated with one particular type of sensilla basiconica, have mechanoreceptors.

In a distinct area of the last flagellar segment small holes are detectable. These are apertures of two other types of sensilla situated below the surface of the cuticle. Depending on their shape they are called sensilla coeloconica or sensilla ampullacea. They are common in the hymenoptera and were first described by Forel in 1884 but he was unable to suggest an adequate stimulus modality for these morphological types [12]. In both cases a peglike structure is located in a cavity with only a small aperture to the outside. The sensillum coeloconicum resides completely within the thick antennal cuticle (Fig. 1 b). In contrast, the sensillum ampullaceum extends further into the lumen of the antenna (Fig. 1 a). Concentrated in an elliptic array on the ventrolateral side of the last flagellar segment, the sensilla coeloconica are situated distally, and the group of 10–12 sensilla ampullacea are proximal to them.

Both types of sensilla are associated with a temperature receptor which is either excited (warm receptor) or inhibited (cold receptor) by increasing temperature. In the distal part of this sensilla arrangement recordings of cold receptors are possible, demonstrating association of these receptors to sensilla coeloconica. In contrast, warm receptors are found in the proximal part. In two experiments we have shown that a
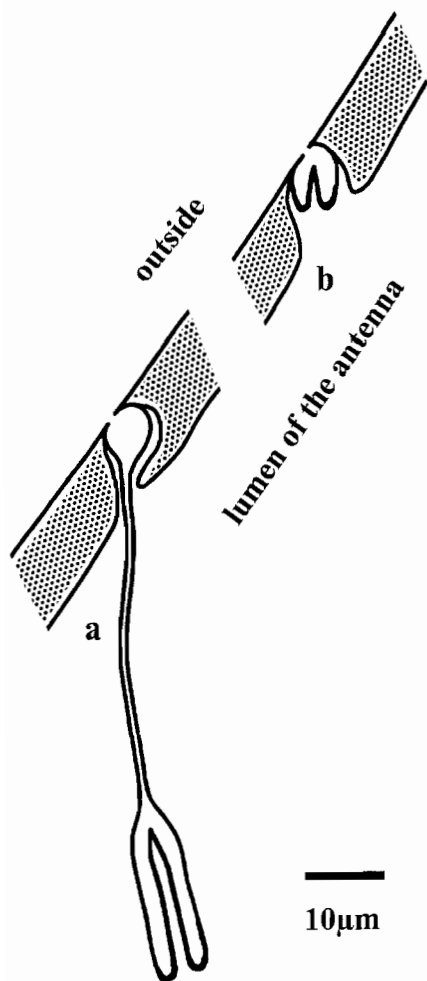
Fig. 1. Sensillum ampullaceum (a) and sensillum coeloconicum (b), schematized from semithin sections of the antenna. *Scale bar:* 10 µm

Fig. 2. Threshold curve of CO_2 receptors. Neuronal activity vs. stimulus intensities, ranging from 0.02% to 4% CO_2 added to a continuous air flow of 0.05% CO_2. Raw data from three different receptor cells

warm receptor sensory neuron is associated together with a CO_2-receptive cell within the same sensillum ampullaceum (identification, see below). The response of the temperature receptors is phasic-tonic, with a resolution of at least 0.2 °C. This accords with behavioral studies of temperature sensitivity in ants [13].

Scanning electron microscopy from the interior of the antenna reveals the extraordinary structure of the sensillum ampullaceum (cover foto). An ampulla, deeply invaginated into the lumen, is connected to a cavity within the thick antennal cuticle by a long and narrow duct (diameter 1.5 µm; Fig. 1a). The length of the ducts varies from 20 to 50 µm in *A. cephalotes* (in a single individuum) and even more obvious length variations occur between differ-
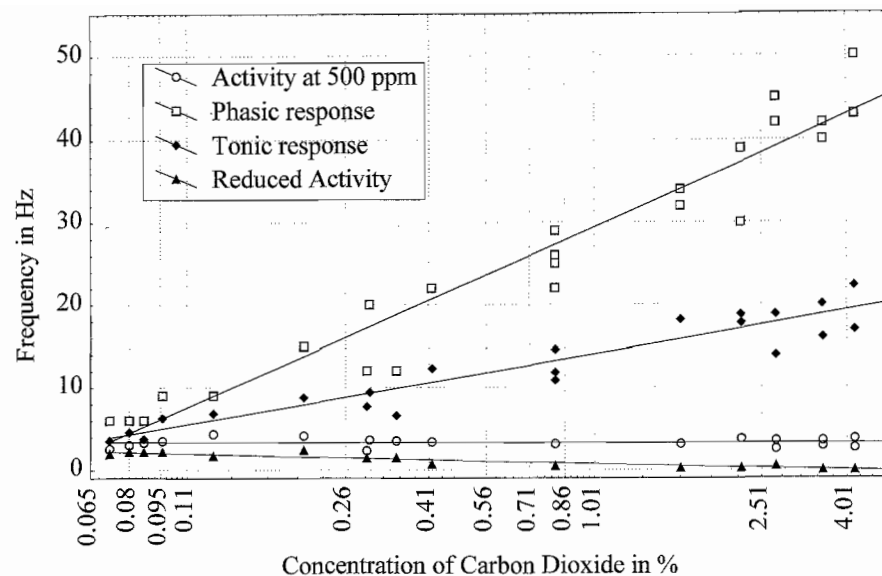
ent ant subfamilies (Ponerinae and Formicinae [5]).

We have identified the sensilla ampullacea as CO_2 receptors. After recording action potentials in response to CO_2 the sensillum was identified by staining with DiI. The lipophilic dye was applied with a glass capillary put onto the aperture of the recorded sensillum. Sectioning afterwards revealed that only a single sensillum of the group was stained. We investigated the stimulus coding properties of the CO_2 receptor cells innervating the sensilla ampullacea in greater detail. The CO_2 stimulus was applied by an injection of CO_2 (10% in N_2 or 100% CO_2) into a continuous air flow (0.05% CO_2, 21% O_2, 78.95% N_2) directed onto the tip of the antenna. The injection of CO_2 was controlled by a magnetic valve. At 500 ppm CO_2 concentration (atmospheric concentration ~ 300 ppm) the frequency of action potentials of the receptor cell is about 4 Hz. With increasing concentration an initial phasic response with a duration of approximately 1 s is followed by a tonic plateau. After termination of the stimulus the activity is reduced, for a period the length of which depends on the intensity of the preceding stimulus. A plot of the mean response frequency vs. stimulus intensities reveals that the phasic

and the tonic components do not saturate in the tested intensity range (0.05 – 4%; Fig. 2).

The threshold curves in Fig. 2 represent raw data from three different receptor cells. The response characteristics of nine other cells in which fewer stimulus intensities were tested, match the curve in Fig. 2 very well. Thus our data indicate that most of the CO_2 receptors work in the same sensitivity range. The CO_2 receptors found in honey bees vary in their sensitivity but have not been identified any further [14]. In addition to the sensilla ampullacea, honey bees have two more types of sensilla below the surface of the flagellum [15]. Normally the half-maximal response is used to compare the sensitivity of receptors, but we did not test stimulus intensities close to saturation. In some lepidopteran species that use CO_2 to locate oviposition sites the half-maximal response occurs at stimulus intensities as low as 75 ppm [16], which is only about 10% of the lowest intensity tested in this study.

The various components (phasic, tonic, rebound) of the sensory response code different aspects of the stimulus. The level of CO_2 concentration is best represented by the tonic part of the response, which shows no adaptation even after a stimulus of 10 min (stimulus intensity of 0.82%). This receptor serves as a logarithmic measuring system in a wide range of intensities and should enable the leaf cutting ant to monitor the current CO_2 concentration continuously in the chambers of their

nest. Due to its initial phasic response component the receptor should be able to assess, in addition to the absolute CO_2 concentration, also the time course of increasing CO_2 concentration. A spatial gradient in CO_2 concentration could be perceived by antennal scanning movements. Inside the nest such gradients may be caused by increased activity of the fungus and ants in the chambers. Outside gradients exist due to sources and sinks of CO_2 [17]. For instance, the nest entrance acts as a large CO_2 source and may establish a gradient which expands near the ground much further than in turbulent air. Orientation to an odor gradient in the laminar boundary layer close to the surface may be possible for ants because mass transport here obeys only the law of diffusion [18]. The initial phasic compound and the rebound characteristics of their CO_2 receptors and the additional fast sampling of different levels in the boundary layer may enhance the resolution of small vertical gradients. Indeed, it has been established that ants use CO_2 as a cue to find the entrance of their nest [19].

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