The Cave-like Sense Organ in the Antennae of Triatominae Bugs

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In the second segment of the antennae of haematophagous reduviids an unusual cave-like organ is found, the function of which was investigated in Triatoma infestans. *The morphology of the organ makes it difficult to ascribe it to a mechano- or chemoreceptive function, but shows some characteristics shared with thermoreceptors of other animals. The electrical activity of sense cells was recorded in the presence of stimuli that evoke behavioural responses in this species, i.e. warm, CO_2, lactic and butyric acids at different concentrations. The three compounds tested failed to evoke a response at all concentrations assayed. Only thermal stimulation evinced a clear modification in the electrical activity of the sense cells. Both the morphological and electrophysiological findings support a thermoreceptive function of this organ, that is only present in insects which rely highly on the thermal cue for food finding, habitat selection and circadian synchronization.*

Key words: Triatoma - thermoreception - chemoreception - sense organs - Chagas' disease

The antennae of Triatominae bugs, vectors of Chagas' disease, play a major role in food-finding and intraspecific communication. Some morphological and physiological studies on the function of the sensilla in Triatominae bugs have been published. The first one was the classical work of Wigglesworth and Gillett (1934) on the function of the antennae in *Rhodnius prolixus*. These authors recognized the importance of the thermal cue in the orientation of the bugs when searching for food. In addition, they described four types of sensilla in the antennae of *R. prolixus*, ascribing them putative functions according to morphological criterion and abundance. Further studies on the antennal sensilla of Triatominae bugs have been carried out by Barth (1952), Mayer (1968), Bernard (1974) and Lazzari (1983, 1990) in *Triatoma infestans*, and Chaika (1980) and McIver and Siemicki (1984, 1985) in *R. prolixus*.

Barth (1952) demonstrated in the distal third of the antennal pedicel of haematophagous reduviids, the existence of an enigmatic sense organ, which he called *cova das cerdas* (cave of bristles). Searching for this organ in related species, this author found it in all larval instars and adults of blood-sucking bugs, but not in those having phytophagous or entomophagous habits. To account for this fact and the morphology of the organ, different to any known chemo- or mechanoreceptor, Barth suggested a thermoreceptive function. By means of ablation experiments, however, this hypothesis could not be corroborated. The cave organ was ignored in the subsequent studies, except in those carried out by Lazzari (1983, 1990) and Catalá (unpubl.).

In this report the question on the function of the cave organ of blood-sucking reduviids is addressed. We studied the morphology of the organ and its electrical activity while stimulating it with different modalities searching for its functional role.

MATERIALS AND METHODS

Adults of *T. infestans* (Klug) (Hemiptera: Reduviidae), the main vector of Chagas' disease in South America, were used throughout. Insects were reared in the laboratory at 30°C, 70 % relative humidity and fed on citrated sheep blood using an artificial feeder (Núñez & Lazzari 1990). Insects were used in the first two days after the imaginal moult, when the cuticle was not fully hardened.

Microscopy - Several light and scanning electron microscopy (SEM) techniques were used for the study of the antennal sensilla: (a) Clearing: heads and antennae were removed and immersed in a solution of NaOH 5% at 70°C for about 2-3 hs, then washed in 70 % ethanol and transferred

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to phenol during 10 min. They were then transferred to creosote, where the final grade of transparentness was controlled with the aid of a stereoscopic microscope. Finally, the antennae were mounted in Canada balsam; (b) Light microscopy sections: antennae were removed and processed following the technique described by Ribi (1987) for electron microscopy preparations. Briefly, pieces were fixed in a buffered solution of glutaraldehyde and paraformaldehyde, and post fixed in 1% OsO_4. After dehydration, small pieces of the antennae were embedded in hard Araldite. Longitudinal and transverse sections of the antennae 0.5 to 5 µm thick were obtained. Sections were stained with 1% methylene blue; (c) Selective staining: crystal violet was applied, after the technique described by Slifer (1960), in order to reveal the presence of pores. Staining was performed at atmospheric, as well as reduced pressure, replacing air with the dye solution, as usually done to fill tracheae; (d) SEM: heads were removed and fixed as done for light microscopy sections, washed five times in basic buffer during 20 min and dehydrated through a graded ethanol series. After critical-point drying, the heads were coated with a layer of gold-palladium 20 nm thick and mounted on steps.

Electrophysiology - Insects were fastened with tape to a cork block and the antennae immobilized by means of metal hooks. An indifferent silver electrode was inserted in a compound eye. The recording electrode, a sharpened tungsten wire, was inserted into the pedicel near to the cave organ. The place of the organ was estimated relative to the distal trichobotrium. After recording, the location of the electrode was verified with the aid of a microscope by clearing the antenna.

Chemical stimulation was tested by presenting substances which probed to evoke a behavioural response in the bugs. For this, carbon dioxide and lactic acid, related to food search (Núñez 1987), and butyric acid, which seems to play a role in intraspecific communication (Ward 1981) were chosen. Lactic and butyric acid were diluted in silicon oil at relations of 1:1, 1:10, 1:100, 1:1000 and 1:10000. The recipient with the sample was placed inside a 60 ml disposable syringe filled with ambient air. The equilibrium between concentrations at the solution and at the air in the syringe was let to be reached by waiting after about 1 hr. Carbon dioxide was assayed at concentrations of 100%, 50%, 25%, 12%, 6% and 3% by mixing volumes of CO_2 with room air (about 0.3% CO_2). The test was performed by injecting the air in the syringe into an air current with constant flow bathing the antenna at room temperature (air flow: 1 ml/sec; injection flow: 1 ml/sec). This method allows only rough semiquantitative measurements, because the actual concentration of the substances, which depends on the vapor tension in both phases, remains unknown. Nevertheless, it was successfully applied to record the response of the whole antenna to chemical stimulation by means of electroantennogram (EAG) (Lazzari 1990, Lazzari & Wicklein unpubl.).

Thermal stimulation was accomplished by bringing a wire loop (wire: 0.1 mm, loop: 1mm diameter) at 30°C (room temperature 20°C) near 1 cm to the antenna for about 0.5-1 sec. We used this rough method instead of a current flow of warm air, because it matches better the natural situation. During food searching, bugs are confronted with warm bodies rather than to warm air currents (Lazzari & Núñez 1989, 1991).

Thirty four records during chemical stimulation and 42 in the presence of thermal stimuli on ten *T. infestans*, males and females, were performed. Controls were accomplished with the sample recipient filled with silicon oil only or with the thermal probe at room temperature.

RESULTS

The morphology of the cave organ - The organ occurs inside the antennae, at a constant position in the distal third of the pedicel, in number of one per antenna. Fig. 1 depicts the location of the organ and the seven trichobothria in the pedicel of *T. infestans*. By clearing the antennae the remaining cuticular structure of the organ has the appearance of a closed sac. The cave cavity is filled with a number of very thin tight packed hairs and communicates to the exterior through a channel (Fig. 1). In *T. infestans* adults, the sac reaches 200-230 µm in the axis parallel to the antenna and 40-45 µm in the transverse one. The channel is 80-90 µm large, 4 µm mean diameter, having very irregular folded walls (Figs 1, 2). It opens to the exterior on the external side, proximal to the insertion of the more distal trichobothrium, by a hole of 3-4 µm diameter (Fig. 1). The length of the hairs filling the sac is not uniform; they are at the proximal and distal ends longer than in the middle region of the sac. A high number of bipolar cells (about 200-300) are distributed around the cuticular sac associated to the base of the hairs, as shown in Fig. 2 (Barth 1952). The axons of the sensory neurons form an unique nerve, which joins the external branch of the antennal nerve (Fig. 2).

By external application of crystal violet many established or putative chemoreceptors of *T. infestans* can be easily stained (Lazzari 1983), but not the *cova das cerdas*. The dye did not reach the inner cavity of the cave, even if a pressure difference was applied. However, when the wax layer of the cuticle was removed by washing the anten-

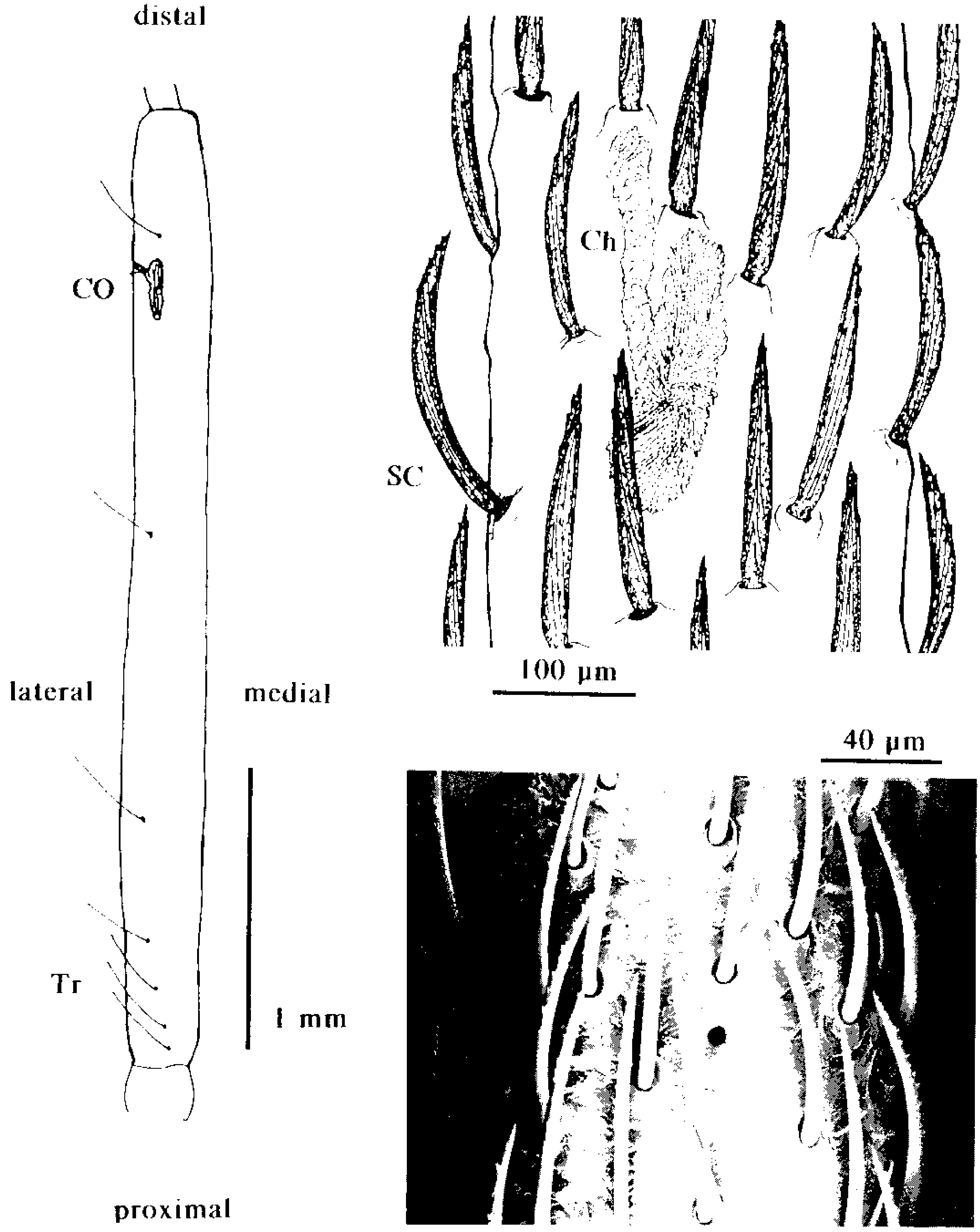

Fig. 1, left: schematic view of the antennal pedicel to show the location of the cave-organ and the seven trichobotria of *Triatoma infestans* adult; right top: the cave-like organ, as seen after clearing the antennae. Only sensilla chaetica are represented on the cuticular surface of the pedicel. Very thin tightly packed hairs fill the inner cavity of the cave which communicates to the exterior through a cuticular channel having irregular folded walls; right bottom: opening of the cave-organ to the exterior as seen by means of SEM. Ch: cuticular channel; CO: cave organ; SC: sensilla chaetica; Tr: trichobotria.

nae in acetone, the dye penetrated the cavity of the organ. This fact suggests that cuticular wax or other acetone soluble material could paste the entrance to the cave.

The electrophysiological response - The electrical activity of the sense cells in the cave organ was not modified by stimulation with CO_2, lactic or butyric acid. The three compounds tested failed to evoke a response at all concentrations assayed. They act, however, as stimuli on the whole antenna, as shown by electroantennogram (EAG) (Lazzari 1990, Lazzari & Wicklein unpubl.), and

Fig. 2, top: sagittal section of the cave organ to show the arrangement of sensory cells around the cuticular portion of the organ and the hairs filling its cavity; bottom: transversal section of the antenna to see the position of the cave-organ relative to the cuticular wall of the pedicel and both branches of the antennal nerve. AN I, AN II: internal and external branches of the antennal nerve; CO: cave organ.

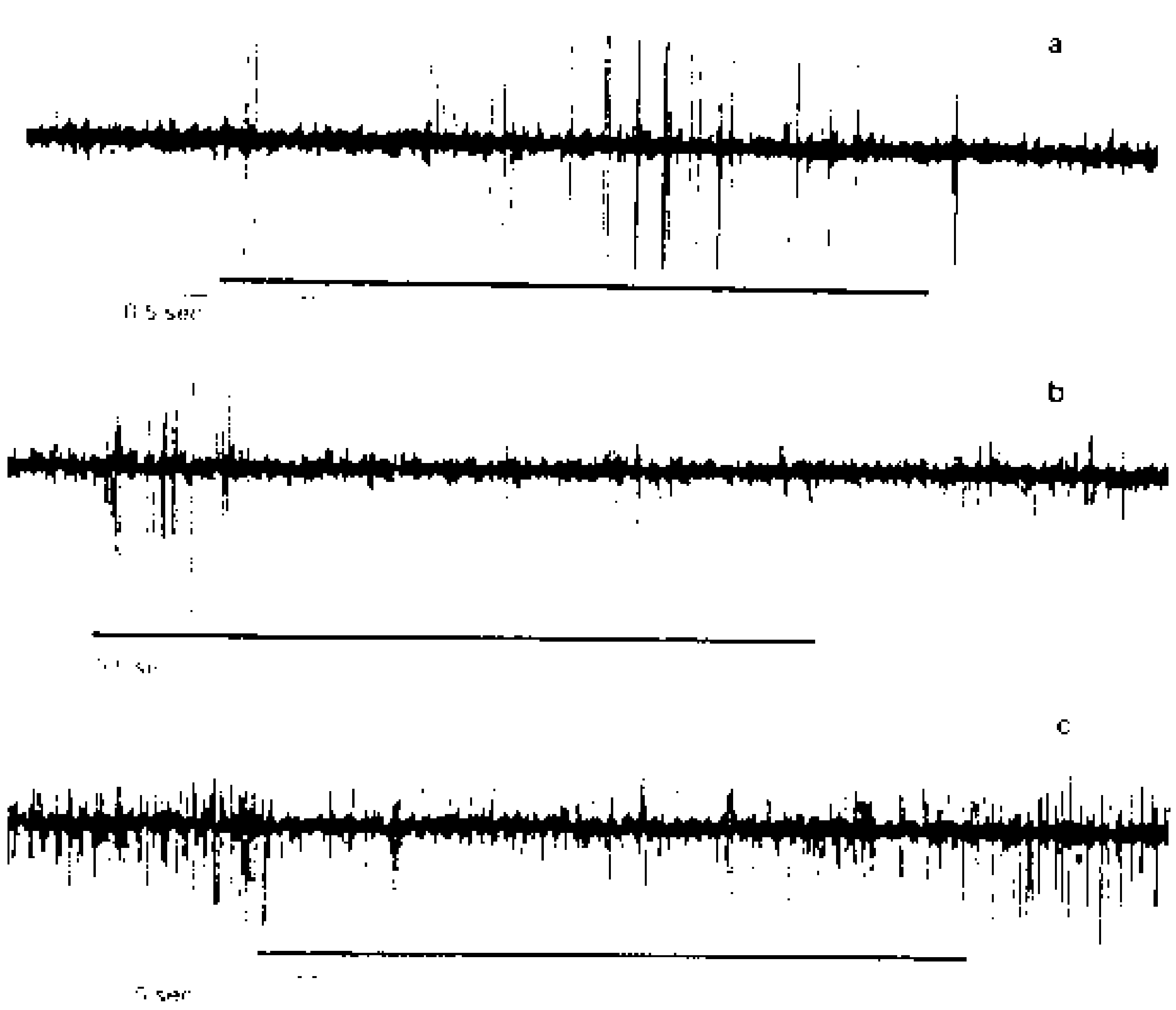

Fig. 3, electrical activity of the sense cell of the cave organ under thermal stimulation. Sample records of the three different response modalities recorded are depicted; a: units firing in a tonic fashion; b: cells responding phasically at the onset of the stimulus and c: units decreasing their firing rate during stimulation.

are able to evoke behavioural responses (Núñez 1987, Ward 1981).

Only thermal stimulation evinced a clear modification in the electrical activity of the sense cells. The typical response corresponded to units which fired in a tonic fashion during thermal stimulation (Fig. 3a). This modality was evident in 14 of 30 records. A low number of cells responding either phasically (3 records) at the onset of the stimulation or tonically decreasing their firing rate (5 records) during thermal stimulation (Fig. 3b, c), were occasionally found. No relation could be established between the modality of the response and the position of the electrode in the organ or the sex of the animals.

DISCUSSION

The thermal cue plays a main role during the orientation of Triatominae bugs to the host (Wigglesworth & Gillett 1934, Nicolle & Mathis 1941, Lazzari & Núñez 1989), in habitat selection (Lazzari 1991) and in the entrainment of the circadian system (Lazzari 1992). On the other hand, *T. infestans* is able to perceive the radiant heat of a host and to estimate the temperature of distant sources (Lazzari & Núñez 1989). By these tasks this species exhibits the highest thermal sensitivity known at present in animals (Lazzari 1990, Lazzari & Núñez 1991).

As a result of his morphological studies, Barth (1952) suggested a thermoreceptive function for the *cova das cerdas*. However, by covering the cave organs of both antennae with paint or cutting the antennae proximal to the organ, this author could not corroborate his hypothesis. Bugs could still successfully orientate towards a warm source. Some years later, Bernard (1974) found that bugs are equipped with sensilla coeloconica sensitive to warmth as well as relative humidity, similar to those present in several other insects (Horn 1982). These organs are distributed not only in the antennae, but also on the whole body surface of the bugs (Lazzari 1983). So, although the cave organ could be a thermoreceptor, Barth's selective ablation, might not render conclusive results. In fact, few days after the ablation of both antennae, *T. infestans* recover their capacity to orientate towards thermal sources (Lazzari unpubl.), evincing the participation of thermoreceptors outside the antennae.

Despite the lack of definitive data, the weight of the morphological as well as the electrophysiological evidence so far suggests a thermoreceptive function for the cave organ of *T. infestans*. The absence of evident flexible or permeable cuticular areas associated to the sense cells, both requisites for mechano- or chemoreceptive functions respectively, in addition to the absence of electrical response to the compounds tested, do not support such capacities for this organ.

Only thermal stimuli were able to modify the electrical activity of the sense cells. Three types of response were observed under thermal stimulation, i.e. tonic and phasic excitation, and inhibition. Artifacts or responses of neighboring sensilla can be discarded for at least two reasons. First, other thermoreceptors in this species are cold receptors (Bernard 1974), i.e. their firing rate decreases when temperature increases. Second, many cells were simultaneously recorded. The cold receptors have only one thermosensitive unit and their distribution is not close enough to allow many elements to be simultaneously recorded (Lazzari 1983).

The cave-like organ shows a series of particularities that deserve to be emphasized, since they could help to understand its function. First, this organ is present exclusively in a group of bugs highly specialized in feeding on warm-blooded animals. It does not occur in close related species having phytophagous or entomophagous habits, namely those that do not rely on the thermal cue by food search. Second, the cave organ shares morphological characteristics with thermoreceptors found in other animals also specialized in the reception of radiant heat. Snakes of the families Crotalidae and Boeidae (Hartline 1974), the buprestid *Melanophila acuminata* (Evans 1966), and *T. infestans* (Lazzari & Núñez 1989) are the only animals in which infrared perception was demonstrated. This capacity was also suggested for the vampire bat *Desmodus rotundus* (Kürten & Schmidt 1982), but the experiments do not allow other mechanism for heat exchange to be excluded. In spite of phylogenetic distances, the thermal sensitive organs of the species named are all pit- or cave-like organs associated to a high number of sensory cells. In addition, a wax layer cover the IR-sensitive thermoreceptor of *M. acuminata*, the same seems to occur in the cave organ of *T. infestans*.

It is important at this stage to say a word of caution. Although the similarities between the cave organ of Triatominae bugs and the IR sensitive organs of other species are quite suggestive, we avoid any claim in this sense until more evidence is obtained. The present study strongly suggests a thermoreceptive function, but it is not conclusive about the question of whether this is the unique adequate stimulus. Although we have tested compounds having established biological significance, there is relative few information about the use of the chemical cue by Triatominae bugs. Even though the morphology of the cave organ seems not to be proper for chemoreception, we can not absolutely exclude this possibility.

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