J Comp Physiol A (1992) 170:665–676

Journal of Comparative Physiology A Sensory, Neural, and Behavioral Physiology

Perception of breath components by the tropical bont tick, *Amblyomma variegatum* Fabricius (Ixodidae)

I. CO_2-excited and CO_2-inhibited receptors

Pascal Steullet and Patrick M. Guerin

Institute of Zoology, University of Neuchâtel, Chantemerle 22, CH–2007 Neuchâtel, Switzerland

Accepted March 23, 1992

Summary. Wall-pore olfactory sensilla located in the capsule of Haller's organ on the tarsus of *Amblyomma variegatum* ticks bear cells responding to vertebrate breath: one of these sensilla contains a CO_2-excited receptor and a second sensillum has a CO_2- inhibited receptor. Each of these antagonistic CO_2-receptors, which display typical phasic-tonic responses, monitors a different CO_2-concentration range. The CO_2-inhibited receptor is very sensitive to small concentration changes between 0 and ca. 0.2%, but variations of 0.01% around ambient (ca. 0.04%) induce the strongest frequency modulation of this receptor. An increase of just 0.001–0.002% (10–20 ppm) above a zero CO_2-level already inhibits this receptor. By contrast, the CO_2-excited receptor is not so sensitive to small CO_2 shifts around ambient, but best monitors changes in CO_2 concentrations above 0.1%. This receptor is characterized by a steep dose-response curve and a fast inactivation even at high CO_2-concentrations (>2%). In a wind-tunnel, *Amblyomma variegatum* is activated from the resting state and attracted by CO_2 concentrations of 0.04 to ca. 1%, which corresponds to the sensitivity range of its CO_2-receptors. The task of perceiving the whole concentration range to which this tick is attracted would thus appear to be divided between two receptors, one sensitive to small changes around ambient and the other sensitive to the higher concentrations normally encountered when approaching a vertebrate host.

Key words: Tick – CO_2-excited receptor – CO_2-inhibited receptor – Haller's organ – Host finding

Introduction

The CO_2 contained in vertebrate breath is an activating stimulus or attractant for most blood-sucking arthropods (e.g. mosquitoes: Gillies and Wilkes 1968; tsetse flies: Turner 1971; *Stomoxys calcitrans:* Warnes and Finlayson 1985; Simulidae: Fallis and Raybould 1975; Tabanidae: French and Kline 1989; Reduvidae: Bernard 1974; Siphonaptera: Osbrink and Rust 1985). Some CO_2-sensitive receptors have been described on palps of mosquitoes (Kellogg 1970) and on antennae of tsetse flies (Bogner 1989). Ticks also respond strongly to breath and CO_2, and Garcia (1962) has shown that CO_2 attracts many different tick species. Since that first report several authors have devised CO_2 baited traps for field sampling (e.g. Garcia 1965; Wilson et al. 1972; Gray 1985; Guglielmone et al. 1985; Norval et al. 1987, 1988), or have studied the effects of CO_2 on tick behaviour in the laboratory (Nevill 1964; Sauer et al. 1974).

In spite of the above, our knowledge of breath and CO_2 perception in ticks is fragmentary. Breath-stimulated ticks lift their first pair of legs in the air to sample the surroundings as insects do with their antennae. From these observations, and various behavioural experiments where different parts of tick appendages were amputated or masked (Hindley and Merriman 1912; Lees 1948), we know of the primordial role of the tarsus of leg pair 1 for host odour perception. A large number of ultrastructural studies have described different kinds of olfactory sensilla located on the tarsus of the first leg pair (reviews: Waladde and Rice 1982; Hess and Vlimant 1986), and the Haller's organ situated on the dorsal side of the tarsus (Fig. 1A and B) bears a significant proportion of all tick olfactory sensilla. Thus, among the 19 tarsal olfactory sensilla of *Amblyomma variegatum,* 3 belong to the anterior pit of Haller's organ and 7 to the capsule of Haller's organ. Nevertheless, few investigations on physiological and functional characteristics of the tarsal olfactory sensilla have been undertaken in relation to host odour perception. Sinitsina (1974) using electrophysiological methods found olfactory cells responding to breath, mice odours, and n-valeric acid in the capsule of the Haller's organ in *Hyalomma asiaticum,* but he failed to account for a CO_2-receptor. On the other hand, Waladde and Rice (1982) mentioned the presence in *Boophilus microplus* of cells responding to

Correspondence to: P. Steullet

breath and cow wash in the anterior pit of Haller's organ, and changes in the activity of cells from the capsule when stimulated with either breath or CO_2. But these studies were mainly qualitative and apparently involved few recordings. The small number of physiological studies on olfactory sensilla of ticks may be ascribed to their limited accessibility, especially for those located inside the capsule, as well as the added complication of the high number of cells in many of these sensilla. Several questions remain unresolved. Do any of these olfactory cells respond to breath? Where are they located? What are the stimuli contained in breath which induce the response? Based on complete ultrastructural studies on all tarsal sensilla of *Amblyomma variegatum* (Hess and Vlimant 1982, 1983, 1986), we have systematically searched for tarsal olfactory sensilla responsive to breath and its components.

Materials and methods

Tick rearing

Experiments were mainly undertaken with unfed *Amblyomma variegatum* males but unfed females were also used for some recordings. Originating from West Africa (Adiopodoumé, Ivory Coast), ticks were reared at the Agricultural Research Centre of Ciba-Geigy Ltd. (St-Aubin, Switzerland). All stages (immatures and adults) were fed on Simmental calves at 22 to 24 °C. Ticks were kept under constant darkness at 28 °C/80–90% RH except during moulting when conditions were 29 °C and 90% RH. Finally, adults were maintained in this laboratory in an environmental cabinet under the following conditions: 10 h of darkness at 18 °C/ 95% RH and 10 h of light at 25 °C/85% RH separated by 2 h "dusk" and "dawn" transition periods.

Light and scanning electron microscopy

Scanning electron microscope examination was made on ticks which were killed and fixed in 80% ethanol for several days, cleaned with ether/chloroform in a soxhlet extractor for 12 h, dehydrated in acetone, and critical point dried in CO_2 with a Balzers CPD device. The mounted specimens were gold sputtered in a Balzers sputtering apparatus, and then observed in a Philips 500 PSEM. Light microscopy examination was made on sections of cut tarsi which were fixed in 2% glutaraldehyde (Sabatini et al. 1963), post-fixed in 2% OsO_4 (Palade 1952), dehydrated in acetone, and embedded in SPURR. Embedded tarsi were cut, at the level of the capsule of the Haller's organ, in either transversal or sagittal sections of 0.5 µm and observed under a light microscope (Vanox-S, Olympus, Japan) after toluidine blue staining.

Tick preparation

The tick was immobilized on a perpex holder on double-sided sticky tape. Pedal nerves were destroyed by pinching coxa of the forelegs with fine forceps; this prevented muscle activity during electrophysiological recordings. To make proper recordings from the 7 olfactory sensilla located in the capsule of Haller's organ (an olfactory pit some 80 µm deep and 60 µm wide), dissection was needed to improve their accessibility as the opening of this capsule is just a narrow slit of ca. 5 µm wide and ca. 50 µm long across the tarsus (Fig. 1B). The cuticular roof was removed (Fig. 1C) with a piece of razor blade in a holder (John Weiss & Son LTD., England) mounted on a Leitz micromanipulator under an Olympus SZH stereomicroscope at a magnification 192× (working distance: 48.5 mm).

Electrophysiology

In order to improve contact, the tips of sensilla not located in the capsule were cut with the flame-pulled tip of a glass rod (1.5 mm dia.) oscillating in the ultrasound frequency range (ca. 120 kHz) as induced by a piezoelectric transducer disk (n° 4322 020 177721, Philips, The Netherlands) (Gödde 1989). The recording glass electrode filled with 0.2 *M* KCl was brought into contact with the cut tip of the sensillum with a Leitz micromanipulator, and the reference glass electrode, filled with 0.2 *M* NaCl, was inserted in the coxa of one of the anterior legs. Electrical activity of capsular sensilla was also recorded with glass electrodes gently introduced into the dissected capsule until cell activity was captured. These recording electrodes also contained 1% polyvinylpyrrolidone K90 (Fluka, Switzerland), in order to prevent electrolyte flowing from the tip. Indeed, tips sometimes broke when they touched cuticular pleomorphs located between sensilla (Fig. 1C). Nevertheless, it was still possible to record properly with broken tips of up to ca. 5 µm. With some experience it was possible to recognize patterns typical of different sensilla according to 1) electrode position, orientation and depth inside the capsule, as the relative position of each sensillum in the cavity was indeed very consistent between individuals, 2) typical spontaneous activity of its cells, 3) spike shapes, and 4) behaviour of these cells to various stimuli. Tungsten electrodes were also used in some cases when the preparation was exposed to a dry air stream.

Recorded signals were fed via a 10^{12} Ω input impedance preamplifier into a universal AC/DC amplifier (UN–03, Syntech, The Netherlands) and registered on video tapes via a PCM–1 Digital VCR-instrumentation recorder adaptator (Medical System Corp. Greenvale, USA) onto a video cassette recorder (Grundig VS540 Monolith, Germany) (Gödde 1985). Records were visualized either by playing them back onto a paper recorder (Graphtec WR7600, Japan) used in memory mode, or by using the plot or the view option of the spike analysis programme SAPID (Smith et al. 1990). For the latter, the recordings were fed into a 386 IBM compatible computer (Mandax) via the DAS16 analogue/digital plug-in board (MetraByte Corporation, USA) at a digitizing rate of 10 kHz. Discrimination for the activated cells according to their amplitudes, shapes, and spike frequencies was made by eye. This simple method was found to be the most appropriate one for the multicellular responses evoked by breath in these sensilla. SAPID was quite inadequate to properly analyse these multicellular responses because of the large number of overlapping spikes and, moreover, because of the change in amplitude and/or shape of some spikes. The length of spike trains employed for determining activity will be indicated for each case in Results.

Nevertheless, for experiments with long CO_2 stimulation, the clear nature of the response of CO_2-excited or CO_2-inhibited receptors in their respective sensilla allowed us to sort spikes of CO_2-receptors with a window discriminator (model 121, W–P Instruments Inc., USA), whose frequency was converted into a DC voltage by a frequency-voltage converter (time constant: 1 s) in the UN–03 amplifier. Visualisation of the window discriminator upper and lower levels on the oscilloscope (Tektronix 5112, USA) allowed us to sort spikes properly for unambiguous discrimination for those of a CO_2-receptor from others in a record. The firing rate of other cells was rather low, thus inducing few or no overlapping spikes. Nevertheless, some rare spikes, not typical of CO_2-receptors, were occasionally counted. But the error was estimated at less than 5%.

Stimulation

Tarsal sensilla of ticks frequently contain receptors for different modalities, i.e. apart from chemoreceptors they may also support thermoreceptors and hygroreceptors other than those reported by Hess and Loftus (1984). In order to discriminate for responses induced primarily by the chemicals being tested here, it was necessary to maintain the sensillum, as far as was practically possible, in

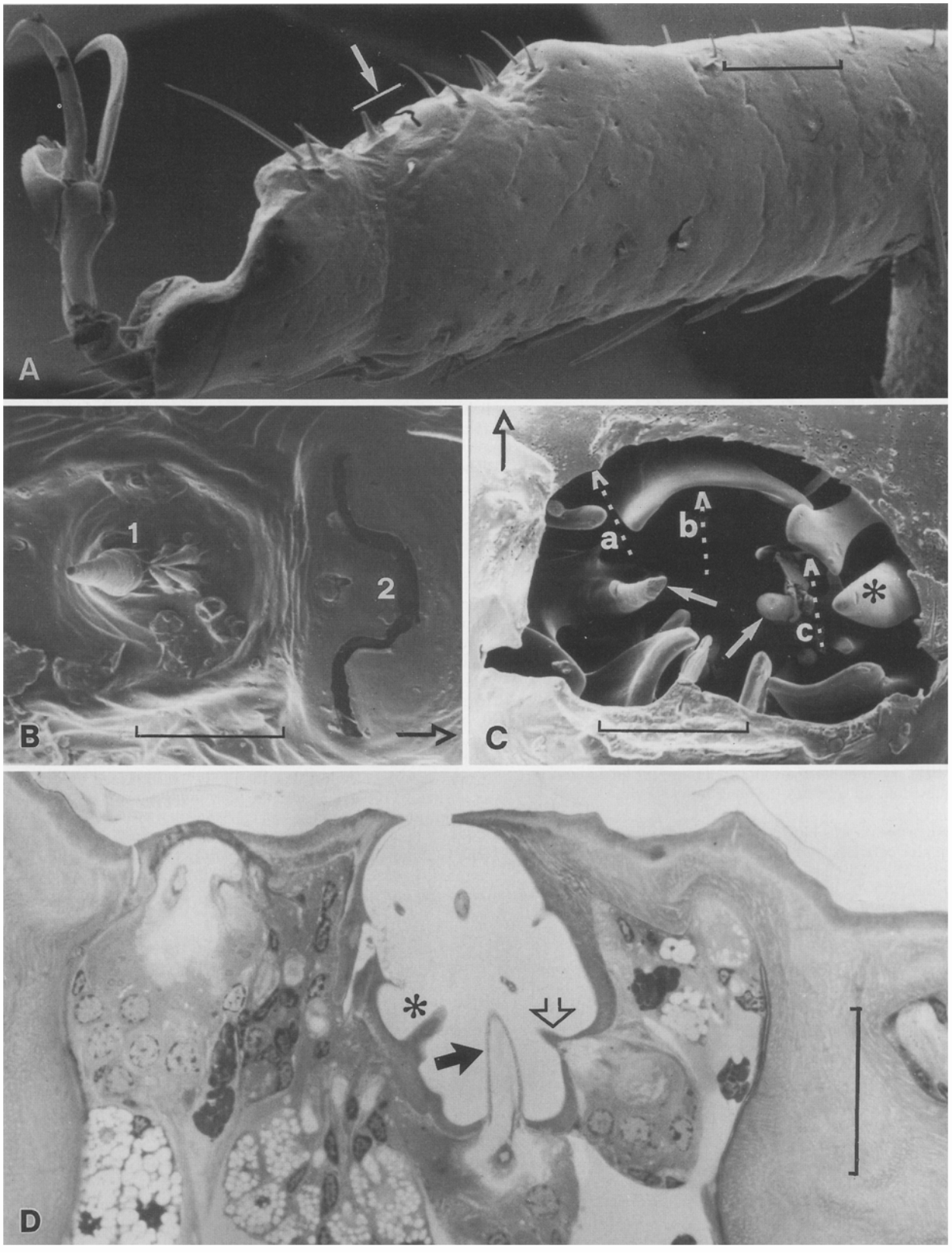

Fig. 1. A Tarsus of the foreleg of a female *Amblyomma variegatum*. The *white arrow* shows Haller's organ on the dorsal side with olfactory sensilla and the opening of the capsule; scale bar: 200 µm. **B** Haller's organ with the group of anterior pit sensilla (*1*) and opening of the capsule (*2*); scale: 30 µm, *arrow:* proximal end of the tarsus. **C** Capsule of Haller's organ with cuticular roof removed revealing two wall-pore capsular sensilla *(white arrows)* and cuticular pleomorphs (*). The 3 *dashed white arrows* indicate approximate electrode positions and angles of approach towards sensilla with breath-sensitive cells: *a* for one inhibited by CO_2, *b* for one excited by CO_2, *c* for one insensitive to CO_2 (described as a H_2S-sensitive cell in Steullet and Guerin 1992); scale bar: 20 µm, *black arrow:* proximal end of the tarsus. **D** Longitudinal section through the capsule showing a sensillum *(black arrow)*; the base of a second sensillum is also visible *(white arrow)*; *: cuticular pleomorph; scale bar: 50 µm. **A, B,** and **C** are scanning electron micrographs, **D** is a light microscope view

a controlled air stream. For that purpose, air scrubbed in charcoal and silicagel, and humidified to 80% RH, at 22±1 °C in a water bath, was continuously blown at 10 ml/s through a glass tube onto the tarsus. The outlet of the tube (3 mm i.d.) was 5 mm from the tarsus, providing an air speed at the level of the preparation of about 1.5 m/s. The tip of a 5-ml polypropylene syringe containing the odour (breath, CO_2, or other volatiles) was introduced through a septum-covered hole in the tube, 3 cm or 25 cm from its outlet, depending of the experiment (see Results). A charcoal-filtered air pulse, delivered by a solenoïd valve, was administered via a stopper at the back of the syringe, so that 2 ml of the syringe content was injected in 1 s into the glass tube. To prevent changes in air flow during stimulation, a charcoal-filtered air flow of 2 ml/s was delivered via another solenoïd valve through a blank syringe into the glass tube, and at the same distance from the preparation, during stimulus off. Stimulations followed at 3 min intervals.

CO_2. A range of concentrations of CO_2 were produced by mixing the manometer-controlled outflows from 100% CO_2 or 5% CO_2/95% O_2 gas cylinders in fixed proportions to pure N_2. A 5-ml syringe with a rubber stopper in place of the plunger was filled with precise concentrations of CO_2, as confirmed with an IR-gas analyser (Binos1, Leybold-Heraeus, FRG). The tip of the syringe was then introduced into the glass stimulus-delivery-tube and 2 ml of its content flushed over the preparation as described above. As the flow rate of the main humidified air flow was 10 ml/s, CO_2 concentration of the stimulus pulse was diluted 6 times in its passage to the preparation to provide a range of concentrations from ca. 0.04% to 5% CO_2. In longer experiments with continuous or pulsed CO_2 stimulation, a mixture of 5% CO_2/95% O_2 from a gas cylinder was injected directly into the glass stimulus-delivery-tube. Various concentrations of CO_2 were obtained by regulation of a voltage-pressure converter which controlled the flow rate of the CO_2/O_2 mixture into either the continuous humidified air stream of ca. 0.04% CO_2 or into a dry synthetic air stream of 20% O_2/80% N_2 which was free of CO_2. In order to prevent changes in air speed at the level of the tarsus, two solenoïd valves operated alternatively, permitting delivery of either the CO_2/O_2 mixture or an equivalent charcoal-filtered air stream into the continuous air flow.

Breath. Human breath was blown into the barrel of a 5-ml syringe used as stimulus cartridge and delivered to the preparation as described above. The CO_2 concentration of breath was likewise measured with the IR–CO_2 analyser. Taking into account a dilution factor of 6 in the delivery tube, the estimated concentration at the level of the tarsus was therefore ca. 0.6%.

Other volatiles tested. The following volatiles were also tested: methane, ammonia, acetone, 3-pentanone, 4-heptanone, g-butyrolactone, g-valerolactone, g-caprolactone, hexanal, pentanol, 1-octen-3-ol, 1-octene, propionic acid, n-butyric acid, iso-butyric acid, n-valeric acid, iso-valeric acid, heptanoic acid, L-lactic acid (all vertebrate-associated volatiles), nonanoic acid, 2-nitrophenol, 2,6-dichlorophenol, methylsalicylate (tick pheromone components), dichloromethane and distilled H_2O (solvent blanks). The purity of these products, except for the first two gases, was >99% as indicated by GC. Ten µl of a 10^{-3} or 10^{-2} *M* stimulus solution in dichloromethane (Merck analytical grade) or in distilled H_2O was deposited on a piece of filter paper and enclosed in a stoppered 5-ml syringe (stimulus cartridge) after evaporation of the organic solvent. Three min later, 2 ml of the syringe content was evacuated into the delivery tube as described above. Methane was taken from the mains. A stock solution of 35% NH_4OH diluted 10 or 100 times was used as ammonia stimulus.

Spatial field of perception of the capsule of Haller's organ

Stimulation of the CO_2-excited receptor found in one of the capsular sensilla was made from different points in space around the tarsus to determine whether preferential directions exist in the capsule's field of perception. For this purpose, female ticks, which are slightly bigger than males, were mounted on a pointed perpex holder allowing stimulation from almost any direction. A tungsten electrode, chosen to economise space in the capsular slit, was gently introduced through the slit-like opening of an undissected capsule until good contact was made with the sensillum bearing the CO_2-excited receptor. The recording electrode did not occupy more than 10% of the slit opening, and thus had a minimal effect on the way air could enter the capsule. The reference tungsten electrode was fixed in the coxa of one of the anterior legs. CO_2 stimulation was administered in 1 s into the delivery stream (40 cm/s) blowing from the directions indicated in Fig. 7 onto the tarsus, which was placed at 15 mm from the orifice of the stimulus-delivery-tube.

Wind-tunnel experiments

Behavioural experiments were made in a wind-tunnel (111 cm long, 45 cm wide, and 28 cm high) at 22±2 °C and ca. 45% RH. Room air was blown down the tunnel by a fan through a charcoal filter (Therma, Switzerland) and a glass-fibre net (1.4 mm mesh). Five resting males or females, with legs folded under the body, were introduced 80 cm downwind from the stimulus source. Ticks were discarded unless they remained immobile during the 2 min prior to stimulation. The stimulus source was a humidified synthetic air flow bearing various CO_2 concentrations which was introduced via a nozzle centrally at floor level in the upwind part of the tunnel. This stimulus was immediately carried by the wind flowing down the tunnel at 20 cm/s to the resting ticks. Laminarity of the flow, wind speed, and plume characteristics at the floor were defined with cigarette smoke. The stimulus was diluted 25 to 30 times as measured by CO_2 indicator tubes ±5–10% error (Dräger, Germany), to give a mean concentration from ambient control of ca. 0.04% to 0.35% CO_2 over the resting ticks at the highest level tested. The mean CO_2 concentration within 8 cm of the source ranged from ambient control to 1.1% at the highest level tested. Nevertheless, it is important to mention that the plume, as indicated by cigarette smoke, had a disrupted structure with some two-fold differences in concentration around the mean concentration. Ticks were observed during 5 min of stimulation. Individuals initiating locomotion as well as those walking upwind to within 8 cm of the source were counted. Experiments were replicated 20 times for each CO_2 concentration and each sex.

Results

Breath only elicited responses in sensory cells of 3 of the wall-pore-single-walled sensilla according to Altner's et al. classification (1977) in the capsule of Haller's organ. Two of the 7 capsular sensilla as well as some pleomorphs are shown in Fig. 1C, along with the approximate orientation of the recording electrodes used to capture electrical activity of cells which were responsive to breath. The activity pattern of cells recorded from each of these 3 electrode orientations was distinctive and consistent between ticks, and between the left and the right tarsus of the same individual in terms of the number of cells, the spike amplitudes and the spike shapes. Other precise orientations of electrode insertion permitted capture of other patterns of olfactory cell activity which were altered by other stimuli such as methylsalicylate (Hess and Vlimant 1986) or by vertebrate body odours (Steullet, unpublished). Indeed, the different orientations of the electrodes to where cell activities were recorded corresponded to the sensilla locations within the capsule as

observed by microscopy. The pattern of cell activity was the same whether the tip of the capillary remained unbroken (tip diameter < 1 µm) or broke on contact (diameter up to 5 µm). This result indicated that simple contact of the electrolyte with the wall of the sensillum sufficed for a good recording. The walls of these sensilla are indeed very thin (0.08–0.14 µm) with large and numerous pores (0.1–0.16 µm dia.) (Hess and Vlimant 1982). Recordings generally displayed activity of 3 to 5 cells, an observation which correlated well with ultrastructural studies showing that capsular sensilla in *A. variegatum* contain 3 to 5 sensory cells (ibid.). This and the fact that the sensilla were never seen to touch one another in sections under high magnification lead to suggest that the spikes observed in a given recording were picked up from a single sensillum. The fact that electrophysiological activity of cells responding to breath was captured with the electrode inserted in 3 different orientations proximally in the capsule suggests that 3 different sensilla were implicated. Each type displayed a characteristic multicellular response. CO_2 excited a cell in one of these sensilla (Fig. 2A), and inhibited a cell in another sensillum (Fig. 6), whereas cells of the third type of breath sensillum did not respond to CO_2. In the latter, one cell is described as being a sulfide-sensitive cell (Steullet and Guerin 1992). Despite the difficulty associated with working blindly within the capsule, many reproducible recordings were obtained by judicious placement of the electrode. This permitted recordings from 65 breath sensilla bearing the CO_2-excited cell, 17 breath sensilla with the CO_2-inhibited cell, and 37 breath sensilla with no CO_2-receptor, in different ticks.

CO_2-excited receptor

Figure 2A illustrates the cell response of one breath sensillum bearing a CO_2-excited receptor to increasing CO_2 concentrations and human breath, all injected 25 cm from the outlet of the stimulus-delivery-tube and subsequently diluted in the humidified air stream. Detailed sections of some of these responses are given in Fig. 3. As the stimulus onset was not sharp, the phasic portion of the response was not so pronounced and the maximum frequency occurred between 200 and 600 ms after a gradual increase in spike frequency. With the absence of a strong phasic part in the response, it was easier to categorize the spikes visually (Fig. 3). The firing rate of the spike numbered 1 (CO_2-excited receptor) induced by human breath diluted in clean air was quite similar to that induced by the equivalent CO_2 concentration (Fig. 3). This suggests that breath contains nothing else capable of modifying the response of this receptor. Moreover, none of the volatiles, listed in Materials and methods, elicited a response from this receptor.

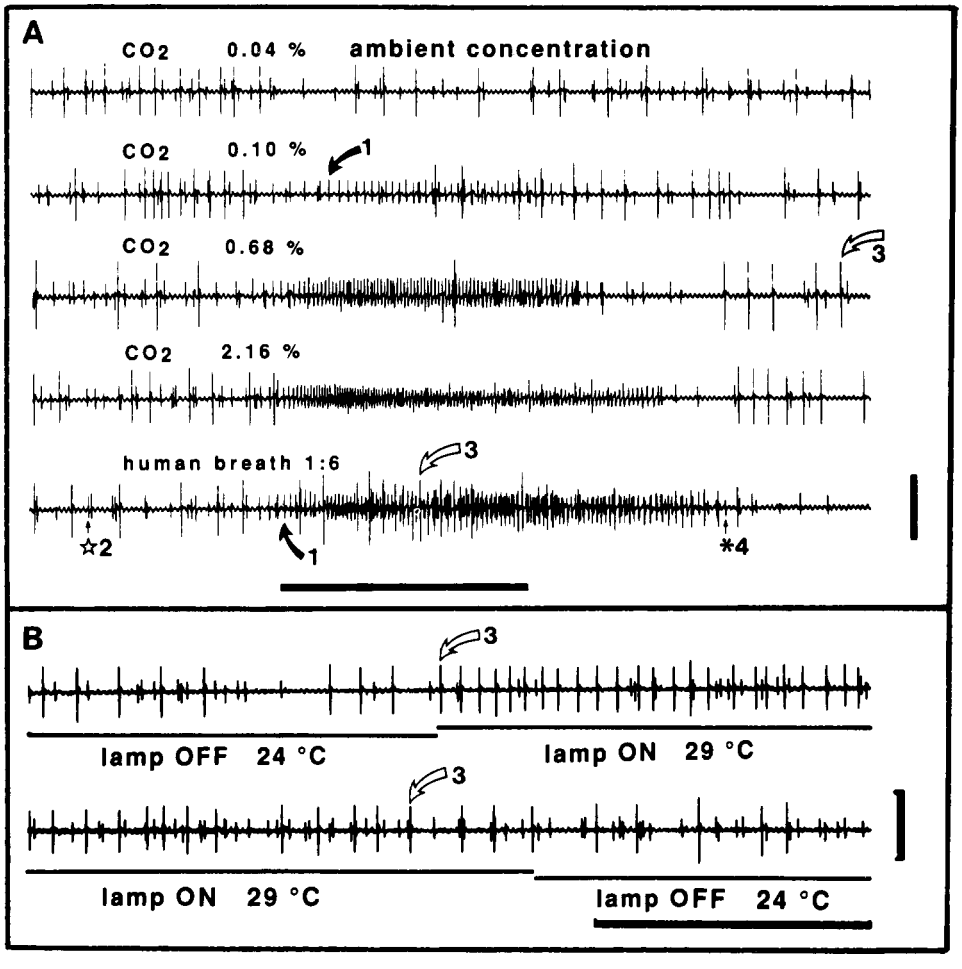

Fig. 2. A Representative responses of a capsular sensillum bearing a CO_2-excited receptor of a male *A. variegatum* (small biphasic spike 1 with *bold arrow*) to increasing CO_2 concentrations and diluted human breath. Stimuli were injected into the stimulus-delivery-tube 25 cm from its outlet, so that the stimulus onset at the preparation was not very sharp, and cell frequencies increased gradually within the first 200 ms of the response. The preparation was maintained in a humidified air stream at ca. 0.04% CO_2. Four spike types are indicated (see text and Fig. 3); numbering of spike types same for Figs. 2 and 3. Spike 4 *(asterisk)* is a sulfide-receptor (according to Steullet and Guerin 1992). Spike 3 *(white arrow)* is a cell inhibited by increasing CO_2 levels, but activated by breath. Its response seemed to be associated more with temperature changes as indicated by its response to turning off and on the microscope lamp (**B**). Thus, a T° increase activated it, whereas a corresponding T° decrease slightly diminished its activity. T° changes were measured with a thermistor put at the place of the preparation. In **A**, horizontal bar, 1 s stimulation; vertical bar, 1 mV. In **B**, horizontal bar, 1 s; vertical bar, 1 mV

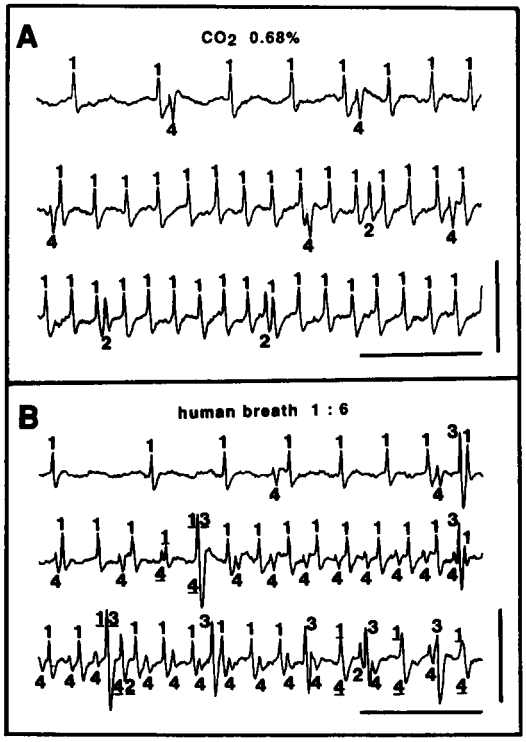

Fig. 3A, B. Detailed sections from the recordings illustrated in Fig. 2 to show how the 4 spike categories were discriminated visually. **A** Recording from the first 750 ms of stimulation with 0.68% CO_2, and **B** recording from the first 750 ms of stimulation with human breath diluted 1:6 in air (ca. 0.6% CO_2). *Spike 1,* activated either by breath or CO_2, is the CO_2-excited receptor. *Spike 2* is another cell with a persistently low firing rate. *Spike 3* may be a thermoreceptor, responding to a slight increase in T° during stimulation with human breath. *Spike 4,* which changed its sign during stimulation with breath, is a sulfide-receptor responding to H_2S (described in Steullet and Guerin 1992). Spike numbers underlined are overlapping events. Numbering of spike types as in Fig. 2. Horizontal bars 100 ms; vertical bars 1 mV

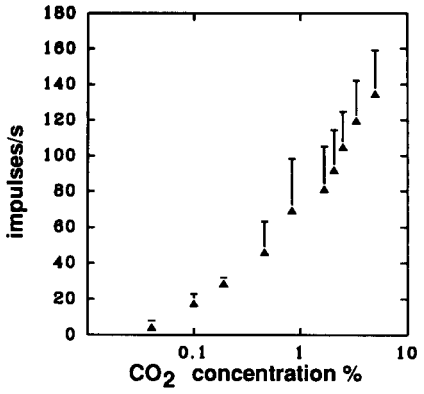

Fig. 4. Dose-response curve of the CO_2-excited receptor of male *A. variegatum* established from the first 160 ms of the response (phasic part). Preparations were maintained in a humidified air stream at ca. 0.04% CO_2 into which CO_2 stimuli were injected 3 cm from the outlet of the tube to the preparation. Data (mean ± SD) have been obtained with 4 CO_2-excited receptors, all from different males. *Abscissa:* estimated concentrations of CO_2 arriving at preparations

Figure 4 shows the dose-response relation established with CO_2-excited receptors from 4 different males which were stimulated with increasing CO_2 concentrations injected 3 cm from the outlet of the stimulus-delivery-tube (sharp stimulus onset). The response magnitude was determined from the first 160 ms of stimulation (phasic portion). The possibility of overlapping spikes was also considered in the determination of the response intensity. The CO_2-excited receptor responded to a concentration range covering some 2 to 3 log orders of magnitude. At ambient concentration of ca. 0.04%, activity was weak at a mean of 4.4 impulses/s, and gradually increased with higher concentrations up to about 140 impulses/s for 5% CO_2. The relation between the CO_2 dose and the tonic

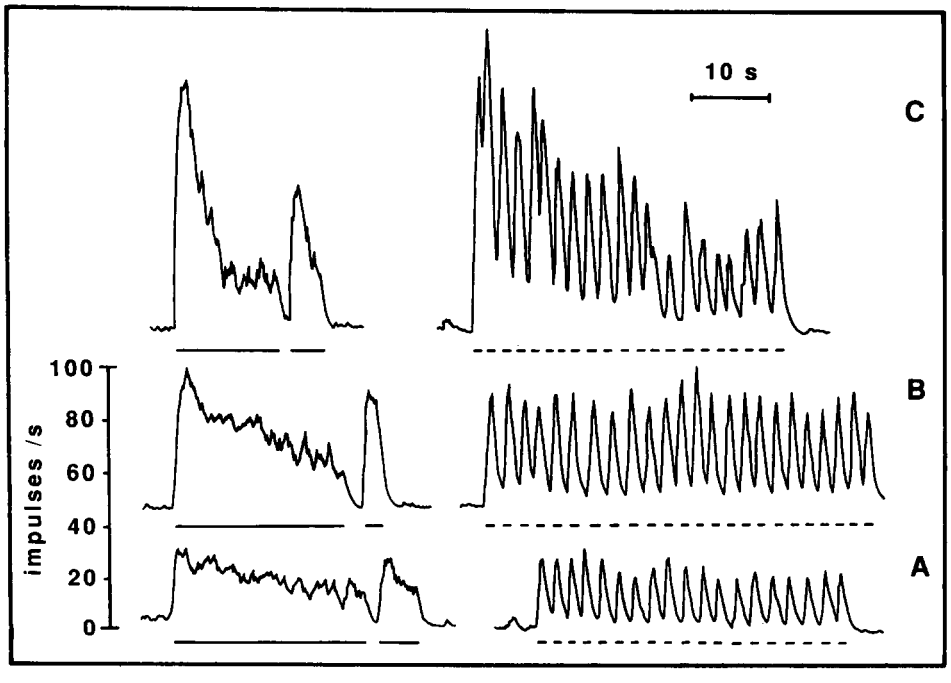

Fig. 5A–C. *Amblyomma variegatum* CO_2-excited receptor response under continuous and repetitive 1 s stimulation: **A** 0.7%, **B** 1.2%, and **C** 1.8% CO_2. *Horizontal bars:* stimulus duration. The preparation was maintained in a humidified air stream at 0.04% CO_2 into which stimuli were injected. These response profiles were obtained by frequency to voltage conversion of the AC signal after sorting spikes of the CO_2-excited receptor from others with a window discriminator (see Materials and methods). Responses were reproducible

portion of the response, established with firing rates observed after 2 min of exposure to a given CO_2 concentration, shows that the CO_2-excited receptor coded rather well constant levels of CO_2 higher than ca. 0.1% (Fig. 8). With repetitive 1 s pulses of CO_2, or with continuous stimulations of more than 10 s, strong adaptation only occurred at levels as high as 1.8% CO_2 (Fig. 5). Even then, however, receptor inactivation was remarkably fast, returning abruptly to its former level of activity after stimulation off (Fig. 2A). At high CO_2 concentrations the spike amplitude of the CO_2-excited receptor sometimes diminished as shown in Fig. 2A, but this did not occur systematically. No difference between CO_2-excited receptors of males and females was recorded.

The sensillum housing the CO_2-excited receptor also had a cell which was inhibited on stimulation with CO_2 in our set-up (cell numbered 3 in Figs. 2A, and 3). Nevertheless, this response was not due primarily to CO_2, since human breath slightly activated this cell. Thermosensitivity may be responsible since a slight increase in T° resulting from switching on the microscope lamp stimulated this cell, whereas switch-off caused some slight inhibition (Fig. 2B). CO_2 stimulation did cause a decrease in T° (<0.5 °C), and breath stimulation an increase in T° (<1 °C) in our set-up.

CO_2-inhibited receptor

This type of receptor was inhibited by an increase in CO_2 concentration and activated by a decrease. Complete inhibition of this receptor (bold arrow in Fig. 6) was achieved by short 1 s stimulation with diluted breath or with the relatively high concentrations of CO_2, i.e. greater than 0.1%, and reactivation of the receptor was clearly delayed after stimulus off. This post-stimulus inhibition was more pronounced with increasing CO_2 concentrations and lasted, in any case, significantly longer than the complete decline of the CO_2-excited receptor. Thus, 1 s stimulation with 0.68% induced a post-stimulus inhibition of 1203 ± 283 ms (mean ± SD) in 6 CO_2-inhibited receptors, whereas the complete post-stimulus decline of 9 CO_2-excited receptors with the same stimulus was reached after 313 ± 126 ms. Reactivation following inhibition due to 1 s CO_2 stimulation was typified by a burst in spike activity which was stronger the higher the CO_2 concentration employed. The frequency of the reactivation, based on the first 400 ms of the response was 22 ± 8 impulses/s (mean ± SD, $n=3$) after a 0.1% CO_2 stimulus, 30 ± 14 impulses/s after 0.68% ($n=6$), and 35 ± 14 impulses/s after 2.16% ($n=2$), whereas activity at ambient was 17 ± 9 impulses/s ($n=18$). As expected, the CO_2-inhibited receptor was also affected by human breath in the same way as with an equivalent concentration of CO_2. Diluted breath containing ca. 0.6% CO_2 elicited inhibition lasting 1170 ± 448 ms (mean ± SD, $n=6$) from stimulation off and a reactivation frequency of 25 ± 11 impulses/s comparable with 1203 ± 283 ms (mean ± SD, $n=6$) post-stimulus inhibition and a reactivation burst of 30 ± 14 impulses/s for an equivalent CO_2 concentration. Nevertheless, as Fig. 7 clearly shows, the same CO_2 drop could induce a very different reactiva-

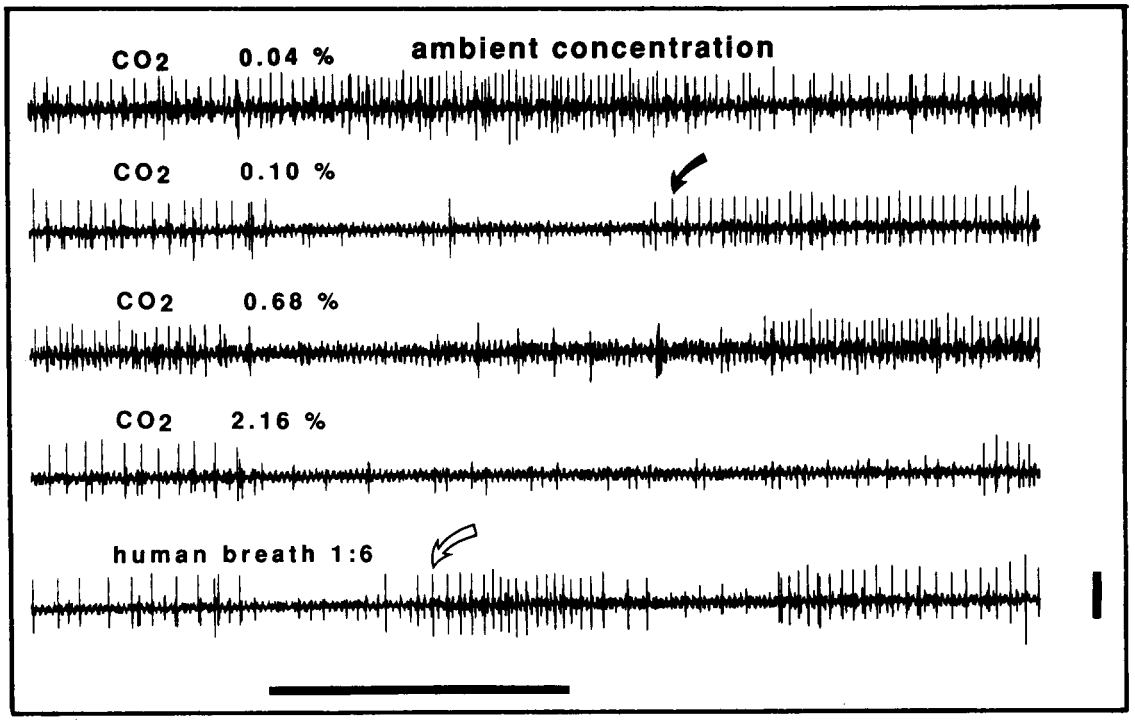

Fig. 6. Representative responses of a capsular sensillum of a male *A. variegatum* bearing a CO_2-inhibited receptor *(bold arrow)* to increasing CO_2 concentrations and diluted human breath. The preparation was maintained in a humidified air stream at ca. 0.04% CO_2 into which stimuli were injected 25 cm from the outlet of the tube. *White arrow:* receptor activated by an unknown breath component with a latency of ca. 300 ms. Horizontal bar 1 s stimulation; vertical scale 1 mV

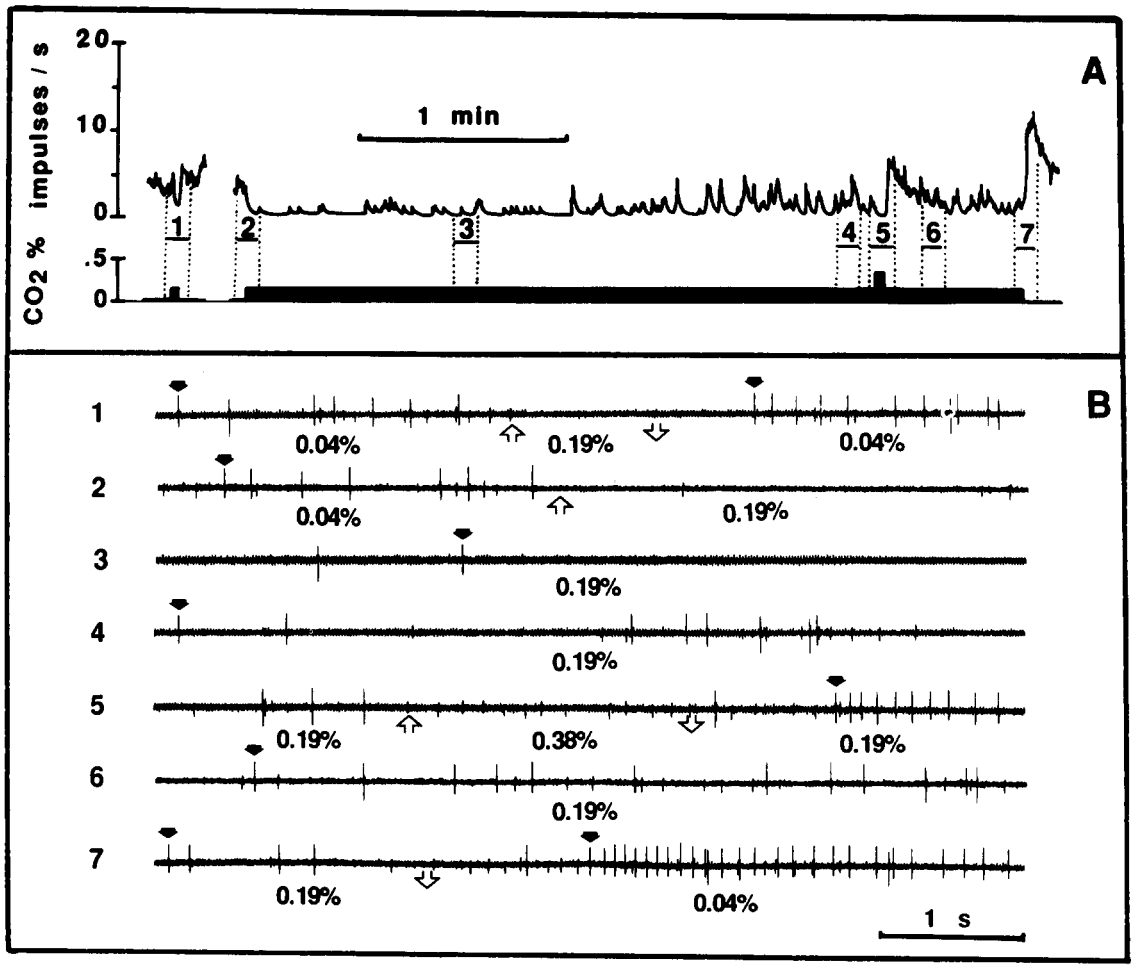

Fig. 7A, B. Adaptation of a CO_2-inhibited receptor *(bold arrow)* of a male *A. variegatum* at different CO_2 concentrations. **A** *Upper trace:* response profile of the CO_2-inhibited receptor, obtained by frequency to voltage conversion of the AC signal after sorting spikes of the CO_2-inhibited receptor from others with a window discriminator (see Materials and methods); *lower trace:* CO_2 concentrations (either 0.04, 0.19, or 0.38%) delivered to the CO_2-inhibited receptor. **B** Detail of the spike pattern underlying the response profile in **A**, where numbers on the left of each record refer to the sections numbered in **A**. *White arrow up,* increase in CO_2 concentration; *white arrow down,* decrease in CO_2, concentration. Responses were reproducible

tion burst in this type of receptor, depending on the CO_2 conditions pertaining before the drop, i.e. the longer the receptor was inhibited by exposure to a given CO_2 concentration then the stronger the reactivation. When the CO_2-inhibited receptor was submitted to long stimulation with CO_2 above ambient as in Fig. 7A, it was first completely inhibited but adapted within minutes to another frequency level. These observations provided evidence for the phasic-tonic characteristic of the CO_2-inhibited receptor.

The tonic responses of both the CO_2-inhibited and the CO_2-excited receptors after 2 min of exposure to stable concentrations of CO_2 ranging from 0% to 5% are shown in Fig. 8. The tonic response of the CO_2-inhibited receptor changes most with concentration between 0% and 0.2% CO_2, i.e. the CO_2-inhibited receptor codes best small shifts in concentration around ambient (Fig. 9). In experiments where the CO_2 level was changed approximately every 5 s, it was clear that the firing rate of the CO_2-inhibited receptor was most affected by shifts of 0.01 to 0.02% CO_2 around the 0.05% level (Fig. 9A), than

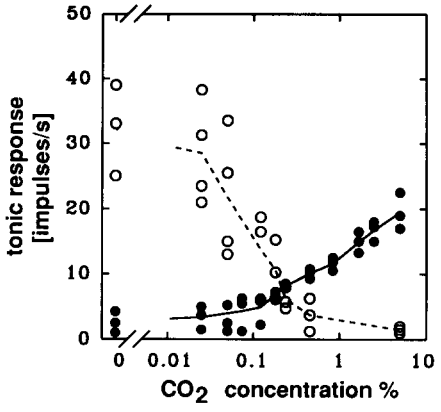

Fig. 8. Dose versus tonic response relationship for both the CO_2-excited *(solid circles)* and the CO_2-inhibited receptors *(open circles)* of male *A. variegatum*. The tonic activity was calculated from a 4000 ms spike train after 2 min of exposure of the receptor to the different CO_2 concentrations, obtained by adding different amounts of 5% CO_2/95% O_2 into a dry synthetic air flow of 20 % O_2/80% N_2 to achieve a CO_2 range of 0% to 5%. Data points were established with, respectively, 3 CO_2-excited and 3 CO_2-inhibited receptors, all from different ticks. *Trend lines* connect mean values

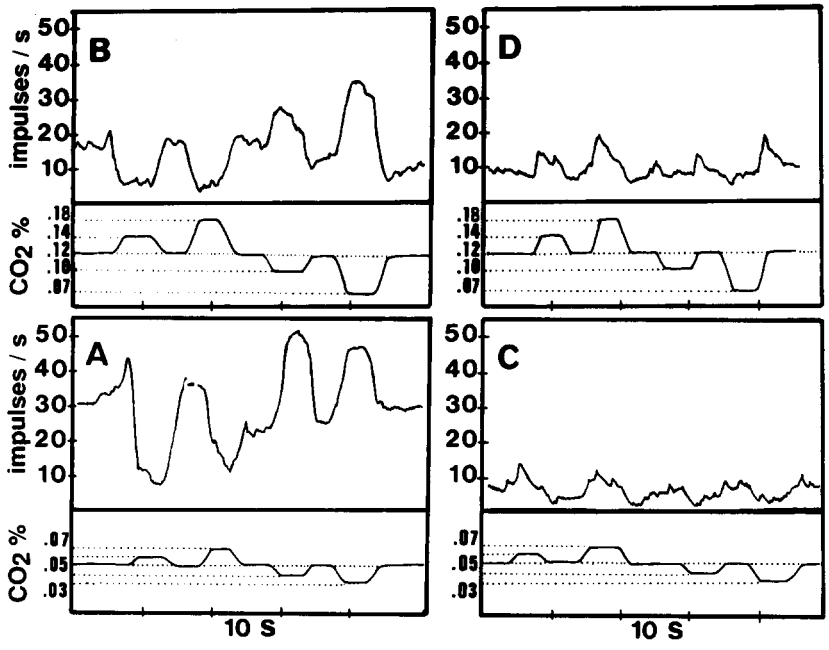

Fig. 9A–D. Modulation of the spike frequency of both a CO_2-inhibited receptor (**A** and **B**) and a CO_2-excited receptor (**C** and **D**) of a male *A. variegatum* by small changes in CO_2 concentration around 0.05% (**A** and **C**) and around 0.12% (**B** and **D**) in a dry synthetic air stream. Changes in CO_2 concentration were obtained by varying the manometer-controlled flow from a gas tank containing 5% CO_2/95% O_2 which was added into the dry synthetic air stream of 20% O_2/80% N_2. Response profiles (*upper trace* in each case) were obtained by frequency to voltage conversion of the AC signal after sorting spikes of either the CO_2-excited or the CO_2-inhibited receptor from others with a window discriminator (see Materials and methods). The *lower trace* in each case is the representation of the stepwise changes made to the CO_2 concentration in time (range 0.03 to 0.07% in **A** and **C**, and 0.07 to 0.18% in **B** and **D**). Time scale on the horizontal axis 10 s/div. Responses were reproducible

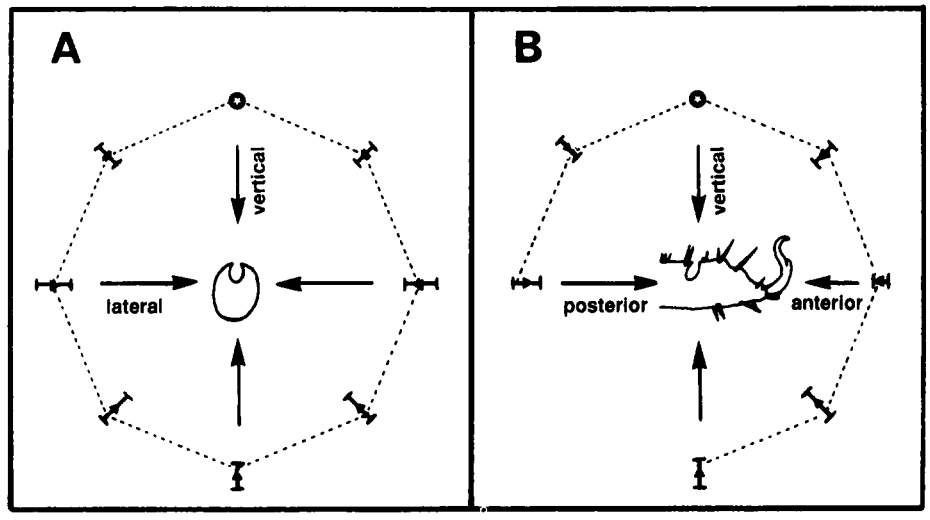

Fig. 10A, B. Field of perception of the CO_2-excited receptor in the capsule of Haller's organ established by the response of the sensilla housing this receptor in 6 female *A. variegatum* to a CO_2 stimulus coming from different directions around the tarsus. Responses (mean ± SD) are given as a percentage of the response to stimulus delivered perpendicularly to the dorsal surface of the tarsus *(star)*. Reference response shown at apex of octagon. **A** Responses to stimulation at various directions perpendicular to the longitudinal axis of the tarsus. **B** Responses to stimulation at various directions on the longitudinal axis of the tarsus. No stimulation was possible from the posterior-ventral direction, where the body of the tick was fixed

by even bigger shifts around 0.12% (Fig. 9B). Even an increase of just 0.001–0.002% CO_2 (i.e. 10–20 ppm) above 0% already elicited a visible decrease in spike activity of the CO_2-inhibited receptor. By contrast, the CO_2-excited receptor coded poorly small shifts around ambient (Fig. 9C, D), but became much more efficient for greater shifts around 0.12% (Figs. 5, 8).

None of the other volatiles listed in Materials and methods influenced activity in the CO_2-inhibited receptor. Nevertheless, this receptor was affected by large T° and humidity changes. A decrease in RH of some 50% or an increase in T° of 5 °C slightly stimulated this receptor, while it was mildly inhibited by a large jump in RH of 50% or a T° decrease of 5 °C. However, the response to stimulation with either breath or CO_2 was not due to T° or humidity shifts, as both breath (T° increase of <1 °C, and RH increase of ca. 2%) and CO_2 (T° decrease of <0.5 °C, and RH decrease of ca. 10%) both inhibited the receptor. Moreover, large T° shifts only elicited slight modulation of the activity of this receptor when it was exposed to CO_2 concentrations near ambient (ca. 0.04%), whereas the same T° shifts did not influence spike frequency in a CO_2-free atmosphere. Because of the extreme sensibility of the CO_2-inhibited receptors to small changes of concentration around ambient, the apparent responses of this receptor to T° or RH shifts are probably due to the influence of these parameters on the CO_2 content of the air. Human breath also activated another cell of the breath sensillum bearing the CO_2-inhibited receptor, but the responsible stimulus is still unknown (white arrow in Fig. 6).

Spatial field of perception of the capsule

The direction from which wind carrying the CO_2 stimulus arrived, as tested in 6 different females, did not have

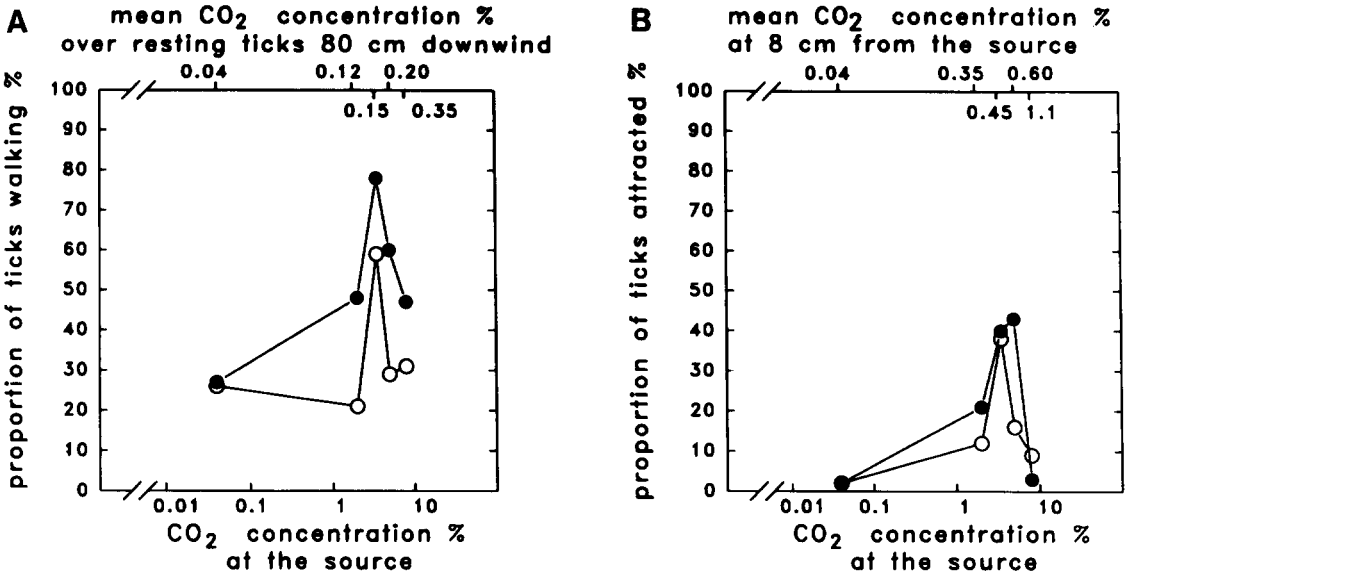

Fig. 11A, B. Behavioural response of male *(solid circles)* and female *(open circles) A. variegatum* to various CO_2 concentrations in a wind-tunnel. **A** Locomotor stimulant effect of various CO_2 levels as measured by proportion of resting ticks induced to commence walking. *Lower abscissa* the CO_2 concentration measured at the source, *upper abscissa* the mean CO_2 concentration as it passed over resting ticks 80 cm downwind from the source. Above 30%, the locomotor effect of the stimulus is significant ($P<0.05$, the exact method for 2×2 tables). **B** Attraction of CO_2 as measured by proportion of ticks walking upwind to within 8 cm of the source. *Lower abscissa* CO_2 concentrations at the source, *upper abscissa* mean CO_2 concentration at 8 cm from the source. Above 10%, attraction is significant ($P<0.05$, the exact method for 2×2 tables). Between 90 and 100 males and females were tested at each CO_2 concentration

any influence on the response of the CO_2-excited receptor enclosed in the undissected capsule (Fig. 10). Indeed, the intensity of the response to stimulation from various directions did not differ from the response to the same stimulus directed perpendicularly to the dorsal side of the capsule, taken here as a reference. Thus, despite enclosure of the olfactory sensilla in a capsule on the tarsus with a narrow slit as an opening to the exterior, *A. variegatum* is able to detect CO_2 effectively, regardless of the direction from which air currents approach it.

Wind-tunnel experiments

CO_2 is a locomotor stimulant and attractant for both sexes of *A. variegatum,* although males respond better than females. Wind-tunnel experiments show that this tick species responded best to a very narrow range of CO_2 concentrations (Fig. 11A, B). Humidified air with a mean concentration of 0.15% CO_2 passing over the resting ticks was the best locomotor stimulant (Fig. 11A). These activated ticks were attracted to within 8 cm of the source when mean CO_2 concentrations were between 0.45% and 0.6%, whereas a mean of ca. 1.1% CO_2 within 8 cm of the source was no longer attractive (Fig. 11B).

Discussion

CO_2 acts as a locomotor stimulant and an attractant for *A. variegatum* in the wind-tunnel. A source of 3.5 to 5% CO_2, diluted by almost a factor of 10 at 8 cm from the source, and 25 to 30 times at 80 cm downwind where ticks were resting, was most attractive. This suggests that the best attractant in the field for this species, which normally lies in wait in the litter zone, would be a respiring host reposing or grazing a few meters away. Indeed, field experiments with the related species, *Amblyomma hebraeum,* have shown that this tick is attracted over a range of a few meters to cattle or sheep (Norval et al. 1987). The upper limit of some 1% CO_2 which still attracted *A. variegatum* to the source in the wind-tunnel, but above which level they were repelled, is not surprising. In this laboratory we have frequently observed that expiring directly onto an individual of this species which is running toward the observer results in repulsion. CO_2 levels which cause activation and attraction of male and female *A. variegatum* in the wind-tunnel correspond to the discriminative range of CO_2-receptors as revealed by the electrophysiology. Nevertheless, CO_2 is a better locomotor stimulant for males than for females. This difference in behaviour should most probably be ascribed to some internal control at the level of the CNS as no differences have been found between the sexes at the level of the CO_2-receptors.

Despite of the crucial importance CO_2 may play in host-finding behaviour of this species, it is surprising that the tick possesses on the tarsus of its forelegs just one CO_2-excited and one CO_2-inhibited receptor in the capsule of the Haller's organ. Stämpfli (1987) showed that *A. variegatum* no longer responded to CO_2 after removal or masking of the tarsus of each anterior leg. As adults of *A. variegatum* are often obliged to wait for months under occasionally quite adverse environmental con-

ditions before being provided the chance to find a suitable host, the few CO_2-receptors need then to remain entirely functional during a long period, ever alert to any abrupt change in the CO_2 level of its environment. It seems therefore quite normal that these cells should be well protected from any physical damage or desiccation inside the capsule of Haller's organ. Local high humidity around sensilla within the capsule permits thinner walls with larger pores than in sensilla not located within a cavity, factors which consequently increase molecular diffusion through the sensillar wall. In Lepidoptera (Bogner et al. 1986; Bogner 1990), CO_2 receptors are also more or less enclosed. Enclosure could furthermore improve CO_2 perception since a higher local RH around the sensillum could enhance better adsorbtion of CO_2 or its derivatives into the sensillar lymph. The rigid architecture of the capsule does not restrict the passage of volatiles, at least highly diffusable CO_2 molecules, to the capsular sensilla, nor is a specific orientation of the tarsus vis à vis the wind direction at all critical. Local air turbulence around the tarsus, combined with molecular diffusion is probably sufficient to permit permeation of CO_2 and other volatiles into the capsule.

The main characteristics of the CO_2-excited receptor of *A. variegatum* are: 1) a phasic-tonic response, 2) a steep dose-response curve covering 2–3 log orders of magnitude from ambient to ca. 5% CO_2, 3) adaptation occurring only at concentrations as high as 1.8%, and 4) a fast inactivation process after stimulus off even at the highest concentrations tested (5%). The steepness of the dose-response relationship is not very different from that established for the CO_2-receptors of *Aedes aegypti* (Kellogg 1970), *Glossina pallidipes* (Bogner 1989), *Lucilia cuprina* (Stange 1974) and for the electroantennogram response of *Stomoxys calcitrans* to CO_2 (Warnes and Finlayson 1986). The CO_2-excited receptor of *A. variegatum* seems, nevertheless, less sensitive to small changes in CO_2 concentration around ambient than those described for haematophagous Diptera and especially mosquitoes (Kellogg 1970). However, as in *A. variegatum,* a fast inactivation process characterizes all of these CO_2-receptors (Kellogg 1970).

A CO_2-inhibited receptor has not, to our knowledge, been reported previously. This cell is extremely sensitive to very small changes in CO_2 levels around ambient concentrations, and the tonic part of the response is most affected by small changes in concentration of between 0% and ca. 0.2%. Shifts of 0.01 to 0.02% CO_2 around ambient are nicely monitored by the phasic part of the response of this receptor. Stimulation with 0.001–0.002% CO_2 (10–20 ppm) in a CO_2-free atmosphere already clearly diminishes the spike frequency of the CO_2-inhibited receptor. This extreme sensitivity explains why large T° or RH shifts, which can certainly modify the CO_2 content of ambient air, can induce small changes in the activity of this receptor. This phenomenon has already been observed for the very sensitive CO_2-receptors of Lepidoptera (Bogner 1990). At concentrations up to ca. 0.2%, the CO_2-inhibited receptor follows changes in the order of 0.01% CO_2 by modulation of spike frequency. At higher levels, the receptor is so strongly inhibited that it can no longer monitor abrupt changes in concentrations. Our experiments have shown that the length of post-stimulus inhibition is concentration dependent. Furthermore, the reactivation burst after stimulus off is not only concentration dependent but also related to the length of inhibition by CO_2. On the other hand, the CO_2-excited receptor responds weakly to small changes in CO_2 concentration near ambient, but perfectly codes for concentration changes higher than ca. 0.1%. In addition, inactivation of the CO_2-excited receptor even after stimulation with high concentrations of CO_2 is very fast, much faster than the post-stimulus recovery of the CO_2-inhibited receptor for the same CO_2 stimulus.

Consequently, *A. variegatum* possesses a CO_2 perception system operating with divaricate CO_2-excited and CO_2-inhibited receptors, each working most efficiently in a different but complimentary concentration range. This evidently permits the parasite to perceive the whole range of CO_2 concentrations to which it is confronted in host-finding, i.e. from ambient concentrations to the 5% level of vertebrate breath. However, since the CO_2-excited receptor operates through the whole range, from ambient to at least 5%, it can not evidently show at the same time a very high resolving power for small changes of concentration around ambient. The CO_2-inhibited receptor compensates amply for this by being very sensitive to small CO_2 shifts around ambient. The task of perceiving the whole CO_2 range to which the tick is confronted is thus divided between the two receptors.

The CO_2 levels in the litter zone where *A. variegatum* lies in wait for its host vary most probably between 0.03% to ca. 0.09% (cf. Holsher et al. 1980), well within the range of the CO_2-inhibited receptor. The latter may thus be used to detect small alterations in CO_2 concentration and alert a resting tick to the presence of a host nearby. Then the CO_2-excited receptor could take over at the higher concentrations encountered during orientation to the host.

Acknowledgements. We are indebted to the Hasselblad, Roche, Sandoz, and the Swiss National Science Foundation (Grant Nos. 3.609–0.87 and 31–28684.90), the Ciba-Geigy-Jubilaeums-Stiftung, and the Swiss Office for Education and Science for funding studies on tick sensory physiology at Neuchâtel. We thank Misters Bouvard, Rohrer and Cesari of CIBA–GEIGY Ltd., St. Aubin, Switzerland for supplying us with adult ticks. We are grateful to Michèle Vlimant for her help and advice with the microscopy, and to Dr. Tilman Haug (Regensburg University) for his useful discussions regarding electrophysiology. This paper is part of the Ph.D. thesis of Pascal Steullet at the University of Neuchâtel.

References

Altner H, Sass H, Altner I (1977) Relationship between structure and function of antennal chemo-, hygro, and thermoreceptive sensilla in *Periplaneta americana.* Cell Tissue Res 176:389–405

Bernard J (1974) Etude électrophysiologique de récepteurs impliqués dans l'orientation vers l'hôte et dans l'acte hématophage chez un hémiptère: *Triatoma infestans.* Thèse Université de Rennes (France)

Bogner F (1989) Single cell recordings of antennal CO_2-receptors in tsetse flies. Verh Dt Zool Ges 82:272–273

Bogner F (1990) Sensory physiological investigation of carbon dioxide receptors in Lepidoptera. J Insect Physiol 36:951–957

Bogner F, Boppré M, Ernst K-D, Boeckh J (1986) CO_2 sensitive receptors on labial palps of *Rhodogastria* moths (Lepidoptera: Arctiidae): physiology, fine structure and central projection. J Comp Physiol A 158:741–749

Fallis AM, Raybould JN (1975) Response of two African simuliids to silhouettes and carbon dioxide. J Med Entomol 12:349–351

French FE, Kline DL (1989) 1-octen-3-ol, an effective attractant for Tabanidae (Diptera). J Med Entomol 26:459–461

Garcia R (1962) Carbon dioxide as an attractant for certain ticks (Acarina, Argasidae and Ixodidae). Ann Entomol Soc Am 14:605–606

Garcia R (1965) Collection of *Dermacentor andersoni* (Stiles) with carbon dioxide and its application in studies of Colorado tick virus. Am J Trop Med Hyg 14:1090–1093

Gillies MT, Wilkes TJ (1968) A comparison of the range of attraction of animal baits and of carbon dioxide for some West African mosquitoes. Bull Entomol Res 59:441–456

Gödde J (1985) Low cost storing of two electrical biosignals from DC to 20 kHz at more than 80 dB dynamic range. Pflügers Arch 403:324–327

Gödde J (1989) Vibrating glass stylets: tools for precise microsurgery on cuticular structures. J Neurosci Methods 29:77–89

Gray JS (1985) A carbon dioxide trap for prolonged sampling of *Ixodes ricinus* L. populations. Exp Appl Acarol 1:35–44

Guglielmone AA, Moorhouse DE, Wolf G (1985) Attraction to carbon dioxide of unfed stages of *Amblyomma triguttatum triguttatum* Koch, under field conditions. Acarologia 26:123–129

Hess E, Loftus R (1984) Warm and cold receptors of two sensilla on the foreleg tarsi of the tropical bont tick *Amblyomma variegatum*. J Comp Physiol A 155:187–195

Hess E, Vlimant M (1982) The tarsal sensory system of *Amblyomma variegatum* Fabricius (Ixodidae, Metastriata). I. Wall pore and terminal pore sensilla. Rev Suisse Zool 89:713–729

Hess E, Vlimant M (1983) The tarsal sensory system of *Amblyomma variegatum* Fabricius (Ixodidae, Metastriata). III. Mapping of sensory hairs and evolution of the relative importance of sensory modalities during post-embryonic development. Rev Suisse Zool 90:887–897

Hess E, Vlimant M (1986) Leg sense organs of ticks. In: Sauer JR, Hair JA (eds) Morphology, physiology, and behavioural biology of ticks. Ellis Horwood, Chicester, pp 361–390

Hindley E, Merriman G (1912) The sensory perception of *Argas persicus* (Oken). Parasitology 5:203–216

Holsher KH, Gearhart HL, Barker RW (1980) Electrophysiological responses of three tick species to carbon dioxide in the laboratory and field. Ann Entomol Soc Am 73:288–292

Kellogg FE (1970) Water vapour and carbon dioxide receptors in *Aedes aegypti*. J Insect Physiol 16:99–108

Lees AD (1948) The sensory physiology of the sheep tick *Ixodes ricinus*. J Exp Biol 25:145–207

Nevill EM (1964) The role of carbon dioxide as a stimulant and attractant to the sand tampan *Ornithodoros savigny* (Audouin). Onderstepoort J Vet Res 31:59–68

Norval RAI, Yunker CE, Butler JF (1987) Field sampling of unfed adults of *Amblyomma hebraeum* Koch. Exp Appl Acarol 3:213–217

Norval RAI, Yunker CE, Gibson JD, Deem SLD (1988) Field sampling of unfed nymphs of *Amblyomma hebraeum*. Exp Appl Acarol 4:173–177

Osbrink WLA, Rust MK (1985) Cat flea (Siphonaptera: Pulicidae): Factors influencing host-finding behavior in the laboratory. Ann Entomol Soc Am 78:29–34

Palade GE (1952) A study of fixation for electron microscopy. J Exp Med 95:285

Sabatini DD, Bensch KG, Barrnett RJ (1963) Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J Cell Biol 17:19–58

Sauer JR, Hair JA, Houts MS (1974) Chemo-attraction in the lone star tick (Acarina: Ixodidae). 2. Responses to various concentrations of CO_2. Ann Entomol Soc Am 67:150–152

Sinitsina EE (1974) Electrophysiological reactions of the neurons of the Haller's organ to the odour stimuli in the tick *Hyalomma asiaticum*. Parazitologiya 8:223–226

Smith JJB, Mitchell BK, Rolseth BM, Whitehead AT, Alben PJ (1990) SAPID tools: microcomputer programs for analysis of multi-unit nerve recordings. Chem Senses 15:253–270

Stämpfli N (1987) Etude des facteurs intervenant dans la phase initiale du comportement de recherche de l'hôte, chez la tique, *Amblyomma variegatum*, Fabricius 1794 (Acarina: Ixodidae). Thèse Université de Neuchâtel

Stange G (1974) Linear relation between stimulus concentration and primary transduction process in insect CO_2-receptors. In: Denton DA, Coghan JP (eds) Olfaction and Taste V. Pergamon Press, London, pp 207–211

Steullet P, Guerin PM (1992) Perception of breath components by the tropical bont tick *Amblyomma variegatum* Fabricius (Ixodidae). II. Sulfide-receptors. J Comp Physiol A 170:677–685

Turner DA (1971) Olfactory perception of live hosts and carbon dioxide by the tsetse fly *Glossina morsitans orientalis* Vanderplank. Bull Entomol Res 61:75–96

Waladde SM, Rice MJ (1982) The sensory basis of tick feeding behaviour. In: Obenchain FD, Galun R (eds) Physiology of ticks. Pergamon Press, Oxford New York Toronto, pp 71–118

Warnes ML, Finlayson LH (1985) Responses of the stable fly, *Stomoxys calcitrans* (L.) (Diptera: Muscidae) to carbon dioxide and host odours. 2. Orientation. Bull Entomol Res 75:717–727

Warnes ML, Finlayson LH (1986) Electroantennogram responses of the stable fly, *Stomoxys calcitrans*, to carbon dioxide and other odours. Physiol Entomol 11:469–473

Wilson JG, Kinzer DR, Sauer JR, Hair JA (1972) Chemoattraction of the lone star tick (Acarina, Ixodidae). I. Response of different developmental stages to carbon dioxide administered via traps. J Med Entomol 9:245–252