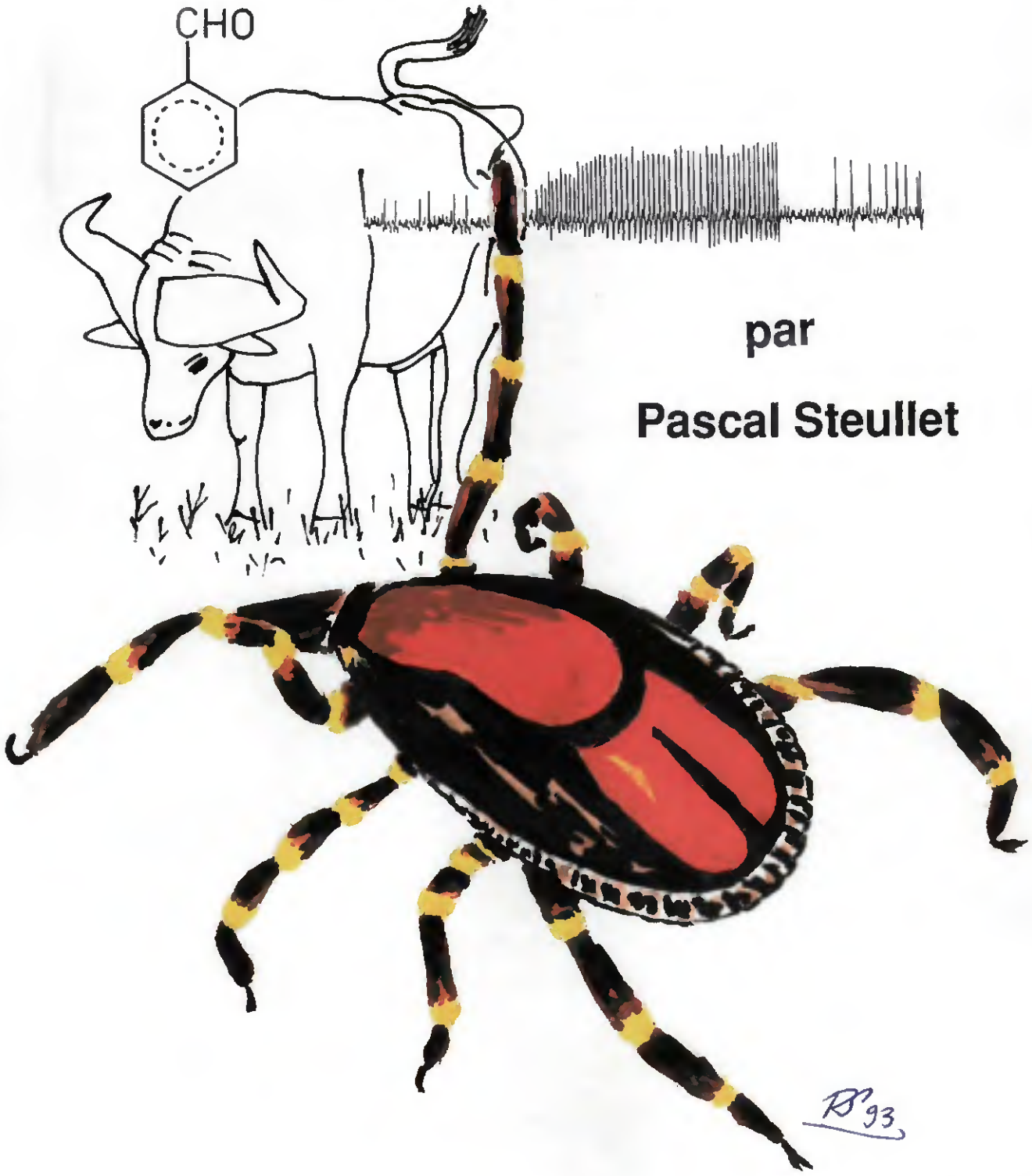
Perception of vertebrate volatiles in the tropical bont tick, *Amblyomma variegatum* Fabricius



par

Pascal Steullet

Thèse présentée à la Faculté des Sciences de l'Université de Neuchâtel
pour obtenir le grade de docteur ès sciences

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IMPRIMATUR POUR LA THÈSE

Perception of vertebrate volatiles in the tropical bont tick, *Amblyomma variegatum* Fabricius

de Monsieur Pascal Steullet

UNIVERSITÉ DE NEUCHÂTEL

FACULTÉ DES SCIENCES

La Faculté des sciences de l'Université de Neuchâtel
sur le rapport des membres du jury,

MM. P. Guerin, P.A. Diehl, E. Städler (Wädenswil) et R.A. Steinbrecht (Max-Planck-Institut, Seewiesen)

autorise l'impression de la présente thèse.

Neuchâtel, le 25 novembre 1993

Le doyen :

H.-H. Nägeli

...La tique solitaire, concentrée et cachée dans son arbre, aveugle sourde et muette, tout occupée pendant des années, à flairer sur des lieues à la ronde le sang des animaux qui passent et qu'elle n'atteindra jamais par ses propres moyens.... ....Mais la tique, butée, bornée et répugnante, reste embusquée, et vit, et attend. Attend jusqu'à ce qu'un hasard extrêmement improbable lui amène le sang juste sous son arbre, sous la forme d'un animal. Et c'est alors seulement qu'elle sort de sa réserve, se laisse tomber, se cramponne, mord et s'enfonce dans cette chair inconnue...

....Il (Jean-Baptiste Grenouille) se mit à s'introduire nuitamment dans les étables, pour y envelopper pendant quelques heures des vaches, des chèvres ou des cochons avec des linges enduits de graisse, ou pour les emmailloter dans des bandages huileux. Ou bien il se glissait furtivement dans un enclos à brebis pour y tondre clandestinement un agneau, dont ensuite il lavait à l'esprit-de-vin la laine odorante.....Les résultats ne furent d'abord guère satisfaisants. Car, à la différence d'objets dociles comme un bouton de porte ou une pierre, les animaux se montraient récalcitrants au prélèvement de leur odeur....

extrait de "Le Parfum"
de Patrick Süskind
(traduit de l'allemand par Bernard Lortholary)
édition Fayard (Le livre de poche)

CONTENTS

I. INTRODUCTION

Whenever the word tick is pronounced, a host of fears, legends and questions about these blood-sucking animals invariably arise. " A *dangerous insect that falls from trees onto humans and mammals to feed on blood* " would likely be the erroneous definition of a tick given by many people. The usual ignorance about how ticks recognize and find a host contributes to the legends on these small ectoparasites. The present work on the chemical ecology of ticks, more precisely on host-odour detection in the tropical bont tick *Amblyomma variegatum*, consequently needs a short introduction on tick systematic, the biology of *A. variegatum*, and different aspects of olfaction and the host-finding strategy in this tick species.

1.1. Systematic position of ticks and *Amblyomma variegatum*

As members of the Order Acari ticks (900 sp.) are distinguishable from mites by stigmata located behind the fourth leg pair, but also have a highly expandible fused body (the idiosoma), and possess mouthparts adapted to haematophagy (the gnathosoma) equipped with 2 sharp chelicerae, 1 hypostome covered with recurved teeth, and 2 palps. These blood-sucking arthropods comprise 3 families: Nuttalliellidae, Argasidae (soft ticks), and Ixodidae (hard ticks) to which the genus *Amblyomma* belongs (Fig. 1.1.). The Ixodidae, subdivided into 5 subfamilies (Fig. 1.1.), are characterized by a sclerotized plate, which in the female and immature stages is limited to the anterior dorsal region, while in males it covers the entire back thus restricting male body extension during a blood meal. The subfamily Amblyomminae includes the genus *Amblyomma* (102 sp) which are considered primitive ticks because of their large size and their three-host life-cycle pattern (Sonenshine 1991).

1.2. Biology of *Amblyomma variegatum*

A. variegatum is a large tick widely distributed in most tropical and subtropical regions of continental Africa, Madagascar, the South-West Arabic Peninsula, and the Cape Verde Islands (Hoogstraal 1956; Morel 1969). This species, also called the tropical bont tick, has been noted in the Caribbean Islands since the end of last century (Morel 1969) and threatens to colonize Central and South America. *A. variegatum* is a typical three-host tick with each stage (larva, nymph, adult) parasitizing mammals for a blood-meal of variable duration (larvae 5-8 days; nymphs 6-13 days; females 14-22 days) (Morel

1969). A blood meal is indeed a requirement for successful moulting of immatures, spermiogenesis and ovogenesis in adults, and for oviposition in females.

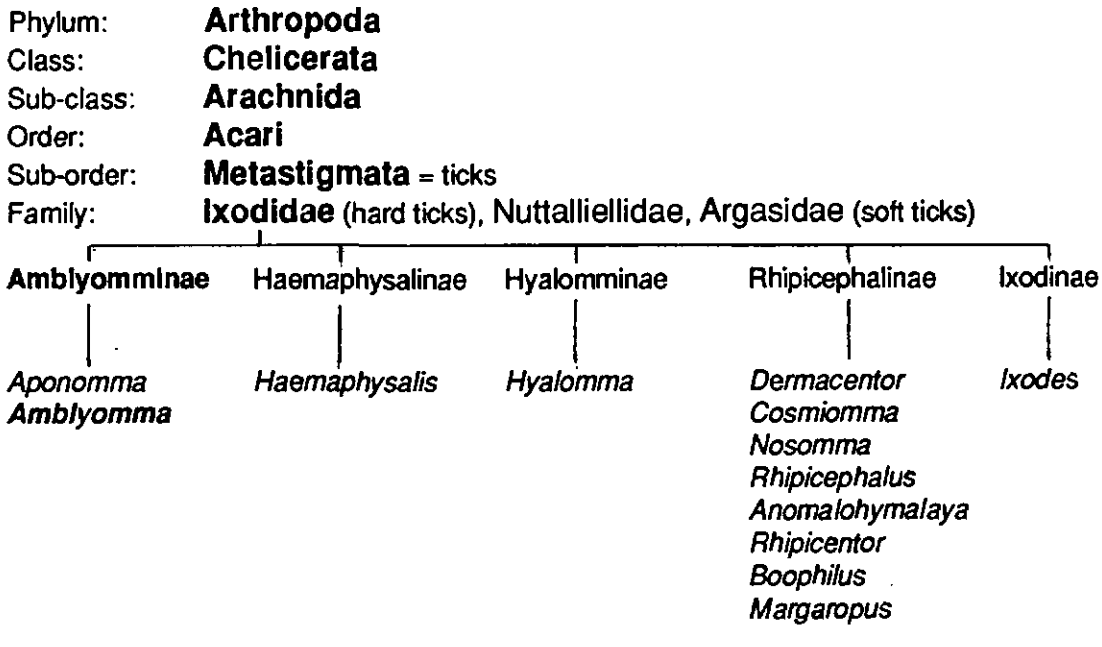

Fig. 1.1. Systematic position of ticks (ca. 900 sp) and the genus Amblyomma (102 sp).

Immature stages of the tropical bont tick parasitize a wide range of reptiles, birds, and mammals (Matthysse and Colbo 1987), but Barré (1989) noticed a preference for ungulates. Adults for their part feed principally on large ruminants (Morel 1969; Matthysse and Colbo 1987; Barré 1989). Although large wild mammals such as buffaloes and antelopes are thought to be the natural hosts, domestic animals (cattle, sheep, goats) are also strongly infested in many areas (Hoogstraal 1956; Matthysse and Colbo 1987). Aeschlimann (1967) suggested that the distribution of this tick species has been expanded by the development of cattle ranching. *A. variegatum,* a vector of various pathogens such as *Cowdria ruminantium* (heart-water), *Coxiella burnetti* (Q-fever) and *Dermatophilus congolensis* (dermatosis), also causes anaemia and skin damage (Aeschlimann 1976, Barré 1989). It has thus become a serious life-stock pest responsible for much animal weakness and illness.

1.3. Host-seeking in *Amblyomma variegatum*

Parasitizing vertebrates is a very tough task for all tick life-stages, since the potential host is much bigger and more mobile. Thus, specific physiological capabilities and adequate host-seeking strategies are the keys to successful completion of the life-cycle. Tremendous resistance to desiccation and starvation allow ticks to survive for months during non-parasitic phases. While some tick species live within nests or burrows and thus maintain a close relation with their host, others, such as *A. variegatum,* quest along pathways or areas frequented by their hosts. Consequently, the latter need well-adapted host-seeking strategies and efficient sense organs to optimize chances of meeting a host. Waladde and Rice (1982) reviewed the different senses involved during the successive behavioural steps of host-seeking, from tick arousal to its attachment on a host. In this context, olfaction is considered a major sense. Transported by wind and air turbulence, odour molecules can be detected over great distances. Odour quality as well as concentration constitute potential sources of information, i.e. estimation of the distance to a host, host recognition, host location.

Immature *A. variegatum* quest on vegetation and grasp any mammal which passes nearby (Barré 1989). Mechanical disturbance causes significant activation of immature stages (Barré 1989). By contrast, adult *A. variegatum,* which are hunter ticks (Morel 1969; Barré 1989), rest in the litter zone until a host in the close vicinity activates them (Fig. 1.2.). Excited ticks then start to walk about on the ground in order to locate the vertebrate, orient to it and grasp it, and then find a suitable feeding-site (Fig. 1.2.). Activation of resting adults is mainly mediated by host odour such as CO_2, while vibrations, temperature, infrared radiation, and visual cues do not play a primordial role during the arousal steps of host-finding (Stämpfli 1987; Steullet unpublished; Poffet unpublished; Kaltenrieder et al. 1989; Yunker and Norval 1991). On the other hand, odours along with vertebrate-emitted infrared radiation (Poffet, unpublished), and visual cues (Kaltenrieder et al. 1989; Kaltenrieder 1990) intervene in host location. Males are first to infest mammals at the end of the dry season (Morel 1969), and females appear a few days later on the infested hosts (Hoogstraal 1956; Morel 1969; see Fig. 1.2.). Others have observed that attached males (the primary colonizers) of different species of *Amblyomma* attract conspecific females and other males (the secondary colonizers) to the same feeding-site (Gladney et al. 1974; Rechav et al. 1977; Norval and Rechav 1979). Schoeni et al. (1984) and Apps et al. (1988) identified the components of

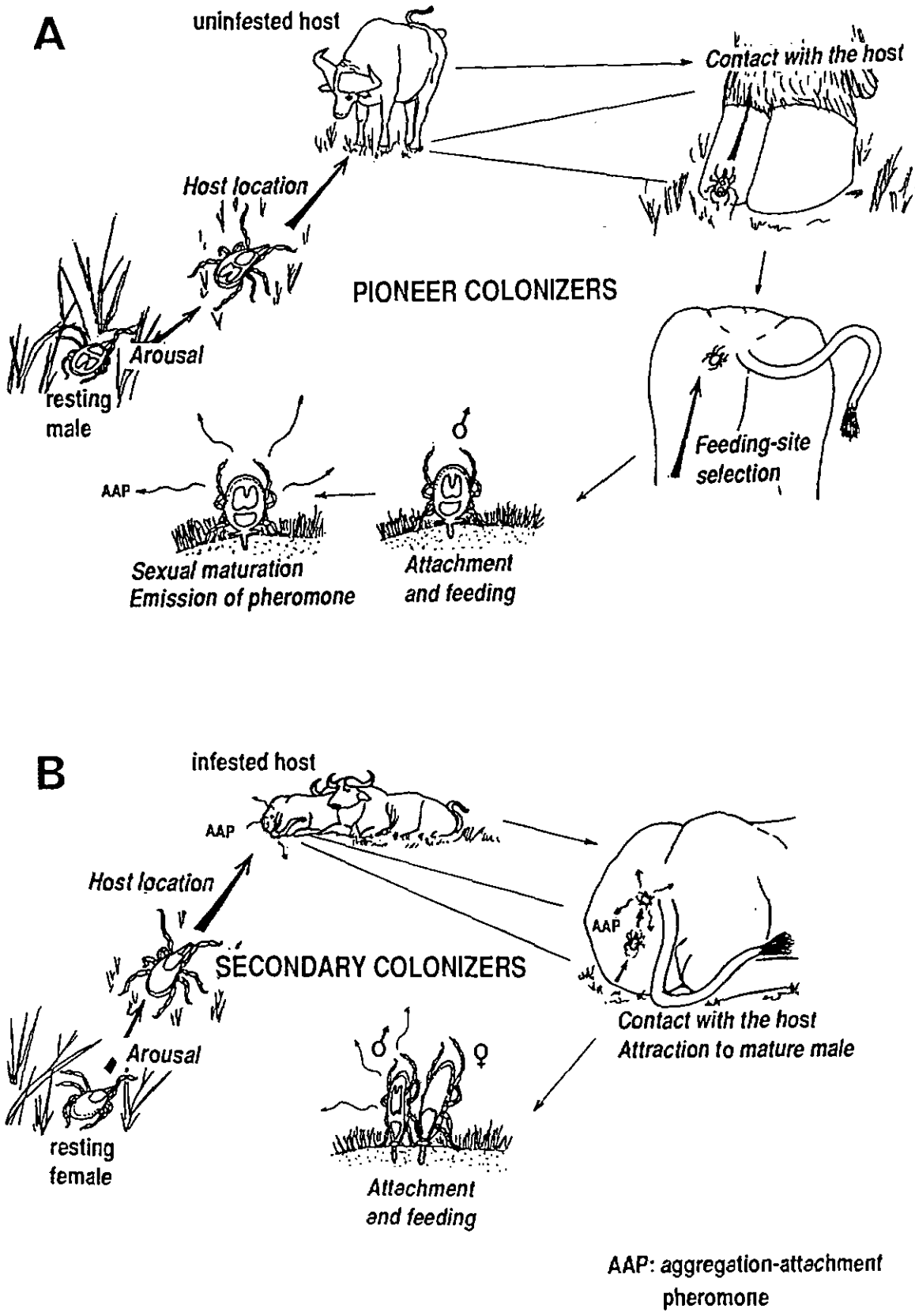

Fig. 1.2. Diagrammatic illustration of host-finding behaviour in adult *A. variegatum*.

the aggregation-attachment pheromone released by fed male *A. variegatum* and *A. hebraeum*. Diehl et al. (1991) found that the amount of pheromone emitted by a male of both species is considerable. Thus, a male *A. variegatum* can secrete in one hour more than 1 µg of 2-nitrophenol, one of the major component of the aggregation-attachment pheromone. The combination of host odours and the aggregation-attachment pheromone leads other members of at least some species of *Amblyomma* to a suitable host on which blood-feeding and mating can be accomplished (Fig. 1.2.). Nevertheless, for pioneer *Amblyomma* males, host odours are the only olfactory cues available during host-seeking. Studies on the sensitivity and the specificity of olfactory sense organs in *A. variegatum* should thus provide information on host-odour cues employed by this parasite.

I.4. Olfactory sense organs in ticks and *Amblyomma variegatum*

With the exception of special olfactory organs such as the sensilla placodea in honeybees (Boeckh et al. 1965), olfactory sense organs in arthropods are normally innervated setae with pore-walls. The latter favour diffusion of molecules from the air to the dendritic membrane of the olfactory receptor cell(s) within the sensillum via the receptor lymph. Sensillum ultrastructure and morphology reviewed by Altner and Prillinger (1980), and Zacharuk (1985), and olfactory transduction processes such as odour molecule diffusion, odour molecule-receptor affinity, enzymatic cascade, electrical events, adaptation and specificity of olfactory receptors have been most extensively investigated in insects (reviews by Kaissling 1986; Kaissling et al. 1987; de Kramer and Hemberger 1987; Vogt et al. 1987a and b; Lerner et al. 1990; Stengl et al. 1992). However, only some works on the ultrastructure of olfactory sensilla and only a few studies on receptor specificity have been published in ticks.

Odour-stimulated ticks often raise the first pair of legs in the air as insects do with their antennae. This behaviour soon suggested to acarologists that the first leg pair bears crucial sense organs. Haller (1881) first described a capsule equipped with numerous setae which he suspected to be a hearing organ on the tarsus of the first leg pair. However, further behavioural experiments in which different parts of tick appendages were amputated or masked revealed that the tarsus of the first leg pair was in fact the main site of odour perception (Hindle and Merriman 1912, Lees 1948). The morphology and ultrastructure of tarsal sense organs, and principally the Haller's organ in ticks have been intensively studied (i.e. Schulze 1941; Leonovitch 1979, 1980;

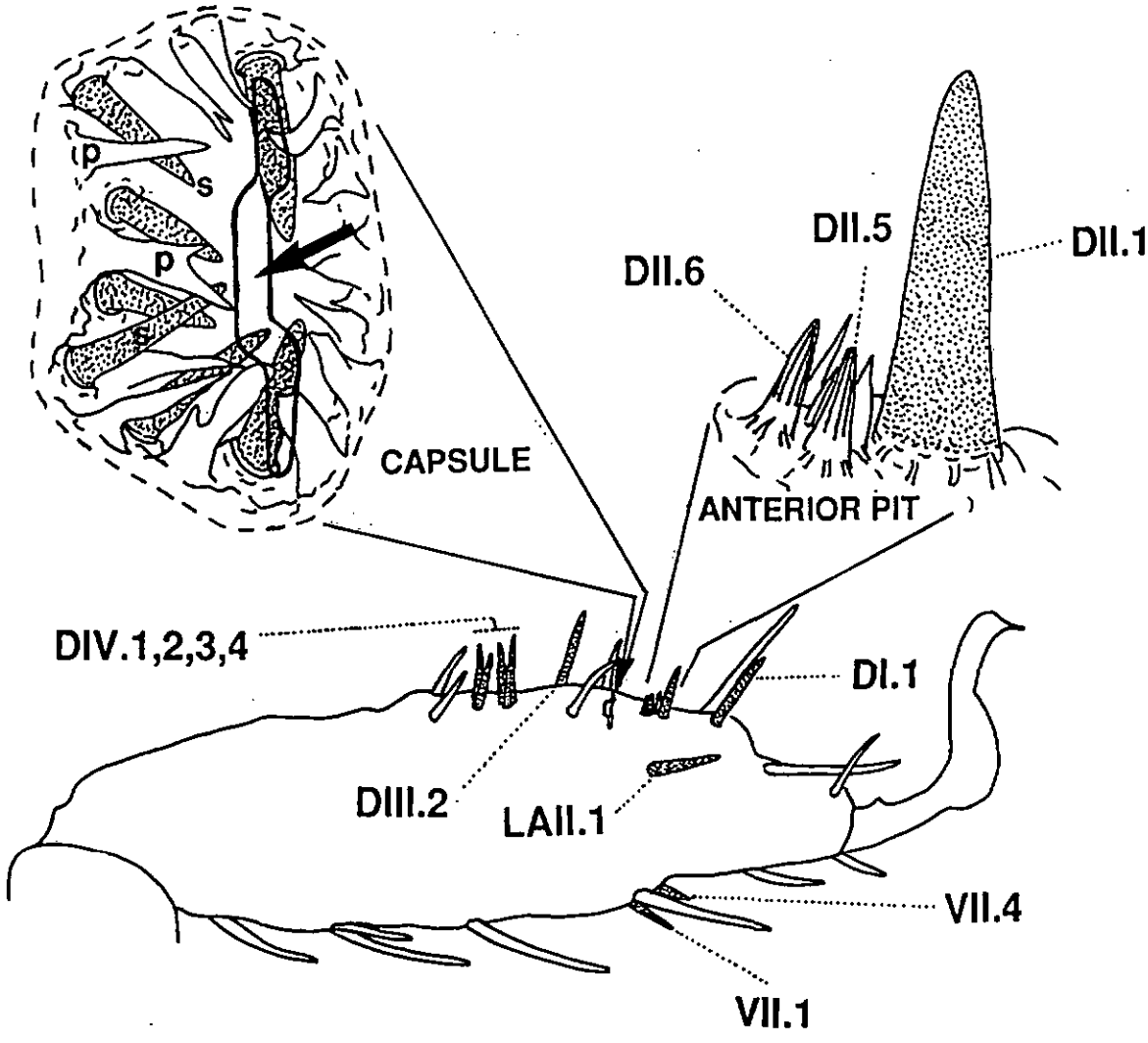

Fig. 1.3. Right tarsus of the first leg pair of an adult *A. variegatum* and location of the wall-pore sensilla with their respective names (according to the nomenclature proposed by Hess and Vlimant, 1982). A brief description of the morphological properties of each olfactory sensillum is given in the text. A detailed diagram of the anterior pit (lateral view) and the capsule (dorsal view) of Haller's organ is also provided. The arrow indicates the slit-like opening of the capsule. The seven wall-pore single walled sensilla (s) and cuticular pleomorphs (p) enclosed in the capsule are represented under the capsule opening.

Roshdy and Axtell 1972; Roshdy et al. 1972; Foelix and Axtell 1972; Waladde 1976, 1977). Haller's organ which contains almost half of all olfactory receptors of the tarsus (Hess and Vlimant 1986) has two dense groups of olfactory sensilla, the anterior pit and the capsule (Fig. 1.3.). Hess and Vlimant (1982, 1983, and 1986) described 5 different types of olfactory sensilla on the tarsus of *A. variegatum*, but none elsewhere (either on the other legs, mouthparts, or on

the idiosoma, Vlimant, personal communication). Three types of olfactory sensilla (wall-pore single-walled A and B; wall-pore double-walled C) are restricted to Haller's organ, whereas two other types (wall-pore double-walled A and B) are located on other parts of the tarsus. Adults and nymphs have 19 olfactory sensilla sharing a total of 68-94 suspected olfactory receptors per tarsus, while larvae possess only 14 olfactory sensilla with 57-77 olfactory receptors (Hess and Vlimant 1986). Furthermore, the number and location of tarsal sensilla is highly conserved between Ixodidae. However, primitive ticks possess slightly more olfactory sensilla, i.e. 20 in *Ixodes ricinus*, 19 in *A. variegatum*, and 17 in *Dermacentor marginatus* compared to 14 in *Boophilus microplus* (Hess and Vlimant 1986). Nevertheless, the total number of olfactory receptors is remarkably similar for the same life-stages of different tick species. Reduction of sensillum number results from the fusion of sensilla (Hess and Vlimant 1982). Figure 1.3. shows the position of each olfactory sensillum on the tarsus of an adult *A. variegatum* and a brief description of these sensilla is provided in the following section (for further details, see Hess and Vlimant 1982, and 1986).

1.5. Ultrastructure of olfactory sensilla in *Amblyomma variegatum*

1.5.1. Wall-pore single-walled A sensilla (L, N, A: 2)[1]

These sensilla basiconica (50-60 µm long) have relatively thick walls (0.6-1 µm thick) with a large number of plugged pores (0.1 µm diameter). One of the two present, the DI.1 sensillum, is located on the dorsal side distally from the anterior pit of Haller's organ (Fig. 1.3.) and includes 5 neurones with branched dendrites. The other, the DII.1 sensillum, belongs to the anterior pit (Fig. 1.3.) and is innervated by 3 sets of neurones (2 x 5 + 1 x 4) with branched dendrites. The latter is thought to result from the fusion of 3 separate sensilla. Indeed, each set of neurones possesses a thecogen cell and a trichogen cell, while the tormogen cells form a common outer receptor lymph cavity (Hess and Vlimant 1982). Interestingly, this fusion is not complete in the related species *A. americanum* where two wall-pore single-walled A sensilla are found in the anterior pit, one with two sets of neurones and the other with only one set of neurones (Foelix and Axtell 1971).

1 number of sensilla per tarsus in larvae (L), nymphs (N), and adults (A)

1.5.2. Wall-pore single-walled B sensilla (L: 4/ N. A: 7)

Wall-pore single-walled B sensilla, occasionally bifid, are deeply enclosed in a cavity, the capsule of Haller's organ (Fig. 1.2). Projecting from the floor or from the proximal or lateral cuticular walls of the capsule towards the slit opening, these sensilla have thin walls (0.08-0.14 µm tick) with wide plugged pores (0.1-0.16 µm) and are innervated by 3 to 5 neurones with dendrites showing various degrees of branching.

1.5.3. Wall-pore double-walled A sensilla (L: 2/ N. A: 4)

Called DIV, 1, 2, 3, and 4 respectively, these sensilla trichodea (35-40 µ m long) are located very close together on the dorsal side of the tarsus, proximally from the capsule (Fig. 1.3.). They are innervated by 1 or 2 neurones with unbranched dendrites and have grooved walls. Radial canals lead from the central cavity of the sensillum to narrow pores, situated in the longitudinal grooves.

1.5.4. Wall-pore double-walled B sensilla (L. N. A: 4)

These sensilla chaetica resemble the wall-pore double-walled A type but have two neurones forming a tubular body (mechanosensory units) and 4 to 7 neurones with unbranched dendrites. One of these sensilla (DIII.2) is located proximally from the capsule, another (LAII.1) is inserted on the lateral side, and the other two (VII.1 and 4) are found on the ventral side of the tarsus (Fig. 1.3.).

1.5.5. Wall-pore double-walled C sensilla (L. N. A: 2)

Both wall-pore double-walled C sensilla basiconica are confined to the anterior pit (Fig. 1.3.) and have walls with longitudinal but interrupted grooves. Three neurones with unbranched dendrites innervate these sensilla.

1.6. Specificity of tick olfactory receptors

Despite several studies, our current knowledge regarding specificity of tick olfactory receptors is rather limited and fragmented. Only a few receptors have been properly characterized. Receptors sensitive to 2,6-dichlorophenol, a tick pheromone component, have been identified in wall-pore single-walled A sensilla of *A. americanum* (Haggart and Davis 1981), and *A. variegatum* (Waladde 1982; Schoeni 1987), and in the wall-pore single-walled C sensillum of *I. ricinus* (Thonney 1987). Wall-pore single-walled A sensilla of *A. variegatum* also contain receptors which respond to 2-nitrophenol (Schoeni 1987) or to short-chain fatty acids (Hess and Vlimant 1980). Other receptors confined to the anterior pit are excited by NH_3 in *Rhipicephalus sanguineus* (Haggart and

Davis 1980), by breath and cattle wash in *B. microplus* (Waladde and Rice 1982). Furthermore, methylsalicylate, an aggregation-attachment pheromone component in *A. variegatum* (Schoeni et al. 1984), stimulates a receptor in the capsule of Haller's organ of *A. variegatum* (Hess and Vlimant 1986), but also in *I. ricinus* (Guerin, unpublished) and *B. microplus* (de Bruyne, unpublished). Other capsule receptors were found to respond to breath and CO_2 in *B. microplus* (Waladde and Rice 1982), while Sinitsina (1974) obtained responses to mouse odour, pentanoic acid, breath, but not to CO_2 in capsular receptors of *Hyalomma asiaticum*. Holsher et al. (1980) also reported presence of CO_2-excited receptor(s) with a tungsten electrode inserted proximally in the capsule of three tick species. Finally Haggart and Davis (1980) found a receptor sensitive to high concentrations of NH_3 in a wall-pore double-walled A sensillum of *R. sanguineus*. The latter is the only report of a response to an olfactory stimulant by receptors in wall-pore double-walled sensilla of ticks.

1.7. Outline of the present study

Although breath, mouse odour, and the pelage wash of cattle can stimulate receptors of some tick species, CO_2, NH_3, and short-chain fatty acids are currently the only vertebrate-associated volatiles known to stimulate tick olfactory receptors. Considering the total number of olfactory receptors present on the tarsus (68-94 in *A. variegatum*), it would be very surprising if no other host-odour compounds could be detected. Although Haller's organ is generally considered as the main site of host-odour detection, the exact location and number of host-odour receptors are not known. The present work aims to systematically investigate the specificity of olfactory receptors of *A. variegatum*, an ideal tick model for such a study. The morphology and ultrastructure of olfactory receptors in this tick species are well known (Hess and Vlimant 1982, 1983, and 1986), the size of *A. variegatum* (up to 1 cm) renders electrophysiological preparation relatively easy, and olfaction plays a primary role in host-finding for this hunter tick. This study should permit identification of different classes of vertebrate-associated volatiles perceived by *A. variegatum* and allow us to understand how finely tuned the olfactory system of *A. variegatum* is to the odour bouquet of various vertebrates. Finally, the host-odour stimulants identified will be considered as behavioural cues in host-finding.

II. METHODS

In this chapter, only the main principles of the Methods used are described. Further details are provided in the corresponding publications presented in chapter 3.

2.1. Animals

Male of *A. variegatum* were principally used because of their pioneer role in parasiting hosts. Rearing conditions are described in chapter 3.1..

2.2. Electrophysiology (chapters 3.1., 3.2., 3.3., 3.4.)

Since Morita and Yamashita's pioneering work on *Bombyx* (1961), classic electrophysiology methods have been used in arthropods to pick up such electrical events as receptor potentials and action potentials generated at the level of olfactory receptors for the study of their specificity and sensitivity. In the present work, electrical activity of tick olfactory receptors has mostly been captured by contacting the cut tip or the pored wall of a sensillum with a glass electrode. A tungsten electrode inserted into the receptor lymph at the base of a sensillum was also employed for specific purposes in this study (see chapter 3.1.). Glass electrodes show several advantages over tungsten electrodes. A recording glass electrode permits recording of shifts in potential and action potentials associated with receptor cells, whereas a tungsten electrode will cut off lower frequencies (slow potential shifts). Furthermore, receptor cells can be more easily destroyed by tungsten than by glass electrodes

Typically, glass electrodes are glass capillaries filled with an electrolyte solution which is electrically connected via Ag/AgCl wires to the amplification circuit. Small voltage changes between the reference glass electrode (ground) inserted in the tick haemolymph and the recording electrode in contact with the sensillum were thus amplified (10 to 1000x) via a 10^{12} Ohm high input impedance preamplifier and an AC/DC amplifier. Signals were then displayed on an oscilloscope, printed on paper, stored on video tapes, or fed to a computer for further analysis (Fig. 2.1.). In many cases, analysis of spike shapes and amplitudes (extracellular recordings of action potentials generated by each receptor cell in response to stimulation with volatiles) permits characterization of the sensitivity and specificity of each responding receptor cell of a sensillum. Indeed, spike shapes and amplitudes generated by the separate receptor cells in the same sensillum often differ. Specific passive properties (impedance and capacitance) of the electrical circuitry within the

sensillum (through receptor cells and accessory cells) may indeed contribute to the shape and amplitude of the extracellularly recorded spikes (de Kramer 1985; Rumbo 1989).

2.3. Stimulation (chapters 3.1., 3.2., 3.3., 3.4.)

Air from a cartridge containing volatiles was briefly swept via electrically controlled valves into a charcoal-filtered air stream leading to the tick preparation. Shifts in potential and spike frequency changes of the olfactory receptors resulting from stimulation were analysed. The specificity of the receptors was tested with a series of well-known vertebrate-associated volatiles representing different chemical classes (CO_2, CH_4, NH_3, acetone, 4-heptanone, γ-butyrolactone, γ-valerolactone, pentanol, 1-octen-3-ol, 4-methylphenol, hexanal, propanoic acid, butanoic acid, 2-methylpropanoic acid, 3-methylbutanoic acid, pentanoic acid, heptanoic acid, L-lactic acid,), with some tick pheromone components (nonanoic acid, 2,6-dichlorophenol, 2-nitrophenol, methylsalicylate), some other volatiles (3-pentanone, 6-caprolactone, 1-octene, octylamine, hexyl acetate) and with vertebrate odours (human breath, human axillary secretions, bovine breath, bovine skin wash, odours collected around steers and rabbits). Vertebrate-associated volatiles were collected on a charcoal trap (for breath), on the porous polymer adsorbent Porapak Q (for breath and odours of steers and rabbits, see Fig. 2.2.), or in a cold trap (for headspace over cotton pads used to wash bovine skin, see Fig. 2.3.) and extracted with solvent for use as host-odour stimuli. Breath as well as cotton pads soaked with human axillary secretions were also introduced into the stimulus cartridge and used directly as stimuli.

2.4. Gas chromatography-coupled electrophysiology (chapters 3.2., 3.3., 3.4.)

Whenever a host-odour extract stimulated receptor(s), the next step consisted of identifying the stimulant(s) among the numerous constituents of the extract. These volatiles were separated by gas chromatography on a high-resolution capillary column coupled to an electrophysiological preparation of a tick olfactory sensillum, thus permitting observation of the effect of each component of the extract on the activity of the receptors (Wadhams 1982). Figure 2.4. provides a diagram of the set-up.

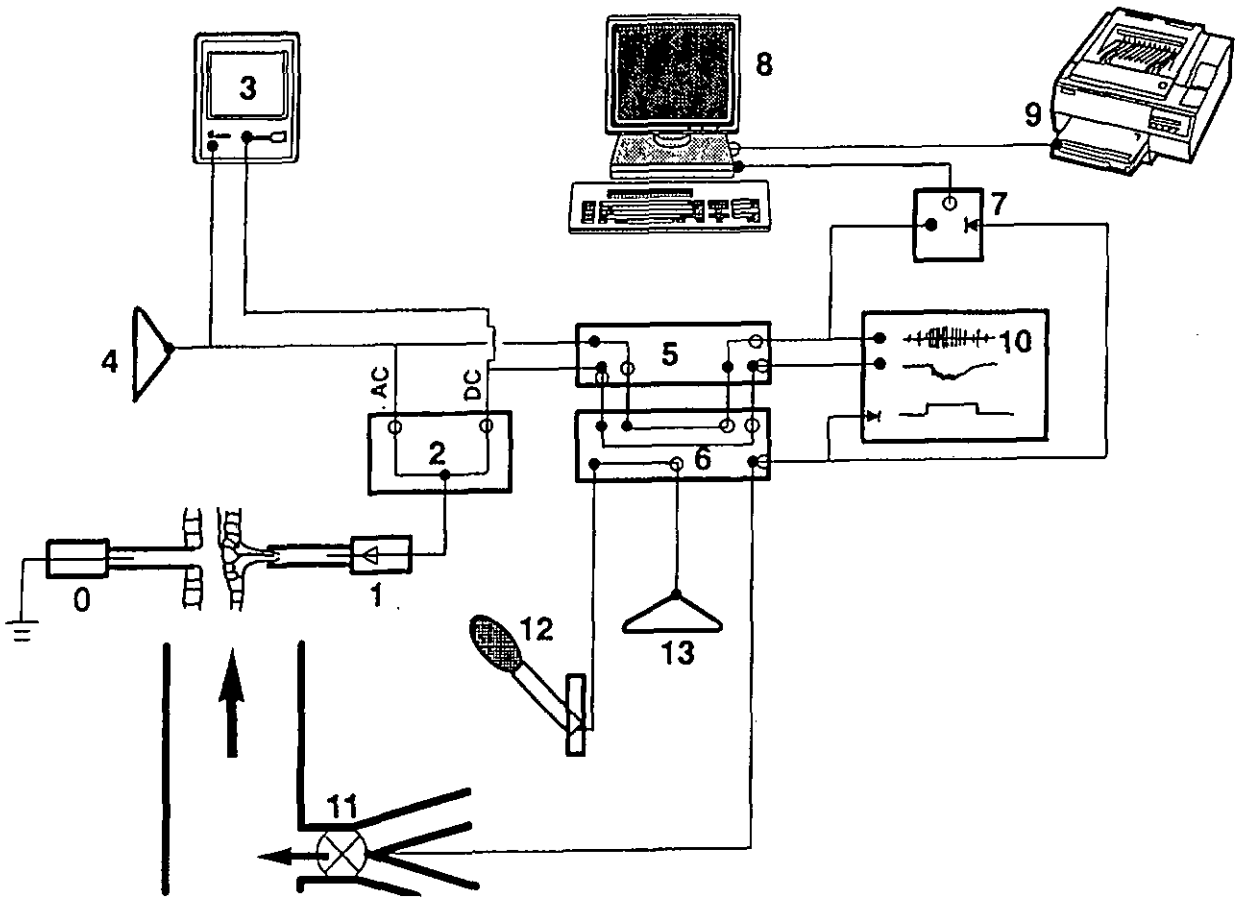

Fig. 2.1. Diagram of the electrophysiology set-up used to study olfactory receptors of *A. variegatum*. (0): reference glass electrode impaled in the coxa of one of the first legs. (1): Recording glass electrode in contact with the cut tip of the sensillum. The electrode is electrically connected via an Ag/AgCl wire to a high input impedance preamplifier (symbolized by a triangle). (2): Universal AC/DC amplifier with separate outputs for the AC and DC parts of the recorded signal. (3): Oscilloscope to visualize the AC and DC signals. (4): Loudspeaker to render spikes audible in the AC signal. (5): PCM-1 Digital-VCR instrumentation recorder adaptator for analogue/digital or digital/analogue conversion of the AC and DC signals. (6): Video tape recorder used to store digitized signals and to play them back for further analysis via digital/analogue conversion in (5). (7): "Go-Box" which contains the circuitry necessary to provide the trigger to start digitization on the computer of the analogue AC signal played back from the video tape recorder via (5). (8): IBM-compatible computer equipped with an analogue/digital board to digitize the analogue AC signal and with software (SAP1D tools) to visualize and analyse the digitized signal. (9): Laser printer to print signals and analyses. (10): Recorder to print signal. (11): Stimulus delivery controlled by solenoid valves. The stimulation period is indicated by a pulse signal which can be stored on an audio channel of (6) and used subsequently as a trigger signal, i.e. in (7) or in (10). (12): Microphone used to add comments stored on the second audio channel of (6). (13): Loudspeaker to listen to comments during play-back. *Bold circles*: inputs to each instrument; *open circles*: outputs of each instrument; *bold triangles*: trigger inputs.

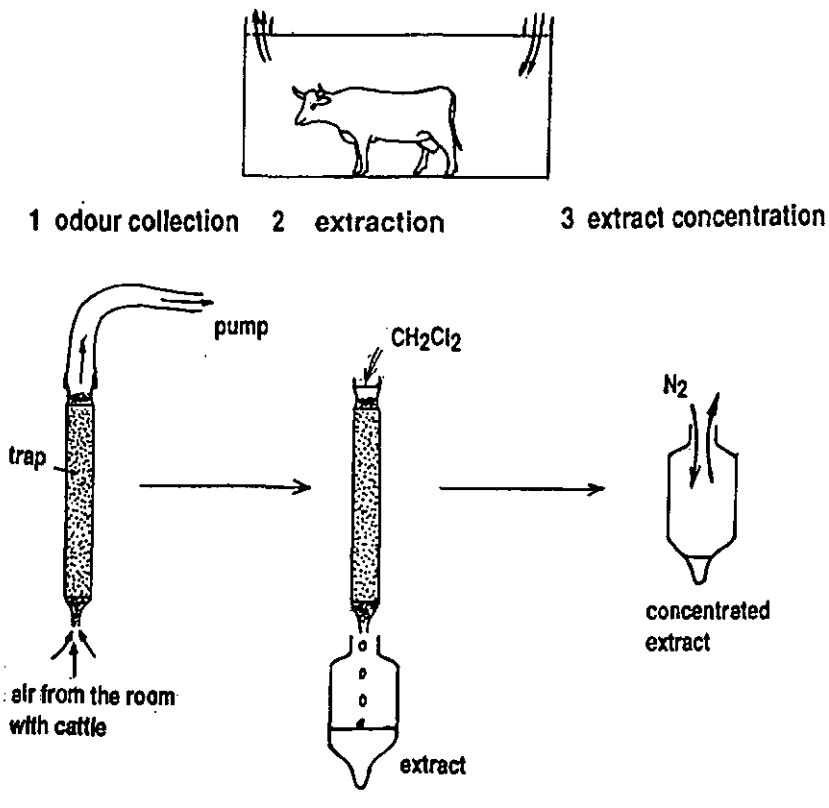

Fig. 2.2. Odour collection using adsorbent material (charcoal or Porapak Q). This method was used to collect volatiles in the air of rooms with either cattle or rabbits.

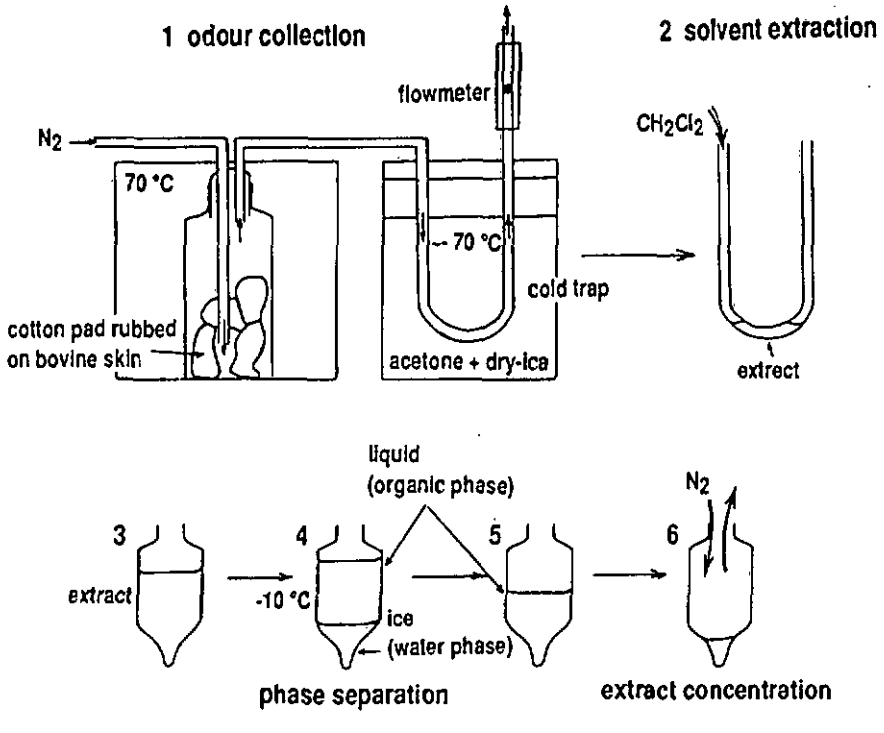

Fig. 2.3. Odour collection using a cold trap. This method was used to collect volatiles from cotton pads rubbed on bovine skin (skin wash).

2.5. Gas chromatography-coupled mass spectrometry (chapters 3.3., 3.4.)

Chemical analysis of the active component(s) of an extract, located by gas chromatography-coupled electrophysiology, was then tackled with gas chromatography-coupled mass spectrometry. After separation of the extract components by gas chromatography, the mass spectrum of the active component(s) (located on the chromatogram with reference to its Kovat's index[1]) was then analysed. Identification of the unknown was based on the match of its mass spectrum with that of a known product stored in a computer-based library. The mass spectrum and the calculated Kovat's index of the unknown were then compared with those of the library-proposed synthetic analogue, injected under the same conditions. Finally, identification was confirmed by testing the electrophysiological activity of the synthetic substance on the relevant receptor.

2.6. Behavioural bioassays (chapters 3.1, 3.2., 3.3., and discussion)

Behavioural bioassays were designed to investigate some identified olfactory stimulants, namely breath stimulants (CO_2, and H_2S), as behavioural cues for adult *A. variegatum*.

2.6.1. Wind tunnel (chapter 3.1.)

Resting adult *A. variegatum* were placed in a wind tunnel and then stimulated with CO_2 as described in chapter 3.1.. Activation as well as positive anemotaxis were observed and quantified as described in chapter 3.1..

2.6.2. Activation bioassay (chapter 3.2.)

Resting adult *A. variegatum* were enclosed in glass flasks continuously swept with charcoal-filtered and humidified air. Every 24 hours, ticks are confronted with short stimulations of two olfactory stimulants identified by electrophysiology, i.e. CO_2, H_2S, and mixture of both. The tick's degree of

[1]The Kovat's index indicates the retention characteristic of a product on a column with respect to n-alkanes. It is expressed as following:

$$I = 100[\, n\{(\log V^x - \log V^c)/(\log V^{c+n} - \log V^c)\} + c]$$

where V^x is the retention time of the unknown compound, V^c is the retention time of the alkane eluting before the unknown, V^{c+n} is the retention time of the alkane eluting after the unknown, c is the carbon number of the alkane eluting before the unknown, n refers to the difference in the number of carbon atoms for the two n-alkanes used as reference.

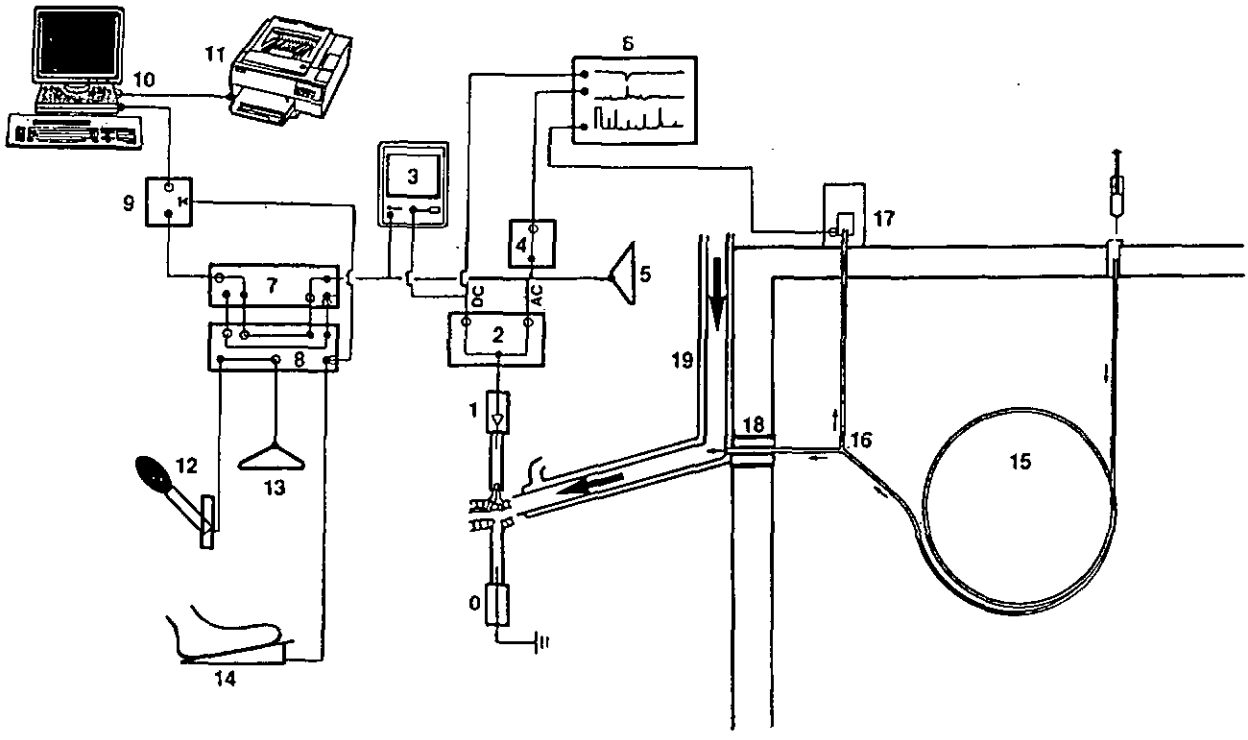

Fig. 2.4. Diagram of the gas chromatography-coupled electrophysiology set-up (GC-EL). (0): Reference glass electrode impaled in the coxa of one of the first legs. (1): Recording glass electrode in contact with the cut tip of the sensillum. The electrode is electrically connected via an Ag/AgCl wire to a high input impedance preamplifier (symbolized by a triangle). (2): Universal AC/DC amplifier with separate outputs for the AC and DC components of the recorded signal. (3): Oscilloscope to visualize AC and DC signals. (4): Window discriminator apparatus and frequency/voltage converter to sort spikes from background noise of the AC signal and to transform the frequency of the spikes into a DC voltage. (5): Loudspeaker to render spikes audible in the AC signal. (6): Chart recorder which printed the DC signal, the frequency to voltage converted signal, and the response of the chemical detector (17) on separate channels. (7): PCM-1 Digital-VCR instrumentation recorder adaptator for analogue/digital or digital/analogue conversion of the AC signal. (8): Video tape recorder used to store the AC signal and to play it back for further analysis via digital/analogue conversion in (7). (9): "Go-Box" which contains the circuitry necessary to provide the trigger to start digitization on the computer of the analogue AC signal played back from the video tape recorder. (10): IBM-compatible computer equipped with an analogue/digital board to digitize analogue AC signal and software (SAPID tools) to visualize and analyse the digitized signal. (11): Laser printer to print signals and analyses. (12): Microphone used to add comments stored on an audio channel of (8). (13): Loudspeaker to listen to comments during play-back. (14): Pulse signal stored on the second audio channel of (8) which is used to mark stimulation period and to subsequently trigger via (9) the digitization of the analogue AC signal in the computer. (15): High-resolution gas capillary column enclosed in the oven of a gas chromatograph. (16): Splitter delivering the column effluent (*thin arrow*) to the chemical detector (17) and the electrophysiological preparation via the heated transfer line (18) of the chromatograph. (19): Water-jacketed glass tube to maintain the humidified main air-stream (*large arrow*) flowing over the preparation at a constant temperature. *Bold circles*: inputs to each instrument; *open circles*: outputs of each instrument; *bold triangles*: trigger inputs.

arousal is evaluated immediately after each stimulation as described in chapter 3.2..

2.6.3. Locomotion compensator (Kramer's sphere) (chapter 4.6.)

Preliminary experiments on the triggering and maintenance of orientation in excited male *A. variegatum* by CO_2, H_2S, and mixture of both were undertaken. Only the most relevant results of these behavioural bioassays are given in chapter 4.6. and briefly discussed. As it is not described elsewhere, details of the method and procedure employed for these preliminary experiments are provided here.

A breath-excited tick was placed on the top of a sphere (50 cm diameter) which rotated so that the animal was always kept at the sphere north pole. A light beam perpendicularly illuminated the sphere pole (field diameter: 3 cm). A small retroflective foil (n° 7610, 3M, Switzerland), glued to the dorsal side of the tick, reflected light back to a sensor which continuously evaluated the deviation of the reflective foil from the sphere pole. According to these deviation evaluations, signals were sent to two servomotors placed orthogonally on the equator of the sphere to recenter the reflective foil and hence the tick (Kramer 1976, Rickli et al. 1992). Displacement of the sphere was registered by two incremental pulse generators with a resolution of 0.1 mm. The X and Y coordinates of the tick's position were sampled at 0.1 s intervals and fed into a computer for track analysis. The sphere was enclosed in a styropore chamber to isolate the experimental area from the open laboratory. An upright aluminium cylinder (12 cm diameter, 11 cm high) with an upwind and a downwind aperture surrounded the north pole of the sphere to render the environment near the tick uniform. Experiments were accomplished during the last 6 hours of light of the daily cycle (L:D 12:12) with unfed 6-7 month old males. Breath-excited individuals were placed on the sphere and allowed to walk for 2 min. Animals which regularly stopped were discarded. Walking responses were then recorded for 90 consecutive s divided into 30 s prestimulation control followed by 30 s of stimulation and a further 30 s poststimulation. The tick behaviour was also recorded on video for further analysis with a CCD camera focused on the tick.

A humidified charcoal-filtered air stream was directed at the north pole of the sphere in a 3.6 cm diameter water-jacketed stainless steel tube to provide a constant air flow of 0.1 m/s at 25 ± 1°C and 70 ± 5% RH. Stimuli carried by solenoid-activated air flow(s) were added to the main air stream at 14 cm from the outlet of the tube situated at 3 cm from where the tick walked. H_2S

stimulation was provided by air (120 ml/min) which passed through a 50-ml gas-wash flask with 1 ml of an aqueous solution of Na_2S (1-100 mg/ml). CO_2 stimulation was made with various additions of a flow from a 5% CO_2/95% N_2 gas cylinder which passed through a 50-ml gas-wash flask with 1 ml distilled water added to provide concentration of 0.15-0.6% CO_2 at the north pole of the sphere. CO_2/H_2S stimulation was made with synthetic 5% CO_2/95% N_2 from a gas cylinder mixed with H_2S vapours from the flask described above. Polluted air was evacuated via a funnel placed 20 cm downwind from where the tick walked and cleaned over charcoal.

III. RESULTS

3.1. Perception of breath components by the tropical bont tick, *Amblyomma variegatum* Fabricius (Ixodidae). I CO_2-receptors. J. Comp. Physiol. A (1992) 170:665-676

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

3.3. Identification of vertebrate volatiles stimulating olfactory receptors on tarsus of the tick *Amblyomma variegatum* Fabricius (Ixodidae). I Receptors within the Haller's organ capsule. J.Comp. Physiol. A (in press)

3.4. Identification of vertebrate volatiles stimulating olfactory receptors on tarsus of the tick *Amblyomma variegatum* Fabricius (Ixodidae). II Receptors outside the Haller's organ capsule. J.Comp. Physiol. A (in press)

J Comp Physiol A (1992) 170:665–676

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

I. CO_2-excited and CO_2-inhibited receptors

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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

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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

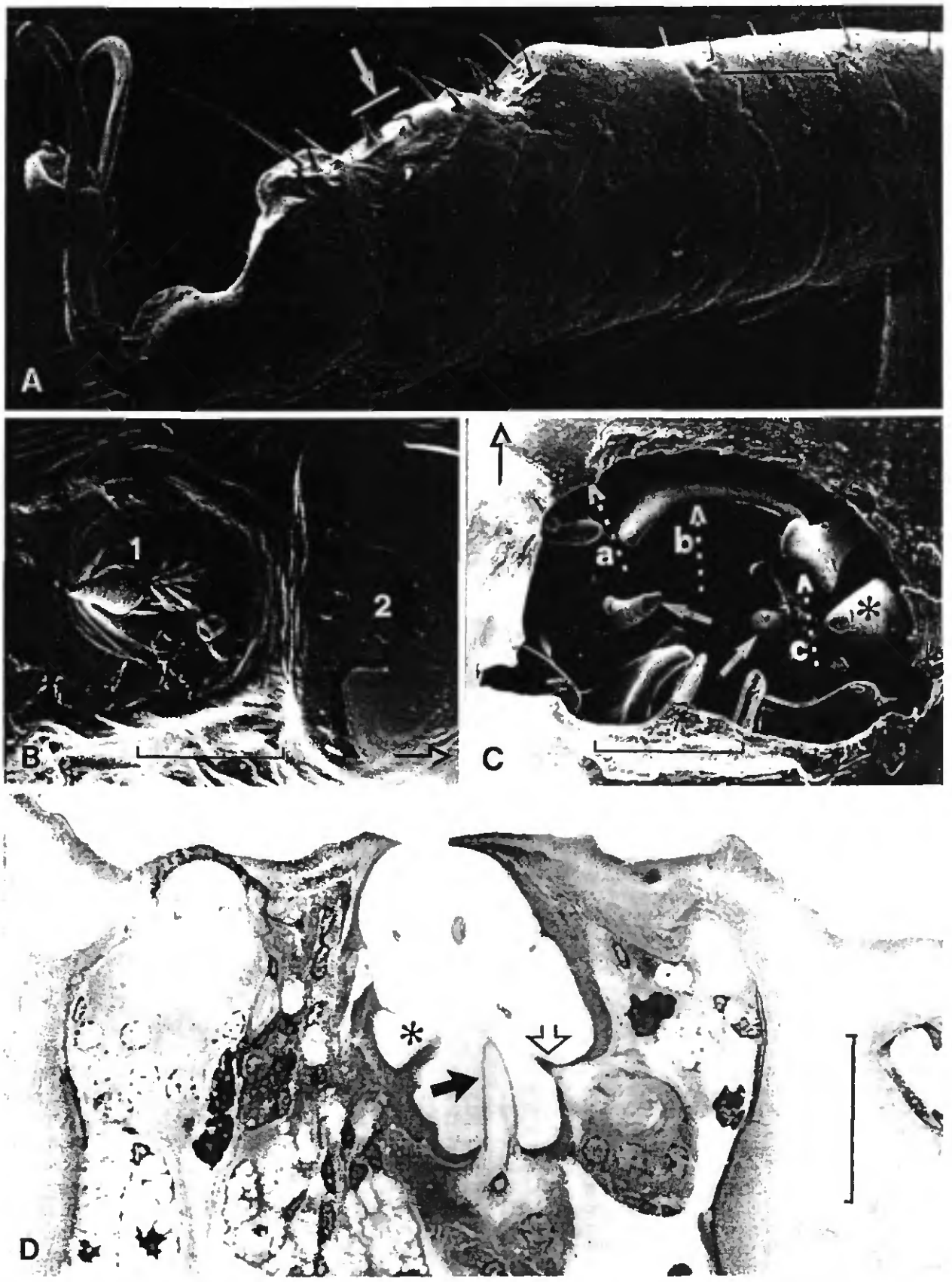

Fig. 1. A Tarsus of the foreleg of a female *Amblyomma variegatum*. The *white arrow* shows Haller's organ on the dorsal side with olfactory sensilla and the opening of the capsule; scale bar: 200 µm. B Haller's organ with the group of anterior pit sensilla (*1*) and opening of the capsule (*2*); scale: 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.

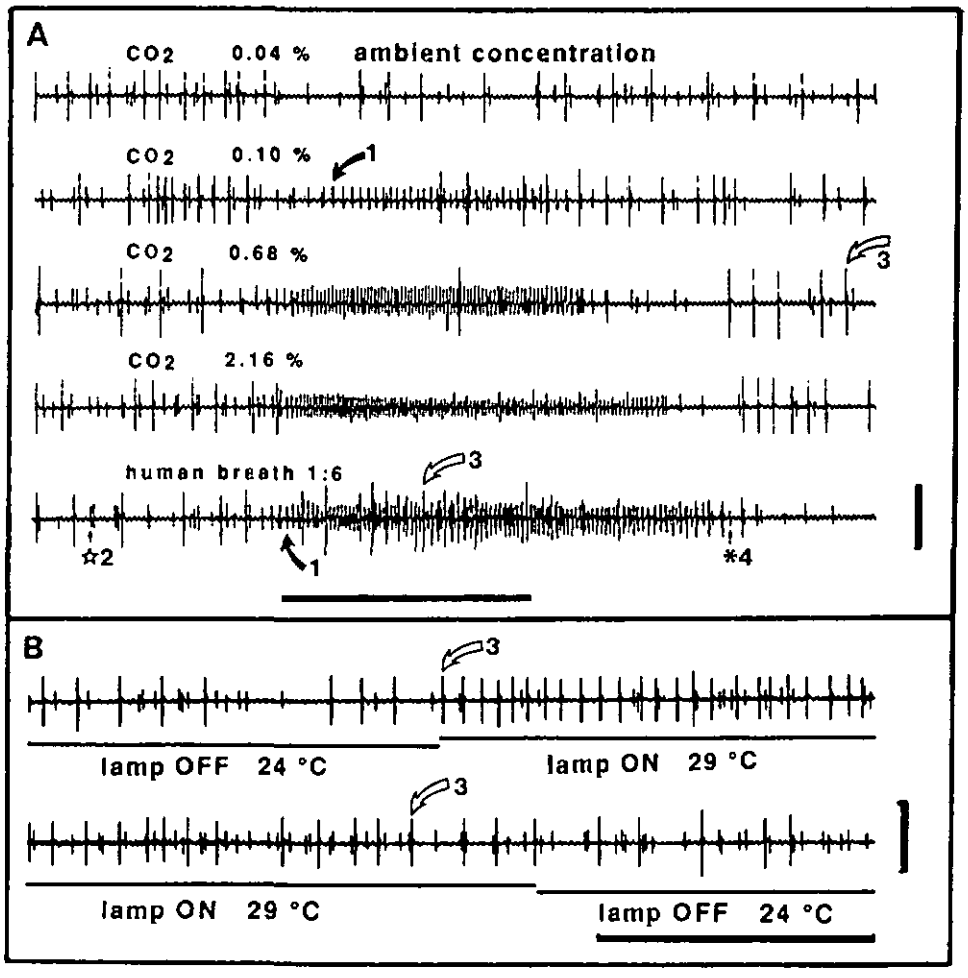

Fig. 2. A Representative responses of a capsular sensillum bearing a CO_2-excited receptor of a male *A. variegatum* (small biphasic spike 1 with *bold arrow*) to increasing CO_2 concentrations and diluted human breath. Stimuli were injected into the stimulus-delivery-tube 25 cm from its outlet, so that 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

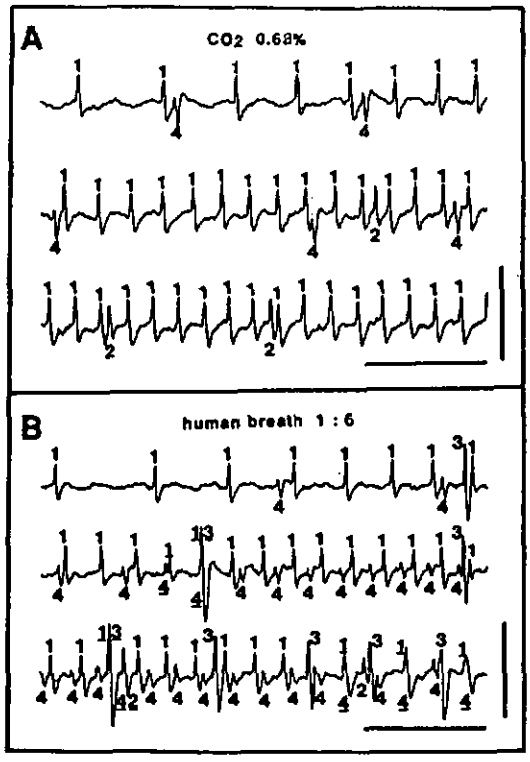

Fig. 3A, B. Detailed sections from the recordings illustrated in Fig. 2 to show how the 4 spike categories were discriminated visually. A Recording from the first 750 ms of stimulation with 0.68% CO_2, and B recording from the first 750 ms of stimulation with human breath diluted 1:6 in air (ca. 0.6% 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

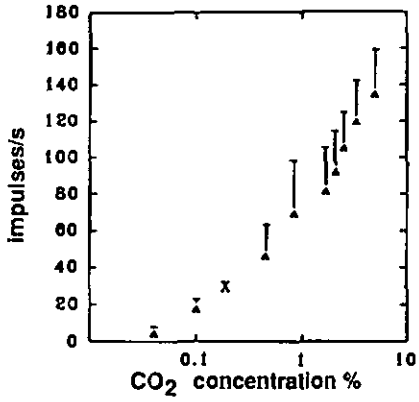

Fig. 4. Dose-response curve of the CO_2-excited receptor of male *A. variegatum* established from the first 160 ms of the response (phasic part). Preparations were maintained in a humidified air stream at ca. 0.04% CO_2 into which CO_2 stimuli were injected 3 cm from the outlet of the tube to the preparation. 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

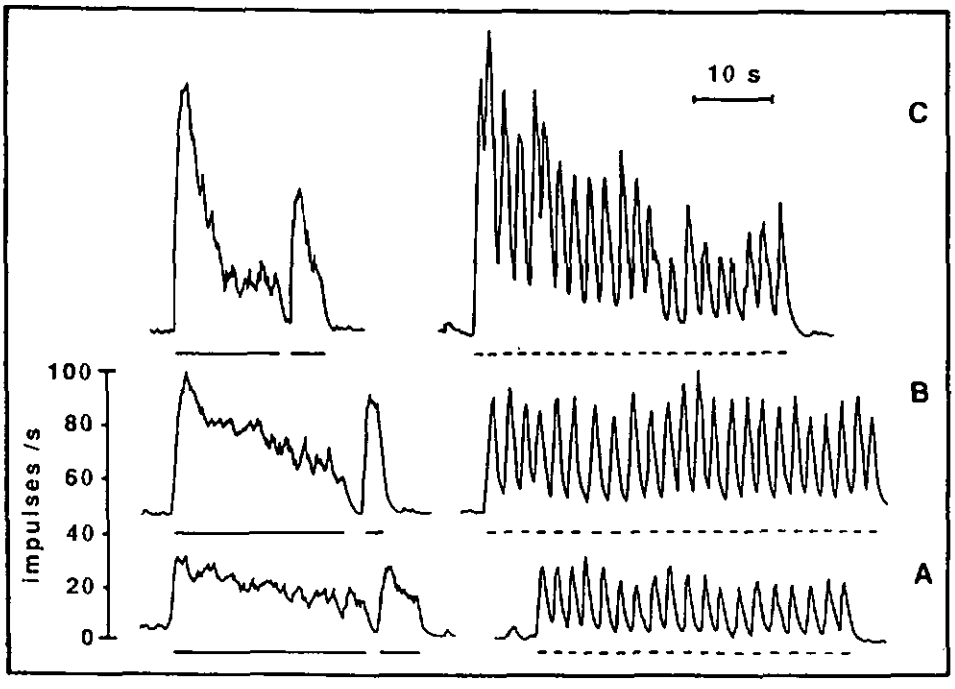

Fig. 5A–C. *Amblyomma variegatum* CO_2-excited receptor response under continuous and repetitive 1 s stimulation: A 0.7%, B 1.2%, and C 1.8% CO_2. *Horizontal bars:* stimulus duration. The preparation was maintained in a humidified air stream at 0.04% CO_2 into which stimuli were injected. These response 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-

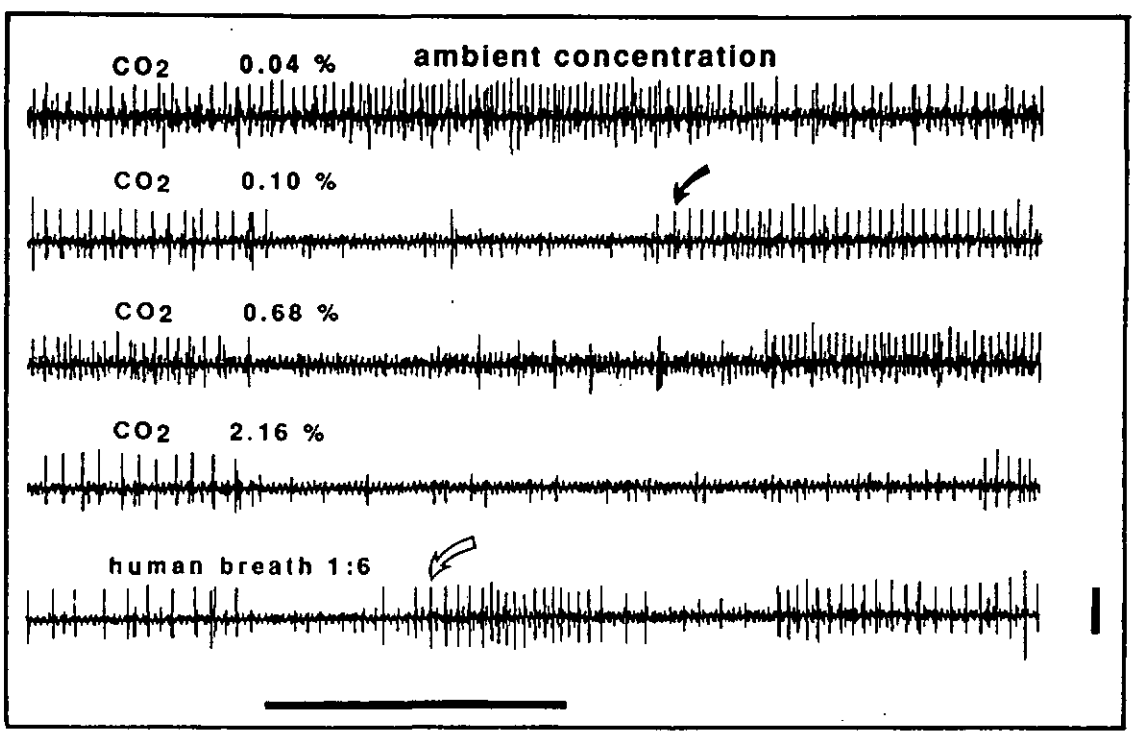

Fig. 6. Representative responses of a capsular sensillum of a male *A. variegatum* bearing a CO_2-inhibited receptor *(bold arrow)* to increasing CO_2 concentrations and diluted human breath. The preparation was maintained in a humidified air stream at ca. 0.04% CO_2 into which stimuli were injected 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

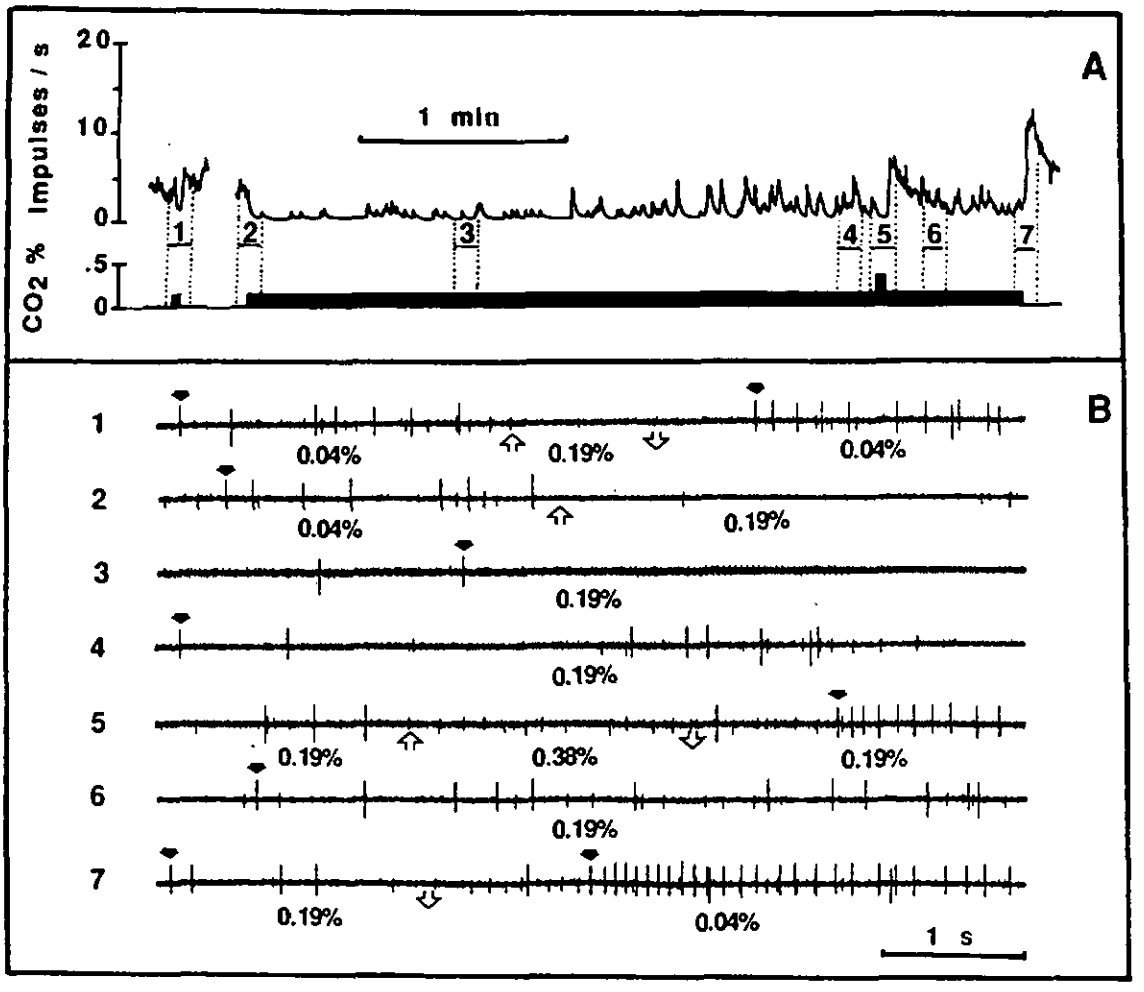

Fig. 7A, B. Adaptation of a CO_2-inhibited receptor *(bold arrow)* of a male *A. variegatum* at different CO_2 concentrations. A *Upper trace:* response profile of the CO_2-inhibited receptor, obtained by frequency to voltage conversion of the AC signal after sorting spikes of the CO_2-inhibited receptor 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

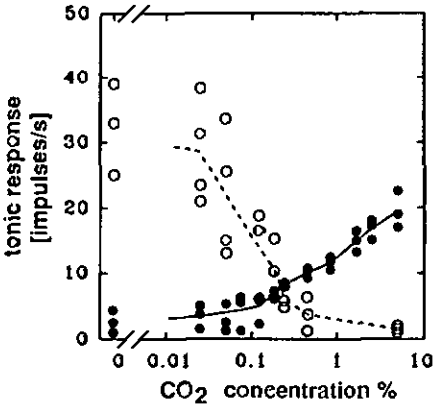

Fig. 8. Dose versus tonic response relationship for both the CO_2-excited *(solid circles)* and the CO_2-inhibited receptors *(open circles)* of male *A. variegatum*. The tonic activity was calculated from a 4000 ms spike train after 2 min of exposure of the receptor to the different CO_2 concentrations, 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

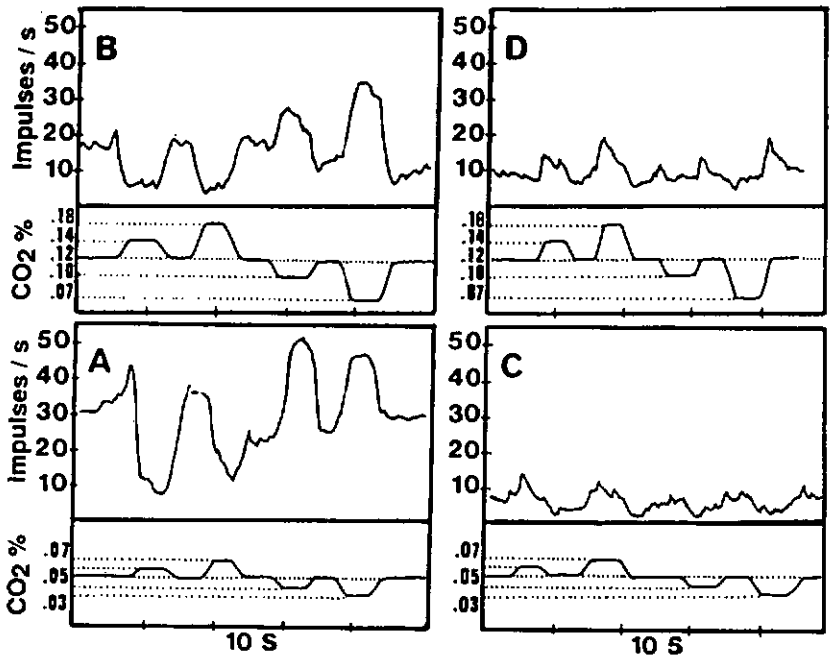

Fig. 9A–D. Modulation of the spike frequency of both a CO_2-inhibited receptor (A and B) and a CO_2-excited receptor (C and D) of a male *A. variegatum* by small changes in CO_2 concentration around 0.05% (A and C) and around 0.12% (B and D) in a dry synthetic air stream. Changes in CO_2 concentration 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

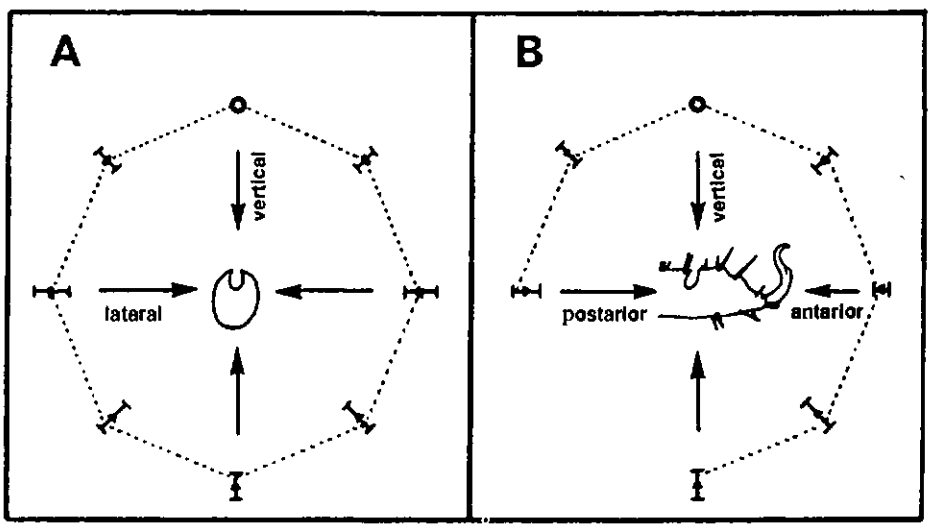

Fig. 10A, B. Field of perception of the CO_2-excited receptor in the capsule of Haller's organ established by the response of the sensilla housing this receptor in 6 female *A. variegatum* to a CO_2 stimulus coming from differeot directions around the tarsus. Responses (mean ± SD) are given as a percentage 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.c. 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

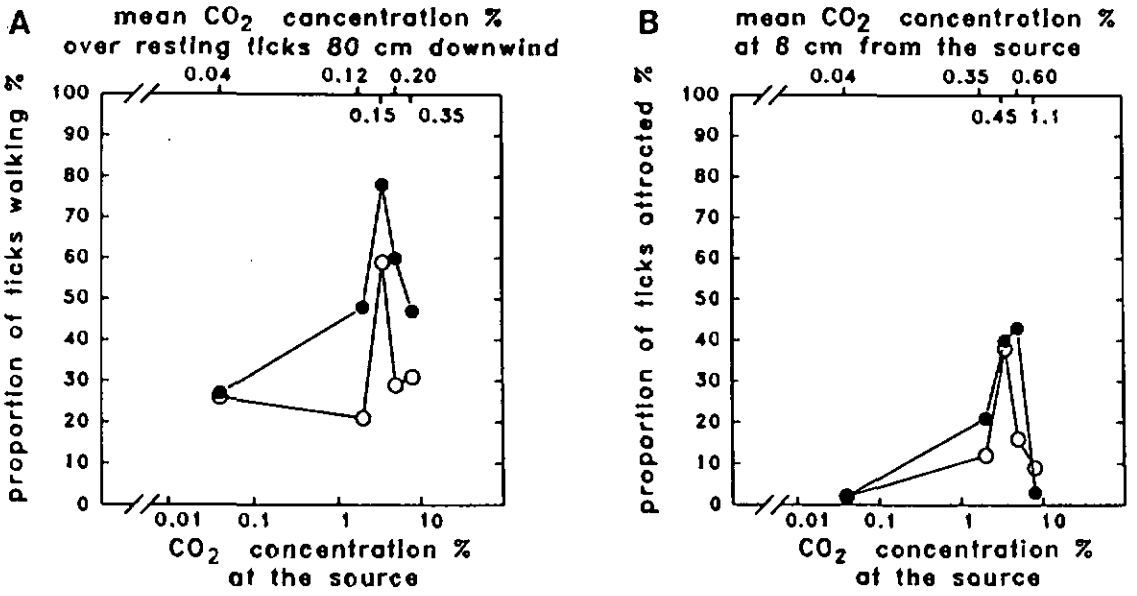

Fig. 11A, B. Behavioural response of male *(solid circles)* and female *(open circles) A. variegatum* to various CO_2 concentrations in a wind-tunnel. A Locomotor stimulant effect of various CO_2 levels as measured by proportion of resting ticks induced to commence walking. *Lower abscissa* the CO_2 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.

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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 oegypti*. 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 sovigny* (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 *Glossino morsitons 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 calcitrons* (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

J Comp Physiol A (1992) 170:677–685

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

II. Sulfide-receptors

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Summary. Wall-pore sensilla in the capsule of Haller's organ on foreleg tarsi of the tick, *Amblyamma variegatum*, show multicellular responses upon stimulation with human and bovine breath. Filtering breath through charcoal removes the stimulant for some of these receptors. Analysis by gas chromatography coupled with olfactory sensillum electrophysiological recordings indicates that an ethanol extract of the breath components trapped on charcoal contains a major stimulant eluting at the same retention time as H_2S. Two types of H_2S-sensitive receptors have been identified. They are housed in separate sensilla, and are called sulfide-receptor 1 and 2.

Although, both receptor types are characterized by a high sensitivity to H_2S with an estimated threshold of ca. 0.1 ppb and a response range covering 5–6 log orders of magnitude, their overall response to sulfides and mercaptans is nevertheless dissimilar. The type 1 receptor fires slightly more upon stimulations with H_2S than type 2, whereas ethylmercaptan induces a stronger response from type 2, and dimethyl sulfide activates only receptor 2.

In a bioassay, H_2S tested at concentrations of ca. 0.02 ppm and 1 ppm equally arouses 60% of resting ticks. Two-thirds of these ticks quest the air with their first pair of legs, and the remainder start active search. By contrast, H_2S at ca. 1 ppm in a mixture with CO_2 severely diminishes the locomotor stimulating effect of CO_2.

Key words: Tick – Host-finding – Vertebrate breath – Hydrogen sulfide – Sulfide-receptors

Introduction

Several investigators have demonstrated that volatiles of vertebrate origin, other than CO_2, act as cues for host-finding by haematophagous anthropods. Despite this, chemical analysis of breath components has been made only in a few cases; 1-octen-3-ol and acetone in cow breath are used for host-location in tsetse flies (Hall et al. 1984; Vale and Hall 1985). Furthermore, octenol and/or acetone improve attractiveness of CO_2 in other blood-sucking Diptera (Culicidae: Kline et al. 1990; *Stomoxys calcitrans:* Warnes and Finlayson 1985a, b; Tabanidae: French and Kline 1989). It is still not clear whether breath components other than CO_2 elicit a response in ticks, although human subjects and CO_2 traps attract roughly the same numbers of *Amblyamma americanum* in the field (Mount and Dunn 1983). Besides, 1-octen-3-ol and acetone do not attract *Amblyomma hebraeum* (Norval et al. 1987). However, there may be other vertebrate associated volatiles which are relevant for *Amblyomma* sp. Indeed, studies in this laboratory on all wall-pore sensilla on the tarsus of the foreleg of *Amblyomma variegatum* have revealed that some olfactory cells in Haller's organ respond to components of vertebrate odour (Steullet, unpublished). Three other sensilla in the capsule of Haller's organ also bear cells which are stimulated by breath (multicellular responses). One of the activated cells is a CO_2-excited receptor, another a CO_2-inhibited receptor (Steullet and Guerin 1992), and the response of two other cells to sulfur components of breath is described here.

Materials and methods

Ticks and electrophysiology

Experiments were undertaken for the most part with unfed *Amblyomma variegatum* males, nevertheless, some unfed females were also used for comparison. Rearing methods, preparation of ticks, electrophysiological set-up, as well as stimulus-delivery system are already described in Steullet and Guerin (1992).

Stimulants

Human breath. Human breath was blown via the needle connexion into the barrel of a 5-ml polypropylene syringe whose other end

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bore a rubber stopper, and was immediately used as stimulus. Breath was collected as a rule in the morning.

Bovine breath. A personal sampling pump (SKC Inc., USA) sucked air into a Tedlar sampling bag (SKC, USA) at a rate of 250 ml/min via a teflon tube placed in the mouth of a 200 kg Simmental steer held in a rearing pen at the Agricultural Research Station of Ciba-Geigy (St-Aubin, Switzerland). Breath was thus transported to the laboratory and used for stimulation 1–2 h later by venting from the Tedlar bag into the barrel of 5-ml syringes which were immediately used as stimulus cartridges.

Porapak conditioning and filtering human breath. Porapak Q, a porous polymer which selectively desorbs water while retaining a large spectrum of volatiles was used to collect breath-borne odours. Conditioning of Porapak Q (50–80 mesh, Milipore Corporation, USA) was carried out by: 1) heating under N_2 (1 l/min) at 200 °C for 24 h, 2) extraction with dichloromethane (Merck, analytical grade) in a Soxhlet extractor for 24 h, and 3) drying under N_2 (1 l/min) at 110 °C for 2 h. About 600 mg of conditioned Porapak Q was then packed into the barrel of a Pasteur pipette (70 mm long, 5 mm i.d.) and human breath was blown directly through this adsorbant trap for 30 s. A portion of the filtered breath was introduced in the barrel of a 5-ml syringe to be used as stimulus.

Filtering human breath through charcoal. Commercially available charcoal air-sampling tubes (coconut-base 50/100 mg in a 6 mm OD × 70 mm long trap, SKC, USA) were also used to collect breath-borne volatiles. Charcoal traps a wide range of volatiles like Porapak Q, but its adsorbtion capacity for small molecular weight compounds is higher. Human breath was blown through the trap for 30 s and a portion of the filtered breath was entrained in a 5-ml syringe to be used as stimulus.

Hydrogen sulfide. Two methods of generating H_2S vapours were employed. A certified H_2S-permeation tube (Dynacal, VICI Metronics, USA) liberating H_2S at 546 ng/min ± 2% at 30 °C was used. Different concentrations of H_2S were obtained by mixing flows of charcoal-filtered air with that passing from a 500-ml gas-wash bottle containing the H_2S-permeation tube held at 30 °C in a constant T°C bath. Precise concentrations were introduced into the stimulus cartridge to cover 4 log orders of magnitude. Considering the dilution which occurred in the stimulus-delivery-tube, H_2S concentrations at the level of the preparation (5 mm from outlet of tube) ranged from ca. 0.003 ppm to ca. 2.6 ppm. Continuous or pulsed stimulations with H_2S were made by passing charcoal-filtered air through the 500-ml flask containing the H_2S-permeation tube, and then directly into the main air stream flowing over the preparation. Air flows were controlled by voltage-pressure converters and the duration of stimulation by solenoid valves. The T° increase (ca. 1 °C) which ensued in the main air stream during a stimulation had no effect on the sulfide-receptor response.

The second method employed to generate H_2S was the use of aqueous solutions of Na_2S. This method was particularly useful for providing H_2S doses higher than that obtainable from the permeation tube. Ten µl aliquots of various Na_2S solutions (10^{-5} mg/10 µl to 10^{-1} mg/10 µl H_2O) were applied to filter paper strips which were then enclosed in the 5-ml syringe. After allowing 3 min for evaporation of H_2S vapour, 2 ml of the stimulus cartridge volume were injected in 1 s into the main air stream flowing over the preparation. The quantity of H_2S leaving the lower Na_2S concentrations was calibrated by comparing the sulfide-receptor responses to these solutions with those obtained with the reference H_2S values from the certified permeation tube.

Other sulfides. A certified ethylmercaptan permeation tube (452 ng/min at 30 °C, Dynacal, VICI Metronics, USA) was employed in the same way as the H_2S-permeation tube to provide a graded concentration series of ethylmercaptan. Dimethyl sulfide (>99% GC, Fluka, Switzerland) was successively diluted in paraffin oil to provide 10^{-1} to 10^{-4} molar solutions. Ten µl of these solutions were applied to filter paper strips, enclosed in the barrel of a 5-ml syringe, and used as stimulus source.

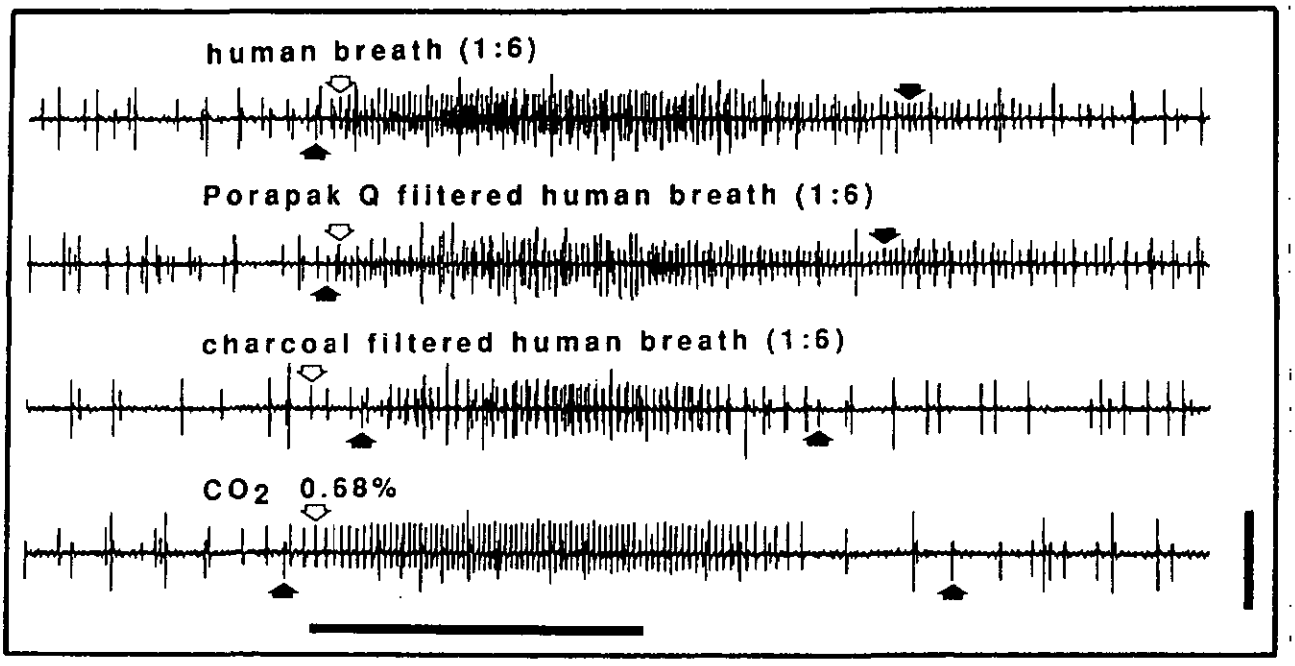

Fig. 1. Representative responses of the *Amblyomma variegatum* breath sensillum 2 to human breath, Porapak-filtered human breath, charcoal-filtered human breath, and CO_2. Filtered and unfiltered breath was diluted by a factor of 6 in the air stream conveying the stimulus to the preparation to give, for unfiltered breath, an equivalent of 0.68% CO_2. Stimuli were introduced, in this case, at 25 cm from the outlet of the stimulus-delivery-tube. *Bold arrow*, sulfide-receptor 2 (cell 4 in Fig. 2), which displays a negative going spike at the beginning of a stimulation, but then becomes biphasic. *White arrow*, CO_2-excited receptor (cell 1 in Fig. 2). Horizontal bar 1 s stimulation; vertical bar 1 mV

Other volatiles tested. The following volatiles were also tested to screen sulfide-receptor specificity: methane, CO_2, ammonia, acetone, 3-pentanone, 4-heptanone, hexanal, pentanol, 1-octen-3-ol, 1-octen, propionic acid, n-butyric acid, isobutyric acid, n-valeric acid, iso-valeric acid, heptanoic acid, L-lactic acid, γ-butyrolactone, γ-valerolactone, γ-caprolactone (all vertebrate odours), nonanoic acid, 2-nitrophenol, 2,6-dichlorophenol, methylsalicylate (tick pheromone components), dichloromethane and distilled water (solvents). Chemical purity of trade products was > 99% as indicated by GC. Chemicals were dissolved in analytical grade CH_2Cl_2 or in distilled water, depending on their nature, at 10^{-3} and 10^{-2} *M*, and prepared for stimulation as for dimethyl sulfide (above). Methane from the mains was entrained in the barrel of a 5-ml syringe for stimulation; for CO_2 stimulation see Steullet and Guerin (1992). A concentrated aqueous solution of 35% NH_4OH was diluted 10 and 100 times in distilled H_2O and used as the ammonia stimulus source.

Coupled gas chromatography – single unit recording

Human breath was blown through a coconut-charcoal trap for 15 min. The trap was then eluted with either dichloromethane or ethanol (Merck, analytical grade). From the first drop of the eluate, 1.5 µl was immediately injected on-column onto a DBWAX fused silica capillary gas chromatography column (30 m, 0.32 mm i.d., 0.25 µm film thickness, G&W Scientific, USA) with H_2 (0.5 m/s) as carrier gas; oven temperature: 30 °C for 5 min, then programmed at 10 °C/min to 230 °C. The column effluent was divided with a glass Y-piece splitter in the ratio 2:1 over, respectively, the flame ionisation detector (FID) and the biological detector. The latter consisted of an electrophysiological preparation of a capsular sensillum known to have cells responding to breath. An air stream, maintained at ca. 80% RH and 22 ± 1 °C in a water-jacketed tube (3 mm i.d.), swept a third of the column effluent from the heated (250 °C) transfer line of the chromatograph to the tick preparation at a speed of 1.5 m/s. The outlet of the tube was 5 mm from the tick tarsus. Column effluent was thus simultaneously monitored by both the FID detector and sensillum in order to locate any electrophysiologically active component in the extract (Wadhams 1982). A 1.5 µl aliquot of the first solvent drop eluting from an unused charcoal trap served as the blank control. A standard H_2S stimulus was obtained by injection of 10 µl of headspace from an aqueous solution of Na_2S (1 g/10 ml) moderately acidified with few drops of 1 N HCl. All spikes were sorted from noise with a discriminator level incorporated in the amplifier (UN-03 Syntech, The Netherlands) and the frequency was converted into a voltage. This converted signal, the receptor potential and FID responses were simultaneously printed on a chart recorder and stored on video tapes (Steullet and Guerin 1992). Play back of parts of the recording where an increase in spike frequency occurred was necessary to identify the responding cell(s). Discrimination for different spike types was made by eye (as described in Steullet and Guerin 1992). Identification of a stimulant in the extract was based on 1) the response of the biological detector to both a component of the extract and the standard eluting at the same retention time on the capillary column, and 2) on the type of cell activated.

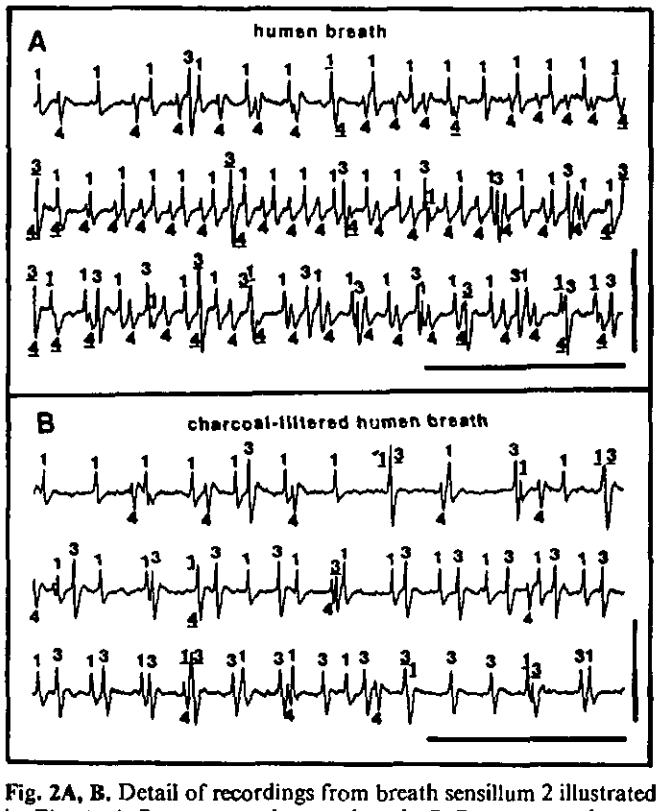

Fig. 2A, B. Detail of recordings from breath sensillum 2 illustrated in Fig. 1. **A** Response to human breath. **B** Response to human breath filtered through charcoal. Spike discrimination was made by eye. Note that unfiltered human breath elicits higher firing rates than charcoal-filtered human breath in cell 1 (CO_2-excited receptor, white arrow in Fig. 1) and cell 4 (sulfide-receptor 2, bold arrow in Fig. 1). Note also that spike 4 has a strong negative phase at the lower frequency in **B**, but becomes biphasic at the higher frequency in **A**. Cell 3, in this particular case, is slightly more excited in B than in A, but no statistical difference exists as a rule (Fig. 3). Cell 2 fires at A very low frequency. It is absent from this example. Enumeration corresponds to cell numbers in Fig. 3, and underlined spikes are overlapping events. Horizontal bars 100 ms; vertical bars 1 mV

Behavioral bioassay

A behavioral bioassay based on the activity level of ticks was developed. During first steps in host-searching by adult *A. variegatum*, 3 distinct phases are observed: 1) resting, where fully inactive ticks keep their legs folded under their body; 2) questing, where

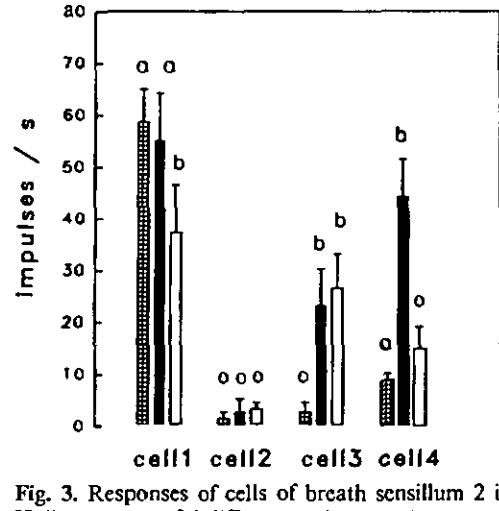

Fig. 3. Responses of cells of breath sensillum 2 in the capsule of Haller's organ of 6 different male *A. variegatum* to human breath, charcoal-filtered human breath, and to CO_2 at a concentration equivalent to the level in unfiltered breath. Stimuli were introduced into the main air stream at 25 cm from the outlet of the stimulus-delivery-tube. Cell frequencies were determined on spike trains of 1 s from the beginning of the response. For each cell, blocks labelled *a* and *b* are significantly different from one another ($P < 0.05$) (Wilcoxon's paired comparison test). Bars associated with each block are standard deviations. See also Fig. 2. ■ carbon dioxide (breath conc.), ■ human breath, □ charcoal-filtered human breath

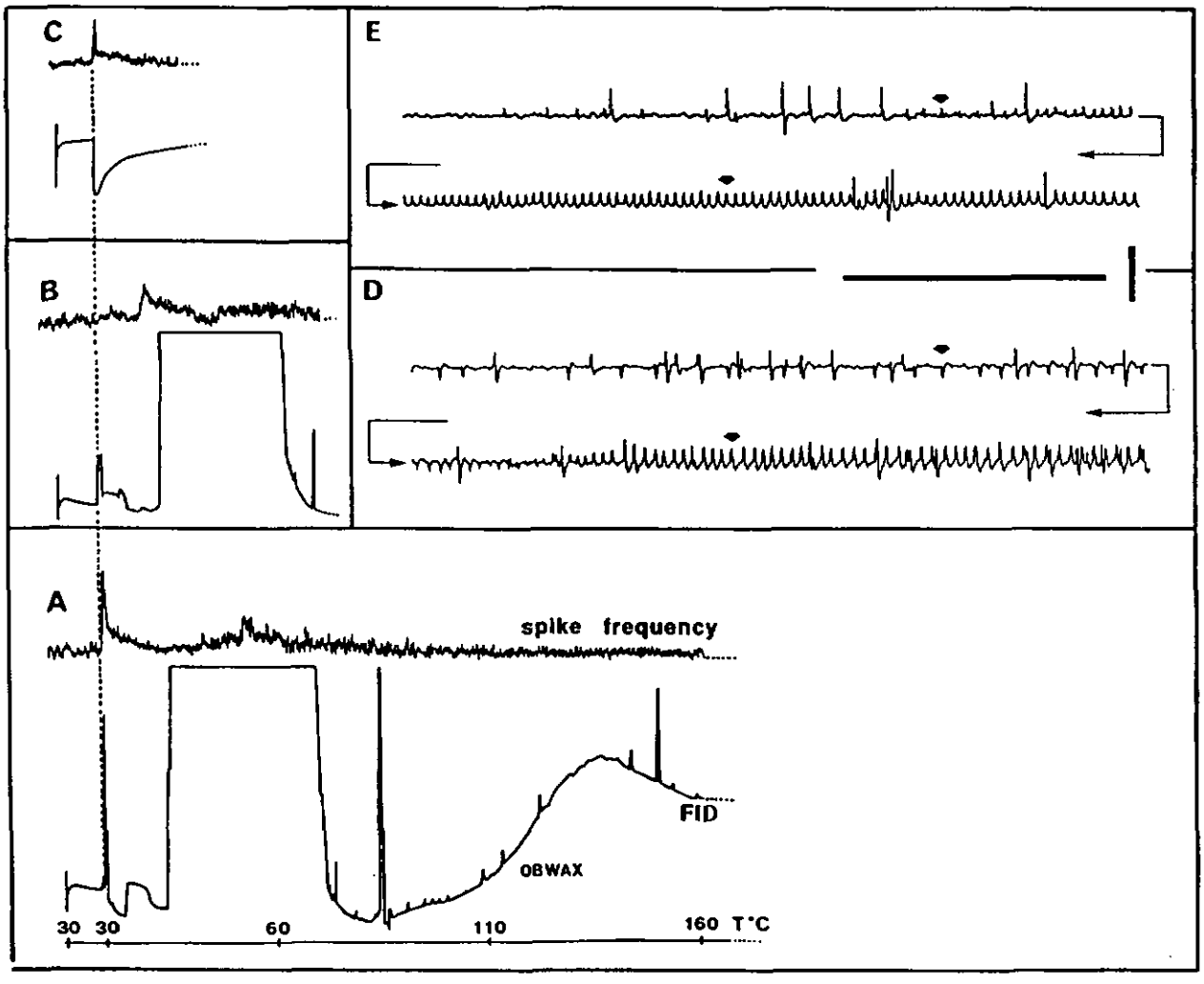

Fig. 4A–E. Analysis of human breath by gas chromatography coupled with breath sensillum 2 electrophysiology recordings from the capsule of Haller's organ of a male *A. variegatum*. A, B, and C spike activity of breath sensillum 2 (*upper trace*, frequency to voltage converted signal) recorded simultaneously with chromatogram (*lower trace* obtained with a flame ionisation detector, *FID*). A Breath components adsorbed on charcoal and extracted with ethanol, B blank control, i.e. an ethanol extract of an unused charcoal trap, and C 10 µl of the headspace over an acidified aqueous solution of Na_2S which generates H_2S. Note that the breath component, which causes an increase in spike frequency of the breath sensillum in A, elutes at the same retention time as H_2S (negative FID response) in C, where a corresponding increase in spike frequency is also observable. *Column: DBWAX* (J & W Scientific, USA) high resolution fused silica capillary column (30 m, 0.32 mm i.d., 0.25 µm film thickness) was temperature programmed after 5 min at 30 °C at 10 °C/min to 230 °C. Temperature scale same for A, B, and C. D Expanded trace of the breath sensillum 2 response, before and during elution of the active breath component in A. E Expanded trace of the breath sensillum 2 response, before and during elution of H_2S in C. For both D and E, horizontal bar is 1 s; vertical bar 1 mV. *Bold arrow*: sulfide-receptor 2 (cell 4 in Figs. 2 and 3). Note how the spike of this receptor changes sign on stimulation in D, and remained biphasic after strong stimulation. This explains why in E (experiment made after D) the sulfide-receptor 2 is already biphasic even before arrival of H_2S at the sensillum

ticks raise at least one of the forelegs; 3) walking, where ticks finally rise to their feet and begin locomotion. Groups of 25 male *A. variegatum* (7–8 months old, all fed on the same steers) were placed in 100-ml glass gas-wash bottles. A charcoal-filtered and humidified air stream (80% RH, 23±2 °C, 3.3 ml/s) passed continuously through each bottle. Blanks or stimuli, contained in 10-ml syringes, were injected at 0.8 ml/s for 10 s and thus diluted by a factor of 5 in the humidified air stream before entering the bottle at 2 cm from the floor, where most of ticks lay. Cigarette smoke indicated that the stimuli were distributed throughout the bottle for ca. 40 s before being flushed out. Only one stimulus was tested per day on the same group of ticks. Ticks had thus a day to return to the resting position (most do so < 1 h after stimulation). On successive days, a series of stimulations was applied to each group of ticks in a different sequence, to avoid any influence of the order in which the stimuli were tested. The number of resting, questing, and walking ticks was recorded just before and after stimulation. Data concerning the same stimulus were pooled and compared by Chi-square with data for other stimuli. The following stimuli enclosed in 10-ml syringes were tested: 1) H_2S produced by 10 µl of an aqueous solution of Na_2S at either 10^{-4} or 10^{-3} mg/10 µl deposited on a filter paper strip and enclosed in the syringe in an atmosphere of either N_2, 5% CO_2, or 1% CO_2; 2) dimethyl sulfide (10 µl of a 10^{-2} *M* solution in paraffin oil) enclosed in a syringe with N_2; 3) 5% CO_2; 4) 1% CO_2; 5) human breath collected in the morning; N_2 (blank control).

Results

Breath-sensitive cells

Three functionally different types of sensilla, whose cell activity was captured with different recording electrode

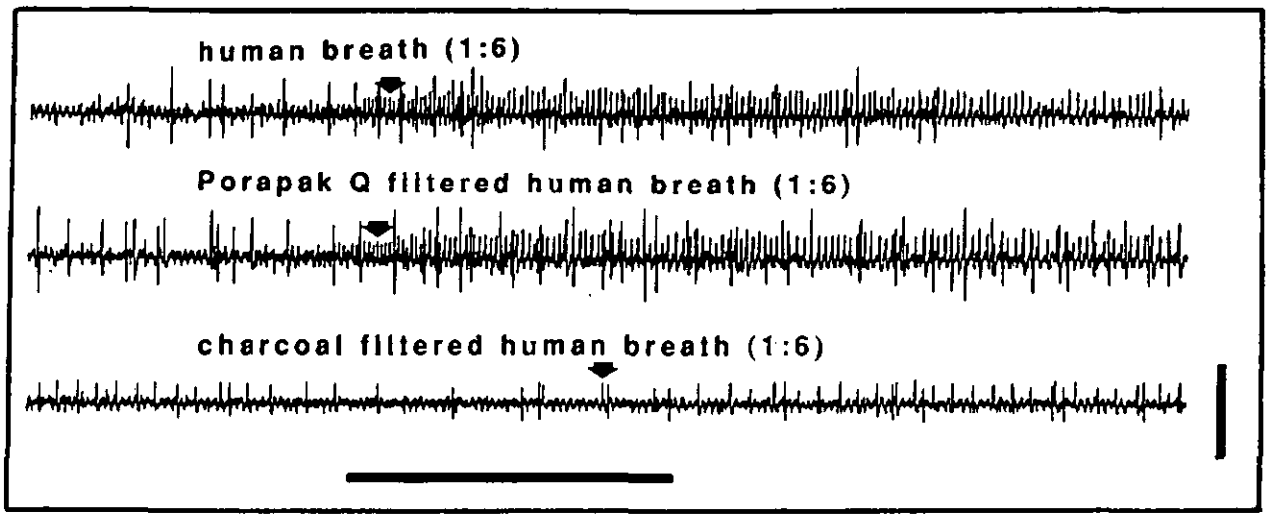

Fig. 5. Representative responses of sulfide-receptor 1 *(bold arrow)* of breath sensillum 1 in the capsule of Haller's organ of a male *A. variegatum* to human breath, Porapak-filtered human breath, and charcoal-filtered human breath. Filtered and unfiltered breath samples were diluted by a factor of 6 in the air stream conveying the stimulus to the preparation. Stimuli were introduced at 25 cm from the outlet of the stimulus-delivery-tube. Horizontal bar 1 s stimulation; vertical bar 1 mV

orientations within the capsule, carry cells responding to breath (Steullet and Guerin 1992). For convenience, these sensilla types are termed here breath sensillum 1, 2, and 3. As previously demonstrated, breath sensillum 2 has a CO_2-excited receptor and breath sensillum 3 a CO_2-inhibited receptor (Steullet and Guerin 1992). Results described in this paper deal with 2 other cells, respectively found in breath sensilla 1 and 2, which were activated by human and bovine breath. These results are based on 37 recordings from breath sensillum type 1 and 65 recordings from breath sensillum type 2 from different ticks. For practical reasons, most experiments were undertaken with human breath. Responses of these cells to breath strongly decreased over the working day, suggesting the active component(s) in human breath was much more prevalent in the morning.

Sulfide-sensitive cell in breath sensillum 2. In breath sensillum 2, Porapak-filtered breath elicited a multicellular response, involving 4 different cells, which was hardly different from that produced on stimulation with human breath. By contrast, the pattern of cell activity induced by charcoal-filtered breath was much simpler (Fig. 1 and details in Fig. 2). Recordings from sensilla of 6 different males indicated that cells 1 and 4 of this sensillum (Figs. 1, 2, and 3) were significantly less activated with charcoal-filtered breath than with unfiltered breath ($P<0.05$, Wilcoxon's paired comparison test), whereas no difference in the response of cell 3 was observed (Fig. 3). Cell 1 is the CO_2-excited receptor described in Steullet and Guerin (1992). A slight retention of CO_2 on charcoal is responsible for the weaker response of this cell to charcoal-filtered breath. On the other hand, the breath component activating cell 4 was not adsorbed by Porapak, but was retained on charcoal. But even on the latter, some breakthrough occurred as evidenced by the fact that air displaced from the charcoal trap, through which breath had been blown for a few minutes, excited cell 4. This suggested that the active breath component was very volatile.

A dichloromethane extract of breath components adsorbed on charcoal did not stimulate cell 4, but an ethanol extract did so strongly. In coupled gas chromatography and breath sensillum 2 recordings, one early eluting component of the latter extract elicited a response as evidenced by an increase in cellular activity (Fig. 4A) and a clear receptor potential. This active component had a retention time of 1 min, eluting before the solvent. A careful examination of the associated spikes established that this response was due to a strong activation of cell 4 (bold arrow in Fig. 4D), characterized by a quite small spike amplitude. An extract of an unused charcoal trap did not stimulate any cell of breath sensillum 2 (Fig. 4B). The retention time of the active breath component matched that of H_2S, which also activated cell 4 (Fig. 4C, bold arrow in Fig. 4E). Figure 6 illustrates the response of this sulfide-receptor to increasing concentrations of H_2S.

Sulfide-sensitive cell in breath sensillum 1. Unfiltered or Porapak-filtered breath was equally effective as a stimulant for a cell in breath sensillum 1, whereas charcoal-filtered breath failed to stimulate the same cell in this sensillum (bold arrow in Fig. 5). However, this cell responded strongly to an ethanol extract of the charcoal trap. Like cell 4 of breath sensillum 2, this cell also responded selectively to H_2S.

Properties and specificity of the two sulfide-sensitive cells

Both the activated cell of breath sensillum 1 (bold arrow in Fig. 5) and cell 4 of breath sensillum 2 (bold arrow in Fig. 1) are sulfide-sensitive. Nevertheless, they differ in important respects and will be termed sulfide-receptor

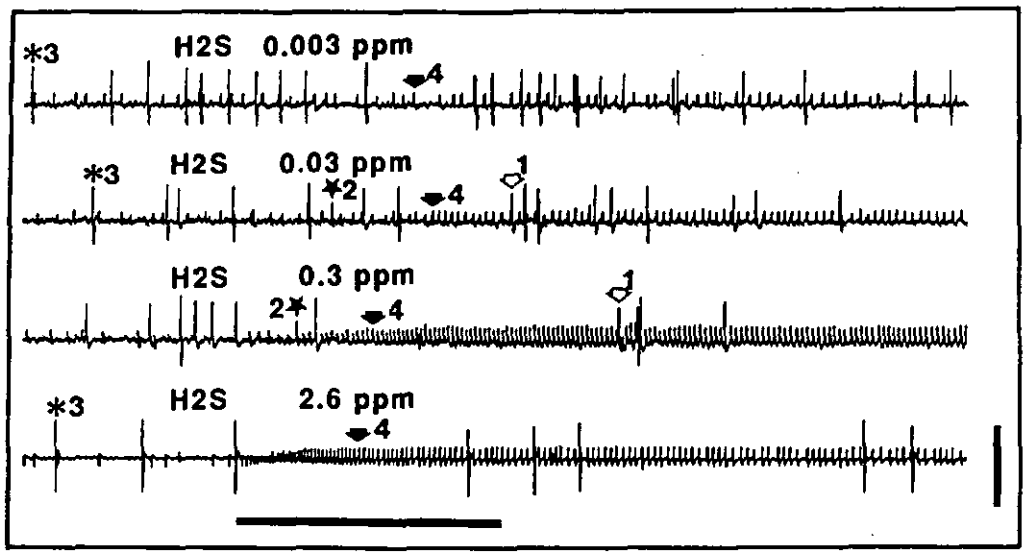

Fig. 6. Representative responses of sulfide-receptor 2 (*bold arraw*, and cell 4 in Fig. 2) of breath sensillum 2 in the capsule of Haller's orgao of a male *A. variegatum* to 4 concentrations of H_2S. Stimulus concentration was estimated at the level of the preparation. H_2S vapours were introduced into the stimulus-delivery-tube at 25 cm from its outlet. *White arrow:* CO_2-excited receptor (cell 1 io Fig. 2); *asterisk:* cell 3 in Fig. 2; *star:* cell 2 in Fig. 2. Note the change in tbe sign of the sulfide-receptor spikes following stimulation with high concentrations of H_2S (see text). Horizontal bar 1 s stimulation; vertical bar 1 mV

1 and 2, henceforth. Spike shapes of both cells were quite similar, characterized by a small amplitude (0.1–1 mV) and a duration of 5–6 ms. Interestingly, the spikes were often negative going at low frequency but became biphasic at a higher frequency (bold arrow in Fig. 4D, and Fig. 6). They occasionally remained biphasic after strong excitation.

Figure 7 illustrates the relationship between H_2S dose and the phasic part of the response of the sulfide-receptor 2 with two different stimulation methods. In Fig. 7a, the H_2S vapour was flushed into the stimulus-delivery-tube at 25 cm from its outlet to the preparation, so that the stimulus onset was not very sharp. This resulted in an attenuation of the phasic part of the response (slope of the logarithmic relation $m = 0.11$). In Fig. 7b, the stimulus was introduced 3 cm from the outlet of the stimulus-delivery-tube, so that the stimulus onset was much sharper. Here, the phasic portion of the response was clearly most modulated by concentration changes between ca. 10^{-2} and ca. 10^{-1} ppm H_2S, and these responses were significantly higher than the responses obtained with the graded stimulus onset ($P < 0.05$, ANOVA test with the General Linear Models Procedure on SAS, Australia). However, the slope of the logarithmic relationship between H_2S dose and the phasic part of the response was also rather flat ($m = 0.21$). With either method of stimulation, the H_2S response curve covered about 6 log orders of magnitude, from an estimated threshold at <0.1 ppb to over 10 ppm. Continuous or repetitive stimulation with ca. 0.1 ppm H_2S is extremely well monitored (Fig. 8); phasic bursts in spike activity followed 1 s repetitive stimulations, and little adaptation occurred over the 10 s continuous stimulation.

Both types of sulfide-receptors show dissimilarity in their respective responses to the 3 sulfides tested (Table 1). Whereas both were very sensitive to H_2S, the firing

Table 1. Responses of sulfide-receptors 1 and 2 to 3 sulfides: hydrogen sulfide, dimethyl sulfide, and ethylmercaptan. Response magnitude was determined on spike trains of 400 ms after the first 200 ms of the response, which corresponded to the period of strongest firing. Responses of sulfide-receptors were compared by ANOVA with the General Linear Models Procedure on SAS: on the right side of the table, a and b signify that responses of receptors 1 and 2 were significantly different ($P < 0.05$) for a given sulfide. § indicates that tbe response frequency of a receptor varies significantly with the concentration of a given compound ($P < 0.05$)

Sulfide-receptor	*n*	Amounts of Na_2S per cartridge				
		10^{-4} mg [imps/s]	10^{-3} mg [imps/s]	10^{-2} mg [imps/s]	10^{-1} mg [imps/s]	
Type 1	12	38.0 ± 21.5	55.4 ± 18.2	68.8 ± 12.4	70.4 ± 15.4	a §
Type 2	12	30.6 ± 16.5	49.2 ± 18.0	61.3 ± 10.8	62.5 ± 12.7	b §

Sulfide-receptor	*n*	molar conc. of dimethyl sulfide in cartridge				
		10^{-4} *M* [imps/s]	10^{-3} *M* [imps/s]	10^{-2} *M* [imps/s]	10^{-1} *M* [imps/s]	
Type 1	9	12.5 ± 7.3	17.8 ± 8.6	14.7 ± 5.7	23.9 ± 9.4	a
Type 2	9	22.9 ± 11.6	23.4 ± 7.9	44.2 ± 13.5	57.8 ± 13.5	b §

Sulfide-receptor	*n*	Ethylmercaptan conc. at preparation			
		10^{-2} ppm [imps/s]	10^{-1} ppm [imps/s]	1.2 ppm [imps/s]	
Type 1	4	13.1 ± 2.4	25.0 ± 16.6	25.0 ± 11.4	a §
Type 2	4	24.4 ± 13.9	36.3 ± 16.6	44.4 ± 11.6	b §

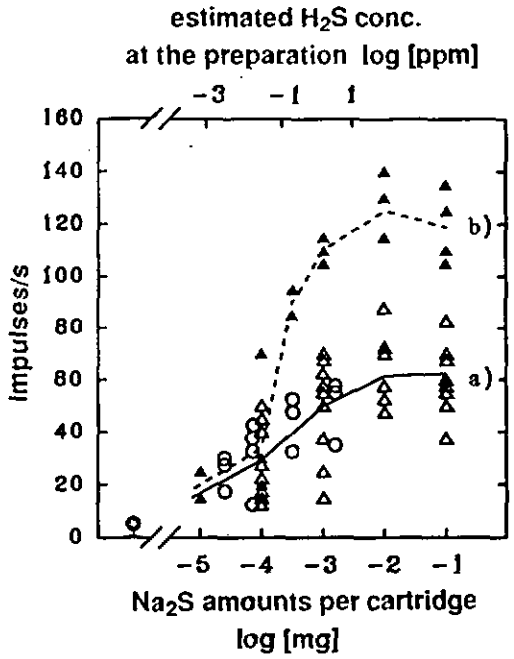

Fig. 7. Relationship between dose of H_2S aod the phasic part of the response of the sulfide-receptor 2 of male *A. variegatum* obtained by two methods of stimulation: a) graded stimulus onset was obtained by introducing the H_2S vapour 25 cm from the outlet of the stimulus-delivery-tube to the preparation, b) much sharper stimulus onset was obtained by introducing H_2S vapour 3 cm from the outlet of the stimulus-delivery-tube. Response magnitude was determined for the period when spike frequency was maximal: for curve *a* for 400 ms after 200 ms from the beginning of the response, and for *b* for the first 200 ms of the response. In curve *a*, hollow triangles ($n=12$) refer to responses obtained with aqueous solutions of Na_2S as stimulus source *(bottom abscissa)*, and *hollow circles* ($n=4$) are responses obtained with the certified H_2S-permeation tube which provided a maximal concentration of 3 ppm *(top abscissa)*. Aqueous solutions of Na_2S as stimulus source were used for curve *b (filled triangles)* ($n=4$). The amounts of H_2S produced by the aqueous solutions of Na_2S was calibrated with the responses of the same receptors to H_2S from the certified permeation tube. *Star (lower left)* is the response to H_2S free air ($n=12$). *Trend lines* connect mean values

rate of the type 1 was significantly higher than that of the type 2 over the concentration range tested, suggesting that type 1 is more sensitive (Table 1). On the other hand, ethylmercaptan as well as dimethyl sulfide elicited a significantly stronger response in the type 2 than in the type 1 over the range of concentrations tested; the firing rate of the type 1 was not modified significantly by increasing dimethyl sulfide concentration (Table 1). None of the other synthetic volatiles listed in Materials and methods activated either of these two sulfide-receptors. Although, the number of animals tested were sufficient to detect only large differences in sulfide-receptor responses between males and females, these did not appear.

Behavioral bioassay

Either 0.02 ppm or 1 ppm H_2S blown into the bottle containing the ticks aroused ca. 60% from rest, approximately 40% raised only their forelegs, and ca. 20% began walking (Table 2). A slightly weaker behavioral response, though not significantly different, was recorded for 10^{-2} *M* dimethyl sulfide (Table 2). Behavior triggered by 10^4 or 2000 ppm CO_2 (1% or 0.2%, respectively) in this bioassay was different: although about 60% of ticks were likewise aroused from rest with CO_2, only ca. 10% remained with raised forelegs, but >50% started to walk

Table 2. Behavioral response of *A. variegatum* males to CO_2, H_2S, mixtures of these two volatiles, dimethyl sulfide, and human breath (sampled early in the morning). Activity states observed (numbers of resting, questing, and walking ticks) following each stimulus are compared pairwise, and are assigned different letters when significantly different ($P<0.05$, Chi-square test). Data are presented here in percentages to make results more comprehensible. H_2S was generated by aqueous solutions of Na_2S at either 10^{-4} or 10^{-3} mg/10 µl, generating vapours in the stimulus cartridge corresponding to ca. 0.1 ppm and ca. 5 ppm H_2S, respectively. Before arriving in the bottle containing the ticks, stimuli were diluted by a factor of 5 in the main humidified air stream

Stimulus in the cartridge	n	% ticks activated	% ticks questing	% ticks walking	
Blank	300	18	17	1	a
CO_2 5%	200	63	7	56	b
CO_2 1%	100	62	10	52	bc
CO_2 5% + H_2S 0.1 ppm	50	72	10	62	bc
CO_2 5% + H_2S 5 ppm	50	58	18	40	cd
CO_2 1% + H_2S 0.1 ppm	50	50	22	28	de
CO_2 1% + H_2S 5 ppm	50	78	52	26	f
H_2S 0.1 ppm	100	61	41	20	efg
H_2S 5 ppm	100	53	38	15	eg
Dimethyl sulfide 10^{-2} *M*	50	48	38	10	g
Human breath	50	66	54	12	fg

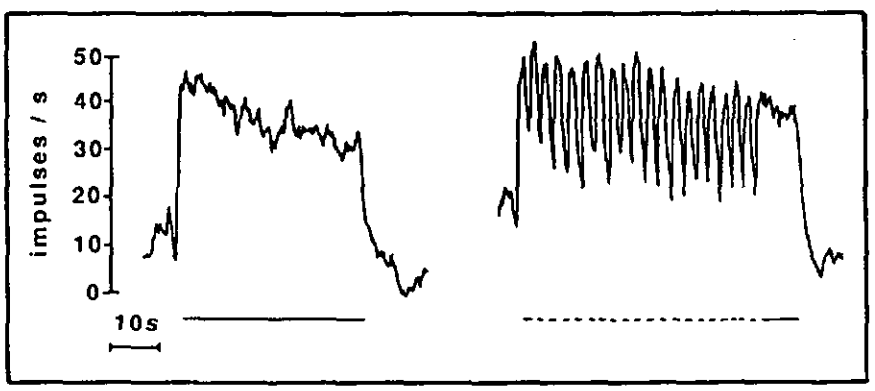

Fig. 8. Representative response of a sulfide-receptor 2 in the capsule of Haller's organ of a male *A. variegatum* to continuous *(left)* and repeated stimulation *(right)* with ca. 0.1 ppm H_2S. The traces are the frequency to voltage converted response of the sulfide-receptor which could be sorted with a window discriminator from other spikes because of its small amplitude. Horizontal bar, stimulation. Responses illustrated here are typical for both types of sulfide-receptors present in the capsule, and were highly reproducible

(Table 2). In mixtures, CO_2 and H_2S appeared to act antagonistically on *A. variegatum* behavior. Depending upon the relative concentrations of CO_2 and H_2S in the mixture, activated ticks tended to either raise their forelegs or begin walking. With CO_2 at 10^4 ppm and H_2S at ca. 0.02 ppm in the mixture, ticks tended to respond as for stimulation with CO_2 alone. By contrast, with a mixtures of CO_2 at 2000 ppm and H_2S at ca. 1 or even as low as 0.02 ppm, ticks responded as for stimulation with H_2S alone (Table 2). Finally, human breath collected early in the morning, when it normally strongly activated sulfide-sensitive cells, evoked a similar response to that of H_2S alone, or to that of a CO_2 and H_2S mixture with a relatively high concentration of H_2S (Table 2).

Discussion

Sulfide-sensitive cells responding to breath have been found in two wall-pore sensilla located in the capsule of Haller's organ of adult *A. variegatum*. Both cells are very sensitive to H_2S, but the type 1 is slightly more sensitive. The H_2S dose-response covers a wide range of concentrations, from <0.1 ppb (estimated threshold) to >10 ppm. The phasic part of the response to H_2S is significantly different depending on whether the stimulus is introduced at 25 cm or 3 cm from the outlet of the stimulus-delivery-tube and proves, as expected, to depend on the sharpness of the stimulus onset. The log/log H_2S dose and phasic part of the response relation does not have a steep slope: 0.21 with a sharp stimulus onset and 0.11 with a graded stimulus onset. Interestingly, the subjective H_2S dose-response relation for humans also has a similar slope (0.11), and the absolute threshold is ca. 10^{-3} ppm (Lindvall 1977). This may suggest equivalent primary transduction processes for H_2S perception in both ticks and humans, an equally high sensitivity most probably imposed by the extreme toxicity of H_2S for cucaryotypes.

The concentration of sulfides in human breath, produced in situ by anaerobic bacteria living on decomposing food remains and saliva in the buccal cavity, is highly variable during the day, ranging from ca. 0.007 ppm to 0.7 ppm for H_2S. Peak levels depend on prolonged periods of reduced saliva flow and abstinence from food or liquid uptake (Tonzetich 1977). Thus, sulfur compound concentrations are generally much higher early in the morning than later in the day. This corroborates our findings that human breath collected from the mouth in the morning stimulated the sulfide-sensitive cells much more than the same collected after lunch. Ruminants, the commonest hosts of adult *A. variegatum*, produce large quantities of H_2S, with concentrations up to about 100 ppm in the rumen (Hungate 1966). The amount of H_2S expelled from the rumen will then vary according to the frequency of eructation events, which is in turn linked to the digestion state. The tick can most probably detect H_2S liberated by an eructating host from significant distances as sulfide-receptors are still sensitive to H_2S levels 10^6 times lower than that found in the rumen.

Concentrations of H_2S expired from vertebrates cover more than 3–4 log orders of magnitude, a range within which the sulfide-receptors of *A. variegatum* can easily discriminate (<0.1 ppb to >10 ppm). This contrasts with the narrow sensitivity range of the CO_2-excited receptor which can only discriminate over 2–3 log concentrations of CO_2, or just 1–2 log concentrations for the CO_2-inhibited receptor (Steullet and Guerin 1992). Moreover, sulfide-receptors are comparatively much more sensitive than the CO_2-receptors (estimated threshold for the sulfide-receptors: <0.1 ppb H_2S, against ca. 10–20 ppm CO_2 for the CO_2-inhibited receptor, and ca. 50–100 ppm CO_2 for the CO_2-excited receptor). Thus, both the CO_2-receptors and the sulfide-receptors are adapted for the perception of CO_2 and H_2S levels given off by vertebrates.

Stimulation with low molecular weight sulfur compounds reveals that the specificity of the two sulfide-receptors differs. The type 1 responds significantly stronger to H_2S, but significantly weaker to ethylmercaptan than the type 2. On the other hand, dimethyl sulfide elicited a strong response in the type 2, but not at all in the type 1. Thus, replacement of one or both of the hydrogens on the sulfur atom by another group (e.g. methyl- or ethyl-) alters perception by sulfide-receptor 1. Although many sulfur compounds other than H_2S have been identified in human breath such as ethyl-3-mercaptoproprionate, methyl-n-propyl sulfide, n-hexylmercaptan, dimethyl sulfide, methyl sulfide, and di-tert-butyl-disulfide (Krotoszynski et al. 1977; Tonzetich 1977), no further active breath component other than H_2S was found for either of the sulfide-receptors during gas chromatography coupled with breath sensillum recordings. Concentrations of sulfides other than H_2S (e.g. dimethyl sulfide) in the breath extracts tested here might have been too low to be detected. Nevertheless, presence of two types of receptors which differ in their specificity suggests that the tick may discriminate for various sulfur compounds. These products abound not only in vertebrate breath but also in general vertebrate body odours (O'Connell et al. 1979; Natynczuk et al. 1989), and a response of these sulfide-receptors to cotton pads impregnated with human axillary secretions was recorded (Steullet, unpublished). Such receptors could provide the tick with specific information about hosts and possibly predilection sites.

Little is known about sulfur compound perception in arthropods. Some carrion beetles (*Necrapharus* sp.) bear carrion sensilla excited by a wide variety of volatiles including H_2S and butylmercaptan (Waldow 1973). On the other hand, some phytophagous insects are attracted by disulfides as well as by thiosulfinates and thiosulfonates (Al Rouz and Thibout 1988; Auger et al. 1989a, b). However, H_2S has never figured to date among the host-finding cues listed for haematophagous artbropods. *Glossina pallidipes* showed no electroantennogram response to H_2S in this laboratory, but recordings in the capsule of Haller's organ of the camel tick, *Hyalomma dromedarii*, provided preliminary evidence for the presence of at least one sulfide-receptor. Kneidel (1984) observed that some American dog ticks, *Dermacentar variabilis*, were attracted by carrion. This behavior could possibly be mediated by sulfides and mercaptans.

In *A. variegatum*, H_2S as well as dimethyl sulfide seem to act in the arousal phase of host-finding, i.e. ticks raise

their forelegs in the air but few start walking. Thus, unlike CO_2, H_2S cannot be considered as a strong locomotor stimulant. In fact, H_2S and CO_2 act antagonistically with regard to activation of *A. variegatum*. Arousal depends greatly on the relative concentration of H_2S in the mixture. A high amount of the latter diminishes the locomotor stimulant effect of CO_2, whereas a smaller quantity of H_2S does not very much alter the response of *A. variegatum* to CO_2. Human breath sampled early in the morning strongly stimulates sulfide-receptors, but does not initiate the same level of locomotion as an equivalent concentration of CO_2 alone. Sulfides as well as other components of vertebrate body odour perceived by the tick (as indicated by gas chromatography coupled to recordings from other olfactory sensilla, Steullet, in preparation) could subsequently intervene as cues bestowing a certain host specificity on *A. variegatum*.

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References

Al Roux H, Thibout E (1988) Analyse en olfactomètre de l'attraction des larves d'*Acrolepiopsis assectella* par des substances allélochimiques. Entomol Exp Appl 47:231–237

Auger J, Lecomte C, Paris J, Thibout E (1989a) Identification of leek-moth and diamondback-moth frass volatiles that stimulate parasitoid, *Diadromus pulchellus*. J Chem Ecol 15:1391–1398

Auger J, Lecomte C, Thibout E (1989b) Leek odor analysis by gas chromatography and identification of the most active substance for the leek moth, *Acrolepiapsis assectella*. J Chem Ecol 15:1847–1854

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

Hall DR, Beevor PS, Cork A, Nesbitt BF, Vale GA (1984) 1-octen-3-ol, a potent olfactory stimulant and attractant for tsetse isolated from cattle odours. Insect Sci Applic 5:335–339

Hungate RE (1966) The rumen and its microbes. Academic Press, New York

Kline DL, Takken W, Wood JR, Carlson DA (1990) Field studies on the potential of butanone, carbon dioxide, honey extract, 1-octen-3-ol, L-lactic acid and phenols as attractants for mosquitoes. Med Vet Entomol 4:383–391

Kneidel KA (1984) Carrion as an attractant to the American dog tick, *Dermacentor variabilis* (Say). J NY Entomol Soc 92:405–406

Krotoszynski B, Gabriel G, O'Neil J, Claudio MPA (1977) Characterization of human expired air: A promising investigative and diagnostic technique. J Chrom Sci 15:239–244

Lindvall T (1977) Perception of composite odorous air polluants. In: Le Magnen J, Macleod P (eds) Olfaction and taste VI. Information Retrievial, London Washington, pp 449–458

Mount GA, Dunn JE (1983) Economic thresholds for Lone Star Ticks (Acari: Ixodidae) in recreational areas based on a relationship between CO_2 and human subject sampling. J Econ Entomol 76:327–329

Natynczuk S, Bradshaw JWS, Macdonald DW (1989) Chemical constituents of the anal sacs of domestic dogs. Biochem Syst Ecol 17:83–87

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

O'Connell RJ, Singer AG, Pfaffmann C, Agosta WC (1979) Pheromones of hamster vaginal discharge. Attraction to femtogram amounts of dimethyl disulfide and to mixtures of volatile components. J Chem Ecol 5:575–585

Steullet P, Guerin PM (1992) Perception of breath components by the tropical bont tick *Amblyamma variegatum* Fabricius (Ixodidae). I. CO_2-excited and CO_2- inhibited receptors. J Comp Physiol A 170:665–676

Tonzetich J (1977) Production and origin of oral malodor: a review of mechanisms and methods of analysis. J Periodontology 48:13–20

Vale GA, Hall DR (1985) The role of 1-octeo-3-ol, acetone and carbon dioxide in the attraction of tsetse flies, *Glossina* spp. (Diptera: Glossinidae) to ox odour. Bull Entomol Res 75:209–217

Wadhams LJ (1982) Coupled gas chromatography-single cell recordiog: a new technique for use in the analysis of insect pheromones. Z Naturforsch 37C: 947–952

Waldow U (1973) Elektrophysiologie eines neuen Aasgeruchrezeptors und seine Bedeutung für das Verhalten des Totengräbers *(Necrophorus)*. J Comp Physiol 83:415–424

Warnes ML, Finlayson LH (1985a) Responses of the stable fly, *Stomoxys calcitrans* (L) (Diptera Muscidae) to carbon dioxide and host odours 1. Activation. Bull Entomol Res 75:519–527

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

J Comp Physiol A (1993) ■:■■■-■■■

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Identification of vertebrate volatiles stimulating olfactory receptors on tarsus I of the tick *Amblyomma variegatum* Fabricius (Ixodidae)

I. Receptors within the Haller's organ capsule

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Abstract. Gas chromatography-coupled electrophysiological recordings (GC-EL) from olfactory sensilla within the capsule of Haller's organ of the tick *Amblyomma variegatum* indicate the presence of a number of stimulants in rabbit and bovine odours, and in steer skin wash. Some of these stimulants were fully identified by gas chromatography-mass spectrometry analysis and by matching electrophysiological activity of synthetic analogues as: 1) hexanal, 2-heptenal, nonanal, furfural, benzaldehyde, and 2-hydroxybenzaldehyde (in all extracts); 2) heptanal, 2-, 3-, and 4-methylbenzaldehyde, and γ-valerolactone (only in bovine and rabbit odour). Careful examination of the electrophysiological responses permit characterization of 6 receptor types: 1) a benzaldehyde receptor, 2) a 2-hydroxybenzaldehyde receptor, 3) three types of receptors responding differently to aliphatic aldehydes, and 4) a lactone receptor.

Key words: Tick – Haller's organ – Olfactory receptors – Benzaldehyde – 2-Hydroxybenzaldehyde – Aliphatic aldehydes – γ-Valerolactone

Introduction

Adults of the tropical bont tick, *Amblyamma variegatum* (Acari, Ixodidae), lie in wait in the litter zone for hosts such as domestic and wild bovidae. The presence of a vertebrate in the vicinity arouses adults of this tick species to initiate active search on the ground in order to locate the host. At the end of the dry season, males of *A. variegatum* are first to find a suitable host, and feed for few days before emitting an aggregation-attachment pheromone (Schoeni et al. 1984), which in turn enhances attractivity of the host for conspecifics (Norval et al. 1989; Barré et al. 1991). This favours meeting of the sexes on the host. While the aggregation-attachment pheromone together with host odour seems crucial for host-seeking and attachment by females (Barré 1989; Barré et al. 1991), host odour alone is important for infestation of the host by pioneer males.

This paper deals with olfactory receptors housed in wall-pore single-walled sensilla within the capsule of Haller's organ on the tarsus of the leg pair I, considered to contain some of the main host-odour receptors in ticks. This supposition was confirmed by the behavioural bioassay of Lees (1948) on *Ixodes ricinus*, and in electrophysiology experiments in which mouse odour was used to stimulate capsule receptors in *Hyalamma asiaticum* (Sinitsina 1974). In addition, breath components CO_2 and H_2S have been clearly identified as olfactory stimulants for receptors in the capsule of Haller's organ of *A. variegatum* (Steullet and Guerin 1992a, b). The present study aims to extend our knowledge on other olfactory receptors (specificity spectrum) responding to host odour within wall-pore single-walled sensilla of the Haller's organ in this tick species. Gas chromatography-coupled electrophysiology recordings of host-odour receptors are then employed to isolate active constituents in vertebrate odour concentrates.

Materials and methods

Tick rearing. *A. variegatum*, originating from the Ivory Coast (Adiopodoumé), have been reared since 1981 at the Agricultural Research Centre of Ciba-Geigy Ltd. (St. Aubin, Switzerland). All stages (immature and adult) are fed on Simmental calves at 22 to 24°C and then kept under constant darkness during moult at 28°C/80–90% RH. Unfed males foreseen for these experiments were maintained in an environmental cabinet: 10 h light at 25°C/85% RH, 10 h darkness at 18°C/95% RH separated by 2 h dusk and dawn periods.

Electrophysiology. Unfed male *A. variegatum* (under 7 months old) were immobilized on a perspex holder with double-sided sticky tape. Pedal nerves of the anterior leg pair were destroyed by pinching the coxa with fine forceps to prevent muscle activity during

Abbreviations: GC-EL, gas chromatography-coupled electrophysiological recording; GC-MS, gas chromatography-coupled mass spectrometry

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electrophysiological recordings. The narrow opening of the capsule (a slit across dorsal side of the tarsus 50 µm long and 5 µm wide) was enlarged to provide better access to the 7 wall-pore sensilla within by using a piece of razor blade mounted on a Leitz micromanipulator.

Recordings from olfactory receptors were accomplished with glass electrodes connected to a high-input impedance preamplifier and an AC/DC amplifier (UN-03, Syntech, The Netherlands). The reference electrode, filled with 0.2 *M* NaCl, was inserted into the coxa of one of the anterior legs, whereas the recording electrode (tip diameter <5 µm), filled with 0.2 *M* KCl and 1% polyvinylpyrrolidon 90 K (Fluka, Switzerland), was mounted on a Leitz micromanipulator and gently introduced into the dissected capsule until cell activity was captured. Contact between the electrode tip and the pore-wall of a sensillum was sufficient to capture cell activity. Recordings from different sensilla within the capsule were made by varying the orientation of the recording electrode in the capsule. Cell activity could thus be consistently recorded at 6 distinct locations (Fig. 1). AC and DC signals were stored on video tapes as in Steullet and Guerin (1992a). AC signals were also fed into a IBM-compatible computer and visually analysed using the view option of the spike analysis programme SAPID (Smith et al. 1990), and displayed on paper with a laser printer.

Stimulus delivery. Air scrubbed through charcoal and silicagel, and humidified to 80% RH at 22°C ± 1°C passed continuously at 40 cm/s over the preparation from a 8 mm diameter glass tube, the outlet of which was about 10 mm from the tarsus. Stimulation was achieved by applying a charcoal-filtered air stream to a 5-ml polypropylene syringe containing the stimulus. A solenoid valve permitted displacement of 2 ml of the syringe content in 1 s into the humidified air stream through a septum-covered hole in the glass tube at 3 cm from its outlet. To prevent changes in air flow during stimulation, a solenoid-controlled charcoal-filtered air flow (2 ml/s) was delivered continuously through a blank syringe into the humidified air stream during stimulus off. Stimulations followed at 3 min intervals.

Different concentrates of host odours and the following synthetic chemicals were at first used to study the specificity of receptors located in the different parts of the capsule: ammonia (3.5% and 0.35% NH_4OH in distilled H_2O), acetone (10^{-3} *M* and 10^{-2} *M* in distilled H_2O), 3-pentanone, 4-heptanone, γ-butyrolactone, γ-valerolactone, 6-caprolactone, pentanol, 1-octen-3-ol, propanoic acid, 2-methylpropanoic acid, butanoic acid, 3-methylbutanoic acid, pentanoic acid, heptanoic acid, L-lactic acid, and 4-methylphenol (all vertebrate-associated volatiles); nonanoic acid, 2-nitrophenol, 2,6-dichlorophenol and methylsalicylate (tick pheromone components); and 1-octene, octylamine, hexyl acetate (others); dichloromethane and distilled H_2O (solvent blanks). Except for ammonia and acetone, all these chemicals (> 98% pure as indicated by GC) were dissolved in dichloromethane (Merck, analytical grade) and tested at 10^{-3} and 10^{-2} *M* dilutions (levels normally evoking clear responses in most responsive receptors). When a receptor responded to a tested chemical, graded dilutions from 10^{-5} to 10^{-2} *M* were delivered to the preparation to determine a dose-response curve. A 10 µl aliquot of the stimulus solution was deposited on a piece of filter paper and placed in the stimulus cartridge after evaporation of the organic solvent. Separate cartridges were employed for each stimulus and each concentration. Each cartridge was only used once. Three min were arbitrarily allowed for stimulus evaporation inside the syringes prior to delivering the volatile to the preparation. CH_4 (neat from the mains) and CO_2 (from a gas cylinder of 5% CO_2/95% O_2) were also tested; stimulus syringes were then directly filled with these gases.

Host-odour stimuli. Human breath, human axillary secretion, and extracts of bovine and rabbit odours were employed as host-odour stimuli. Human breath was blown into a 5-ml syringe used as stimulus cartridge (for further details, see Steullet and Guerin 1992b). Human axillary secretion was collected with a dry-acetone-washed cotton pad (7 × 7 cm) rubbed on the axillary area of a 28 year-old male and then enclosed in a stimulus cartridge. The axillary region was not treated with deodorants or perfumes, and was not washed for 24 h prior to secretion collection. The stimulus blank consisted of a dry-acetone-washed cotton pad.

Air from a metallic cage containing a single tick-naive rabbit (New Zealand), a white strain sometimes used in this laboratory to feed *A. variegatum*, was pumped for 24 h at 500 ml/min through ca. 600 mg of conditioned Porapak Q (60–80 mesh) packed in a glass tube 7 cm long × 4 mm diameter (Steullet and Guerin 1992b). The cage was located in an animal room with 20 other rabbits of the same strain. Volatiles were desorbed with 3 ml dichloromethane (Merck, analytical grade) and the extract was then slowly concentrated under N_2 to ca. 50 µl. One or 10 µl of the concentrated extract was enclosed on filter paper in the stimulus cartridge. Air from adjacent rooms without animals (blank control) was also collected as described above on Porapak and analysed by GC-MS.

Air from a 30 m^3 stall occupied by 2 tick-naive Simmental steers (about 200 kg each), a race frequently used to rear *A. variegatum*, was pumped for 24 h at 500 ml/min through 600 mg of conditioned Porapak Q. Solvent desorbtion and concentration were achieved as for rabbit odour and 1 or 10 µl of the concentrated extract was used as stimulus. Extract of air from a washed stall unoccupied for a month was used as a blank control and analysed by GC-MS. Collection of rabbit and bovine odour was undertaken on several occasions with different rabbits and steer. The concentrated extracts smelled very similar to the natural odours.

Different body parts (head, shoulder, side, dewlap, chest, belly, legs, armpit, and perianal area) of two tick-naive Simmental steers were rubbed with acetone-washed cotton pads (7 × 7 cm) soaked with dichloromethane (analytical grade). Gloves were used for this operation. The cotton pads were placed in a 500-ml gas-wash bottle, held at 70°C, through which N_2 passed for 1 h at 100 ml/min. Released volatiles were held up in a cold trap (4 mm diameter, 20 cm long glass U-tube steeped in a dry ice/acetone mixture) in a Dewar flask. Dichloromethane (2 ml, analytical grade) was used to extract trapped volatiles, water was removed by lowering the extract to −10°C and removing the solvent from the ice. The extract was subsequently concentrated under N_2 to ca. 50 µl. One or 10 µl of the concentrate was then used as stimulus.

Gas chromatography-coupled electrophysiological recordings (GC-EL). Olfactory receptors, characterized as responding to vertebrate odours, were subsequently employed to locate active product(s) among the many constituents of odour extracts by GC-EL. Components of an active extract (bovine or rabbit odours collected on Porapak, skin wash of steer) were separated on a high-resolution capillary gas chromatography column (chromatograph: Carlo Erba Instruments HRGC 5160 with an on-column injector; fused-silica column: 30 m DBWAX, internal diameter 0.32 mm or 0.25 mm, 0.25 µm film thickness, G&W Scientific, USA; carrier gas: H_2 at 0.5 m/s at 40°C; temperature programmed: 60°C for 5 min, 8°C/min to 230°C, and held for 10 min). The column effluent was split (glass Y-splitter), 2/3 being sent to the flame ionisation detector (FID) and 1/3 (longer arm) to an electrophysiological preparation with receptor(s) sensitive to host odour (biological detector). An air stream (1 l/min), maintained at ca. 80% RH and 22 ± 1°C in a 7 mm diameter glass water-jacketed tube, swept one third of the column effluent to the tick preparation 30 cm away from a heated transfer line (250°C) in the wall of the chromatograph. The outlet of the glass tube (reduced outlet of 3 mm diameter) was 5 mm from the tick tarsus where the air speed was 1.5 m/s. Column effluent was thus simultaneously monitored by the FID and the activity of the receptors recorded to locate possible active component(s) of the extracts being analysed (Wadhams 1982).

All spikes from what usually amounted to multicellular recordings (AC signal) were sorted from background noise with a level discriminator incorporated in the UN-03 amplifier, and the sum of the frequencies of all firing cells was continuously converted to a voltage (time constant of the frequency to voltage converter: 1 s). This signal was printed on a multichannel chart-recorder simultaneous with the FID response. An electrophysiological response was

indicated by a sudden change in the overall activity of the olfactory cells recorded. Time delay (about 3 s) between the FID response and the biological response, due to the added travel time of substances in the longer arm of the splitter and delivery tube to the electrophysiological preparation, was estimated by recording activity of an easily accessible 2-nitrophenol-sensitive receptor located in a sensillum on the anterior pit of Haller's organ during elution of 10 ng 2-nitrophenol (Diehl et al. 1991). A difference in the delay between the FID response and the receptor response was never observed between the synthetic compounds tested. Variation in the response latency of the different receptors studied was negligible compared to the passage time of the substances to the preparation. The Kovat's index for each active component detected was calculated with reference to alkanes (C_{10} to C_{20}) injected under the same GC conditions.

Gas chromotography-coupled mass spectrometry (GC-MS). Extracts analysed by GC-EL to locate active constituents were subsequently concentrated about 5 times and analysed on the same GC phase by GC-MS (Hewlett Packard 5890 series I I chromatograph – mass selective detector 5971A) to identify the active products. One µl of extract was injected on-column to the DBWAX capillary column (30 m, 0.25 mm internal diameter, 0.25 µm film thickness, G&W Scientific, USA) connected via 1 m of deactivated fused-silica capillary to the MS (ionisation chamber temperature 180°C; ionisation energy 70 eV). Helium was used as carrier gas under constant pressure (velocity 0.3 m/s at 40°C) and separation was achieved with the same temperature programme as in GC-EL. Active components of the extracts located by GC-EL were relocated in GC-MS from the calculated Kovat's index, and by comparison of the chromatogram profiles obtained in GC-EL and GC-MS.

Identification of the active components. Identification of an electrophysiologically active peak in an extract was first based on the match of its mass spectrum with that of a known product stored in a computer-based library of the GC-MS. The mass spectrum and the calculated Kovat's index of the extract-unknown were then compared with those of the library-proposed synthetic analogue injected under the same conditions. Biological activity of the identified product was subsequently tested with the synthetic analogue by electrophysiology experiments on the olfactory receptor concerned. Full identification based on the mass spectrum was not always feasible because of the small amount of compound present and/or because of coeluting products which obscured the spectrum. In some extracts, compounds either suspected or clearly identified in other extracts, such as methylbenzaldehyde isomers and γ-valerolactone, were nevertheless detected by searching at the retention times of the volatiles in question for some of their characteristic fragment ions whose mass to charge ratios (M/Z) were 65, 91, 119, and 120 for methylbenzaldehyde isomers and 56, 85, and 100 for γ-valerolactone. The following synthetic chemicals were employed as standards in GC-MS and electrophysiology: hexanal, heptanal, nonanal, (E)-2-heptenal, furfural, benzaldehyde, 2-methylbenzaldehyde, 3-methylbenzaldehyde, 4-methylbenzaldehyde, 2-hydroxybenzaldehyde, and γ -valerolactone. In addition, (E)-2-hexenal, 3-hydroxybenzaldehyde, 4-hydroxybenzaldehyde, 1-phenylethanone and cyclohexanone were used because of their relatedness to suspected active products. Dilutions of these chemicals (≥ 98% purity) from 10^{-5} to 10^{-2} *M* in dichloromethane were tested on the receptors concerned as described above.

Blank extracts (air from rooms without rabbits or steer) were also analysed by GC-MS to check for the possible presence of stimuli previously identified in bovine and/or rabbit odour extracts. For this purpose, 2-bromophenol (1.64 µg) was added as internal standard to 1.5 ml rabbit, bovine, and blank extracts before concentration. Using the single ion monitoring facility (SIM) of the mass selective detector, the presence of a stimulus was searched for, at the retention time of the synthetic analogue, by one of its characteristic fragment ions with a mass to charge ratio (M/Z) of 72 for hexanal, 70 for heptanal, 83 for (E)-2-heptenal, 98 for nonanal, 96 for furfural, 106 for benzaldehyde, 119 for methylbenzaldehyde isomers, and 122 for 2-hydroxybenzaldehyde. Quantification was achieved by peak integration for the characteristic fragment ion chosen. Abundance of each stimulus was then normalized with reference to the amount of 2-bromophenol calculated from the peak area of one of its characteristic fragment ions (M/Z = 172 corresponding to M^+-1).

Olfactory receptor characterization. After identification of the host-odour stimulants, attention was focused on the olfactory receptors concerned. Dose-response curves based on the first 200 ms of the response to synthetic stimulants and thus the specificity spectrum of the responsive receptors were studied. To discriminate between different receptor cells of similar spike shape and amplitude in multicellular recordings, double successive stimulation was sometimes necessary. This consisted of delivering an active compound A for a few s to the preparation interrupted for 1 s by stimulation with active compound B, and vice versa. No change in response, decrease or increase in firing of cell(s), cessation of firing in cell(s) or excitation of new cell(s) during the transition from compound A to B indicates if the same cell is responsive to the two substances or if different cells are excited. Possible cross-adaptation between receptor cells was not examined.

Results

Activity of olfactory receptors was captured at 6 distinct locations in the capsule of Haller's organ as revealed by hundreds of recordings. Results presented here deal with receptors recorded from within regions I and VI of the capsule (Fig. 1). Whereas one receptor in region I was

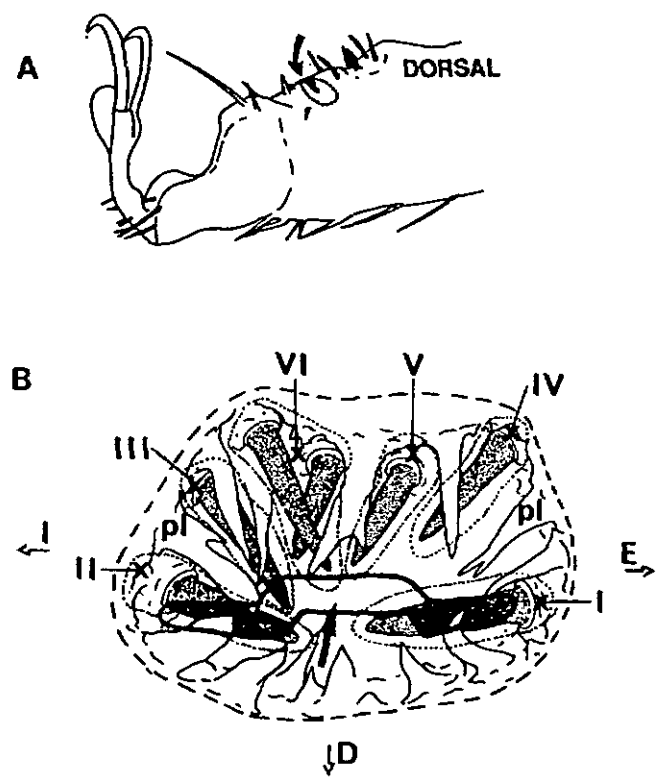

Fig. 1. A Distal end of the tarsus of the leg I of adult *A. variegatum* with the slit opening (*arrow*) to olfactory sensilla within the capsule of Haller's organ. B Diagrammatic view of the lay-out of olfactory sensilla in the capsule as shown by microscopic studies. The capsule is divided here into 6 regions (I-VI) from which olfactory responses were obtained. An outline of the slit opening is surimposed (see *arrow*). *D* distal; *E* external; *I* internal; *pl* pleomorph (for more details, see also Steullet and Guerin 1992a)

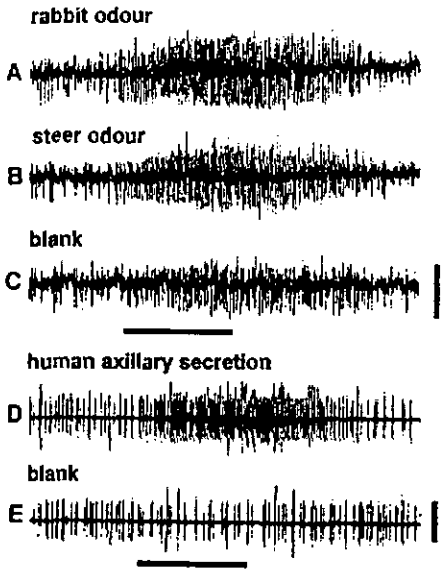

Fig. 2A–E. Responses recorded from olfactory sensilla in region VI (Fig. 1) of the capsule of Haller's organ in male *A. variegatum* where several receptors are excited by vertebrate odours. In preparation one: responses to rabbit odour (A), steer odour (B) both collected on Porapak Q, and to a wash of conditioned but unused Porapak Q (blank odour) (C). The response to stimulation with the blank odour was much lower than that due to either bovine or rabbit odour. In preparation two: responses to human armpit secretion collected on cotton (D), and to cotton alone (blank) (E). A slight inhibition occurs during stimulation with the blank. *Horizontal bars* 1 s stimulation; *vertical bars* 1 mV

sensitive to γ-valerolactone, a compound detected in traces in bovine and rabbit odours, the other receptors stimulated by bovine and rabbit odours collected on Porapak (Fig. 2A–C) were all found in the proximal region of the capsule (region VI in Fig. 1). Receptors within the latter region were also regularly excited by human axillary secretion (Fig. 2D, and E), but did not respond clearly to either human breath or to physiological relevant levels of the synthetic chemicals cited in Materials and methods under stimulus delivery.

Identification of some vertebrate volatiles stimulating olfactory receptors in region VI of the capsule

Gas chromatography-coupled electrophysiological recordings (GC-EL) revealed that several components of bovine and/or rabbit odours stimulated olfactory receptors in region VI of the capsule (Table 1 and Figs. 3, 4, and 5). Gas chromatography-coupled mass spectrometry (GC-MS) subsequently permitted identification of some of these active volatiles, often in minor amounts in both bovine and rabbit odours collected on Porapak. These volatiles were hexanal, heptanal, 2-heptenal, nonanal, furfural, benzaldehyde, 2-, 3-, and 4-methylbenzaldehyde, and 2-hydroxybenzaldehyde (Table 1). Hexanal, 2-heptenal, nonanal, furfural, benzaldehyde, and 2-hydroxybenz-

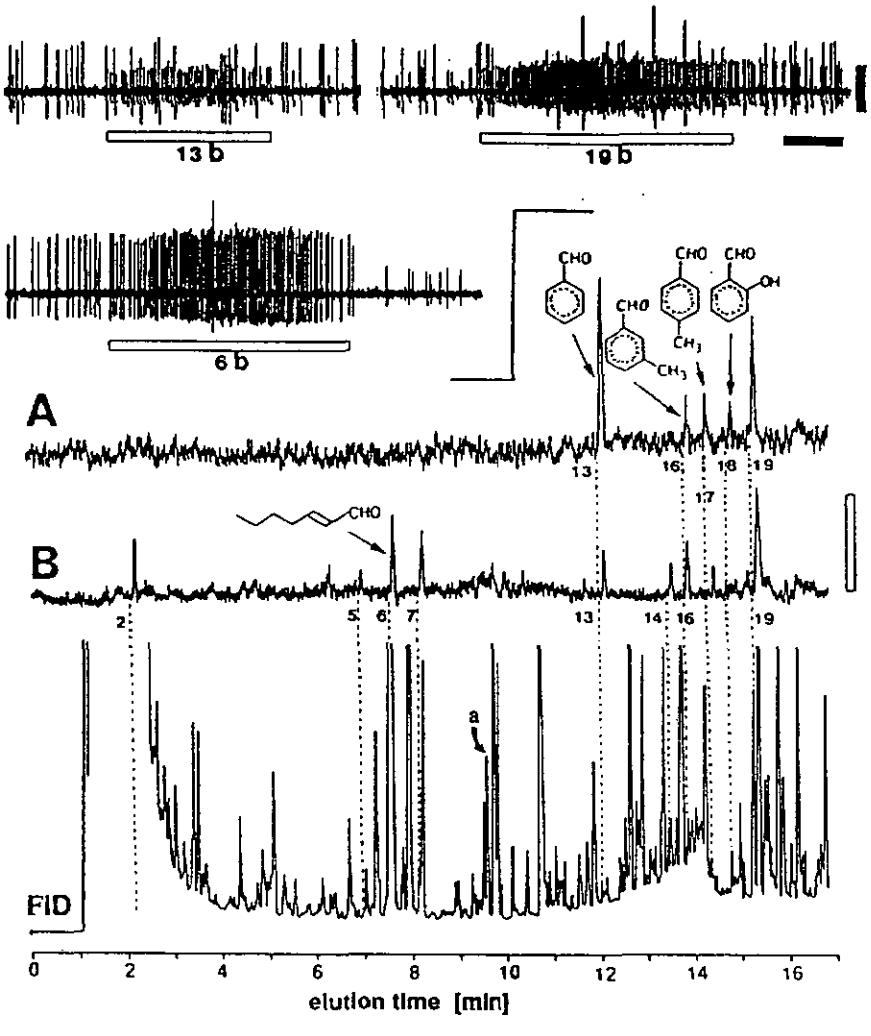

Fig. 3A, B. Analysis of a bovine odour, collected on Porapak, by gas chromatography-coupled electrophysiology of olfactory receptors within region VI of the capsule of Haller's organ in male *A. variegatum*. The *bottom trace* is the flame ionisation detector response (FID). The *upper traces* A and B represent the summed frequency of all firing cells (frequency to voltage converted signal) in two different recordings; hollow vertical bar is 50 impulses/s. Difference in response to the odour constituents between A and B was due to the different population of olfactory receptors captured in each case, although the recording electrode was placed in the same region of the capsule for both analyses. Two olfactory sensilla occur in this region of the capsule (Fig. 1). GC-MS analyses of the active peaks indicated that responses were recorded in both A and B for benzaldehyde (13), 3-methylbenzaldehyde (16), and the unknown peak 19, but only in A for 4-methylbenzaldehyde (17) and 2-hydroxybenzaldehyde (18). Receptors responding to (E)-2-heptenal (6) and the unknown peaks 2, 5, 7, and 14 were present in B but not in A. Other minor responses recorded once or only very occasionally were not accounted for. Active constituents are numbered as in Table 1. The FID trace indicated 0.5 to 1 ng for benzaldehyde (13) in the odour extract injected. Spike trains generated in B during elution of (E)-2-heptenal (6b), benzaldehyde (13b), and the unknown peak 19b are given at the top of the figure. *Hollow horizontal bars* depict the approximate time taken by the products to elute from the GC column; *solid horizontal bar* 1s; *solid vertical bar* 1 mV

Table 1. Identified constituents of bovine and rabbit odour which stimulated olfactory receptors located within the proximal-internal region VI of the capsule of Haller's organ in male *A. variegatum*

Peak number	Olfactory stimulant	a) Identification criteria	b) Odour source	c) Kovat's index in GC-EL	d) Kovat's index in GC-MS	e) Kovat's index of standards in GC-MS	f) Response occurrence
3	hexanal	MKE	steer	1085±0	1077	1079	2/9
			rabbit	1084±8	1081	1079	6/10
4	heptanal	MKE	steer	-	1188	1186	0/9
			rabbit	1184±5	1184	1184	3/10
6	2-heptenal	MKE #	steer*	1336±0	1337	1336	4/9
			rabbit	1338±4	1338	1336	5/10
8	nonanal	MKE	steer	1400±0	1398	1394	3/9
			rabbit	1396	1396	1396	1/10
11	furfural	MKE	steer*	1475±7	1469	1469	2/9
			rabbit	1469±7	1470	1469	3/10
13	benzaldehyde	MKE	steer*	1535±5	1531	1532	5/9
			rabbit	1528±5	1532	1532	6/10
15	2-methylbenzaldehyde	MKE	steer	-	1622	1621	0/10
			rabbit	1600	1622	1621	1/10
16	3-methylbenzaldehyde	MKE	steer	1623±3	1627	1626	2/9
			rabbit	1611±5	1628	1626	3/10
17	4-methylbenzaldehyde	MKE	steer	1657±3	1656	1653	2/9
			rabbit	1643±3	1655	1653	3/10
18	2-hydroxybenzaldehyde	MKE	steer*	1677±3	1686	1682	5/9
			rabbit	1675±5	1689	1682	10/10

This table is based on gas chromatography-coupled electrophysiology (GC-EL) and gas chromatography-coupled mass spectrometry (GC-MS) analyses of bovine and rabbit odour collected on Porapak Q, and the skin wash of steer (both types of analyses were made on the same GC phase DBWAX). a) Different criteria on which identification of a particular vertebrate volatile was based: M – matching mass spectra, K – matching Kovat's index, and E – matching electrophysiological activity with that of the synthetic analogue (# the trans isomer of 2-heptenal was employed). b) Analyses were made of bovine or rabbit odour as collected on Porapak, and * indicates that the active compound was also detected by GC-MS in a bovine skin wash. c) Mean Kovat's index (±standard deviation) of active peaks in GC-EL analyses. d) Kovat's index of the active peak located in GC-MS. e) Kovat's index of the synthetic product corresponding to that of the biologically active peak in GC-MS. f) Number of GC-EL analyses in which a response was observed/out of the total number of analyses with receptors from within region VI of the capsule. Other components of bovine and/or rabbit odours (GC peaks 1, 2, 5, 7, 9, 10, 12, 14, and 19 listed in the text and figures) occasionally activated receptors in GC-EL analyses, but could not be identified by GC-MS

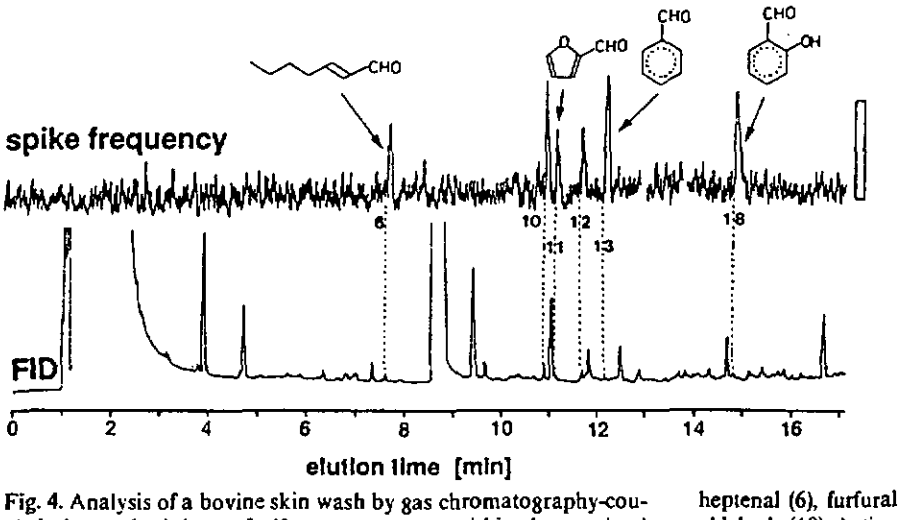

Fig. 4. Analysis of a bovine skin wash by gas chromatography-coupled electrophysiology of olfactory receptors within the proximal region VI of the capsule of Haller's organ in a male *A. variegatum*. The *lower trace* is the flame ionisation detector response (FID) and the *upper trace* is the summed frequency of all firing cells (frequency to voltage converted signal). *Hollow vertical bar* is 50 impulses/s. Active components are numbered as in Table 1. GC-MS analysis of the active peaks indicated that responses were obtained for (E)-2-heptenal (6), furfural (11), benzaldehyde (13), and 2-hydroxybenzaldehyde (18). Active constituents 10 and 12 were not characterized. Although the mass spectrum and retention time of peak 10 suggests 1-octen-3-ol, response to the synthetic analogue was never recorded from this region of the capsule. Furthermore, the response to component 12 always accompanied that to furfural (11) in GC-EL experiments. The FID trace indicated (E)-2-heptenal (6) at 0.1–0.5 ng in the extract injected

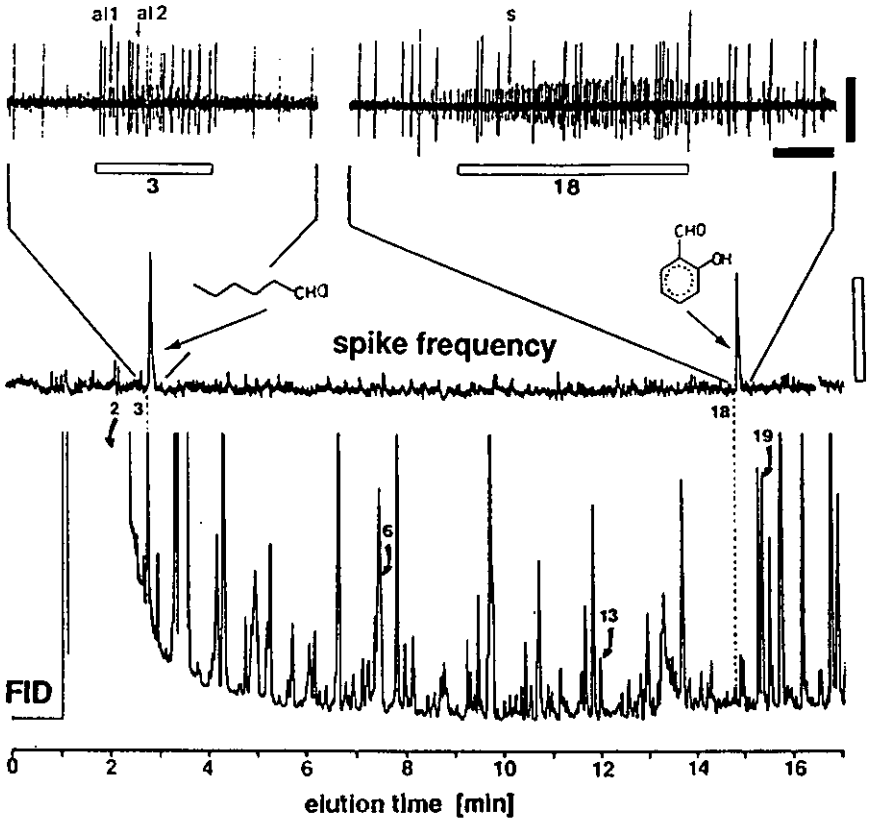

Fig. 5. Analysis of a rabbit odour, collected on Porapak, by gas chromatography-coupled electrophysiology of olfactory receptors within the proximal region VI of the capsule of Haller's organ in a male *A. variegatum*. The *lower trace* is the flame ionisation detector response (FID); the *middle trace* is the summed frequency of all firing cells (frequency to voltage converted signal), *hollow vertical bar* is 25 impulses/s; the *upper traces* are the actual spike trains generated during elution of hexanal (3) and 2-hydroxybenzaldehyde (18). The aliphatic aldehyde receptors type 1 (al1) and type 2 (al2) were both stimulated by hexanal (3), but only the 2-hydroxybenzaldehyde receptor (s) responded to 2-hydroxybenzaldehyde (18). *Hollow horizontal bars* indicate the elution time of the active peaks. *Solid horizontal bar* 1 s; *solid vertical bar* 1 mV. Active components are numbered as in Table 1. Receptors sensitive to 2-heptenal (6), benzaldehyde (13), and the unidentified peak 19, known to occur in this region of the capsule, were not captured in this recording

Table 2. Olfactory receptor types in the capsule of Haller's organ of *A. variegatum* which responded to specific classes of vertebrate volatiles

Receptor type	Location in the capsule	Best stimulant	Number of observations*
Benzaldehyde receptor	region VI	benzaldehyde	25
2-hydroxybenzaldehyde receptor	region VI	2-hydroxybenzaldehyde	23
Aliphatic aldehyde receptor 1	region VI	hexanal	12
Aliphatic aldehyde receptor 2	region VI	heptanal	11
Aliphatic aldehyde receptor 3	region VI	(E)-2-heptenal	13
Lactone receptor	region I	γ-valerolactone	15

* Indicates the number of times the receptor was recorded from in either gas chromatography-coupled electrophysiology analyses of vertebrate volatiles or in classic single-unit recordings

aldehyde were also discovered in steer skin wash. Although hexanal, nonanal, furfural, and benzaldehyde were also detected in blanks, they were more abundant in areas permeated with vertebrate odours, i.e. 2 to 4 times more benzaldehyde and furfural, >4 times more nonanal, and >20 times more hexanal than in blanks.

Characterization of receptors responding to the identified vertebrate volatiles

The responses recorded to the host-odour components by GC-EL recordings from region VI of the capsule were sometimes very variable. Olfactory receptor(s) responding to 2-heptenal (6) and the unidentified components 2, 5, 7, 14 of bovine odour in recording B of Fig. 3 were absent in recording A, whereas activity of the receptor sensitive to 2-hydroxybenzaldehyde (18) was only captured in recording A. In another GC-EL experiment (Fig. 5), no responses were recorded from receptors for 2-heptenal (6), benzaldehyde (13), and the unidentified components 2 and 19, although these constituents of this rabbit extract proved active on olfactory receptors from approximately the same location within the capsule in other GC-EL recordings. This was due to the fact that the different types of olfactory receptors from within region VI of the capsule from which recordings were obtained were probably distributed in two adjacent sensilla (Fig. 1), and the chance of picking up activity of any given receptor was dependent on the exact location of the electrode. In this study, we were unable to determine to which sensilla the different receptors captured within region VI of the capsule belong. Careful examination of spike shape and amplitude of receptors responding to

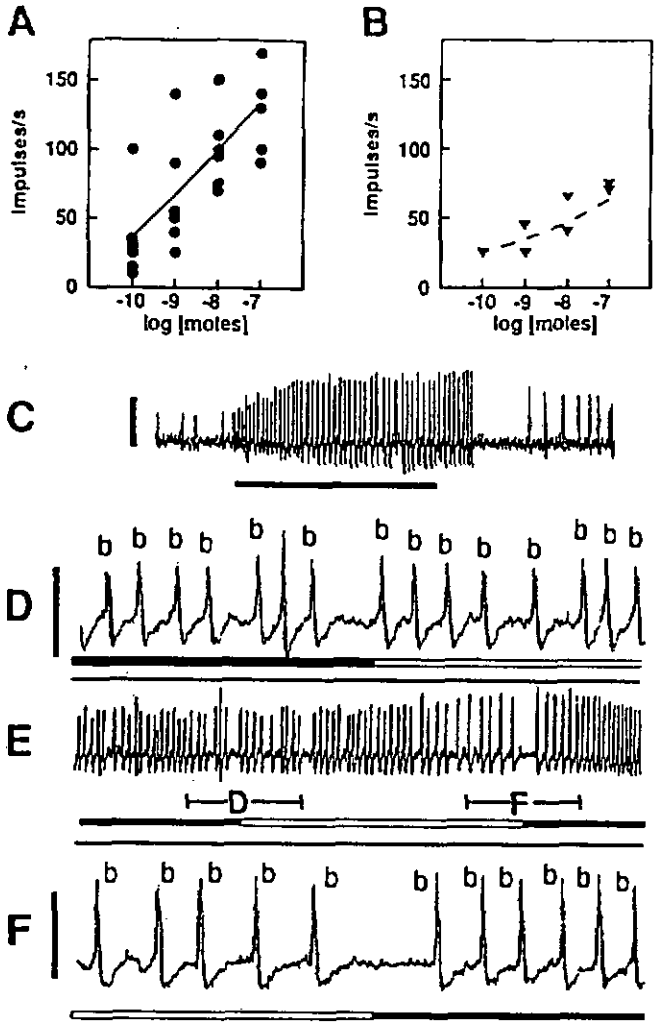

Fig. 6. A–C Response of a benzaldehyde-sensitive receptor in the proximal region VI of the capsule of Haller's organ in male *A. variegotum* to benzaldehyde and furfural. A Dose (moles in stimulus cartridge) of benzaldehyde ($n = 6$ different ticks) and B dose of furfural ($n = 2$ different ticks) plotted against spike frequency of the responding receptor calculated from the first 200 ms of the response. *Trend lines* connect mean values. In A and B the response of each receptor increased with increasing doses throughout the range tested. C Representative response of the receptor to benzaldehyde at 10^{-9} moles in the stimulus cartridge. D–F Double successive stimulation of the benzaldehyde-sensitive receptor (*b*) with 10^{-8} moles benzaldehyde in the stimulus cartridge (*solid horizontal bar*) interrupted with 10^{-7} moles furfural in a second stimulus cartridge (*hollow horizontal bar*). Absence of a second excited cell at onset of stimulation with furfural suggests that furfural activated the same receptor as benzaldehyde. D Spike train illustrating response to last 200 ms of stimulation with benzaldehyde and corresponding period from onset of stimulation with furfural. F Spike train resulting from reverse of situation in D, i.e. last 200 ms of stimulation with furfural and restart of stimulation with benzaldehyde. *Solid horizontal bar* in C and *hollow bor* in E represent 1 s; *vertical bars* 1 mV

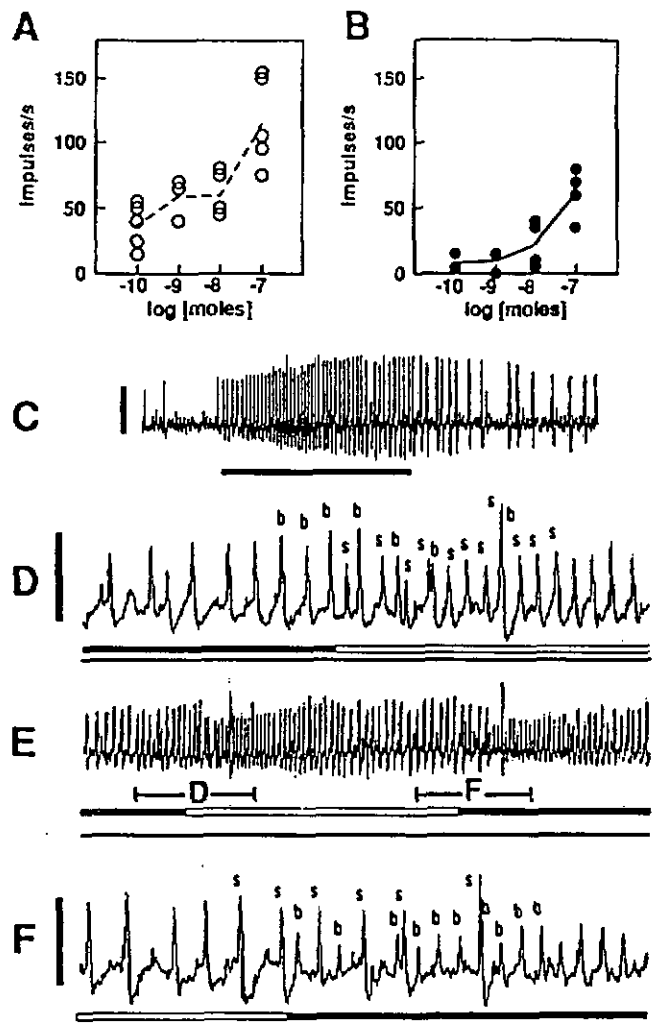

Fig. 7. A–C Response of receptors sensitive to 2-hydroxybenzaldehyde and benzaldehyde in the proximal region VI of the capsule of Haller's organ in *A. variegatum*. A Dose (moles in stimulus cartridge) of 2-hydroxybenzaldehyde ($n = 5$ different ticks) and B dose of benzaldehyde ($n = 4$ different ticks) plotted against spike frequency of the responding receptor calculated from the first 200 ms of the response. *Trend lines* connect mean values. In A and B the response of each receptor increased with increasing doses throughout the range tested. C Representative response of the receptor to 2-hydroxybenzaldehyde at 10^{-9} moles in the stimulus cartridge. D–F Double successive stimulation while recording from a receptor responding to benzaldehyde (*solid horizontal bar*) interrupted for 1 s (*hollow horizontal bar* in E) by stimulation with 2-hydroxybenzaldehyde (both products at 10^{-8} moles in separate stimulus cartridges). A phasic burst in spike frequency of a second excited cell at onset of stimulation with 2-hydroxybenzaldehyde and at restart with benzaldehyde indicated presence of separate receptors (*b* and *s* respectively, in D and F) for these two products in region VI of the capsule of Haller's organ. D Spike train illustrating response to last 200 ms of stimulation with benzaldehyde and corresponding onset period with 2-hydroxybenzaldehyde. F Spike train resulting from reverse of situation in D, i.e. last 200 ms of stimulation with 2-hydroxybenzaldehyde and restart of stimulation with benzaldehyde. *Solid horizontal bar* in C and *hollow bar* in E represent 1 s; *vertical bars* 1 mV

single and/or double stimulations with synthetic analogues of the identified stimulants (benzaldehyde, 2-hydroxybenzaldehyde, furfural, and aliphatic aldehydes) permitted us to clearly discriminate at least five types of receptors in the region VI of the capsule (Table 2). Proper characterization of receptors responding to methylbenzaldehyde isomers was not undertaken because of the great difficulty in obtaining reproducible recordings. GC-EL analyses and double successive stimulations permitted characterization of separate receptors for the aromat-

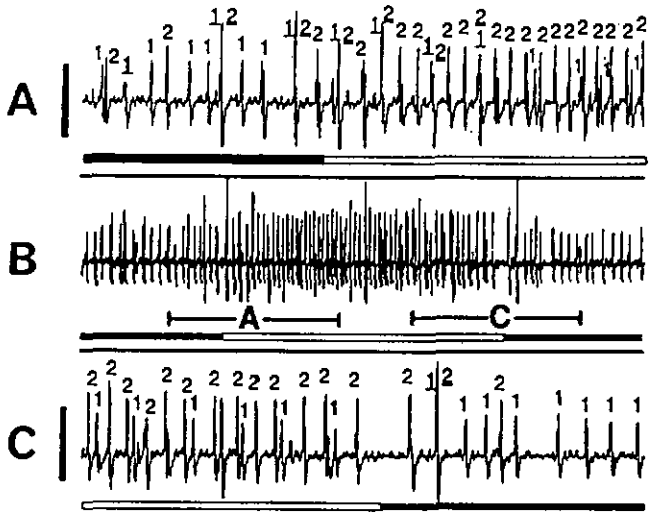

Fig. 8A–C. Response of olfactory receptors located within the proximal region VI of the capsule of Haller's organ in a male *A. variegatum* to the short-chain aliphatic aldehydes (E)-2-hexenal and hexanal (each at 10^{-8} moles in the stimulus cartridge). B Double successive stimulation with (E)-2-hexenal (*solid bar*), which stimulated both receptor 1 and receptor 2 weakly, was interrupted for 1 s by stimulation with hexanal (*hollow bar*) which activated receptor 2 more than (E)-2-hexenal did. Sensitivity of receptor 1 to both aldehydes was approximately the same. A Spike train illustrating response to last 300 ms of stimulation with (E)-2-hexenal and corresponding period from onset of stimulation with hexanal. C Spike train resulting from reverse of the situation in A, i.e. last 300 ms of stimulation with hexanal and restart of stimulation with (E)-2-hexenal. Spikes with underlined numbering are overlapping events; *vertical bars* 1 mV

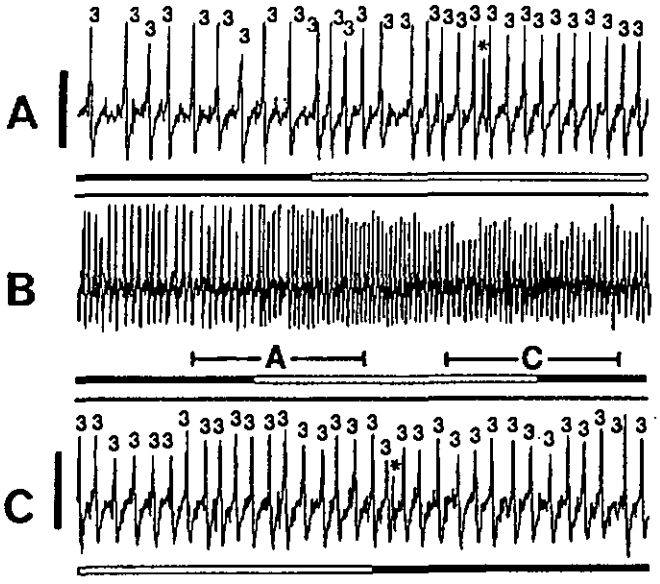

Fig. 9A–C. Response of an olfactory receptor (aldehyde receptor type 3) located within the proximal region VI of the capsule of Haller's organ in a male *A. variegatum* to the unsaturated aldehydes (E)-2-hexenal and (E)-2-heptenal (each at 10^{-8} moles in the stimulus cartridge). B Double successive stimulation with (E)-2-heptenal (*solid bar*) interrupted for 1 s by stimulation with (E)-2-hexenal (*hollow bar*). Absence of a second responding unit at onset of stimulation with (E)-2-hexenal or at restart of stimulation with (E)-2-heptenal indicated that both products activated the same receptor. This receptor type did not respond to short-chain saturated aliphatic aldehydes. A Spike train illustrating response to the last 300 ms of stimulation with (E)-2-heptenal and corresponding period from onset of stimulation with (E)-2-hexenal. C Spike train resulting from reverse of the stimulation in A, i.e. last 300 ms of stimulation with (E)-2-hexenal and restart of stimulation with (E)-2-heptenal. At least one other receptor with a spike of smaller amplitude (*star*) occurs in these traces. *Vertical bars* 1 mV

ic aldehydes (benzaldehyde and 2-hydroxybenzaldehyde) and for different aliphatic aldehydes.

Benzaldehyde receptor. One type of receptor in region VI of the capsule was sensitive to benzaldehyde (Fig. 6A and C) and to furfural as suggested by double successive stimulations (Fig. 6D–F). The dose-response curve for furfural was however much flatter than that for benzaldehyde (Fig. 6A and B). Since in GC-EL an estimated concentration of ca. 6×10^9 molecules of benzaldehyde/cm^3 air arriving at the tick preparation increased the base firing of this receptor from 5 to ca. 50 impulses/s (calculated on a spike train of 1 s during the peak of the response), the threshold of this receptor for benzaldehyde is still much lower than this level. Concentration of benzaldehyde at the preparation was estimated on the amount injected onto the GC column, the width of the benzaldehyde eluting peak, the fraction of the column effluent directed to the preparation, and the dilution factor in the air stream carrying the product to the preparation. Furthermore, responses to furfural were always accompanied by responses to a later eluting product in GC-EL experiments (peak 12 in Fig. 4) which excited spikes of the same shape and amplitude as furfural and benzaldehyde. This suggested that the unidentified component 12 may stimulate the same receptor as benzaldehyde and furfural, and thus share common chemical properties with these two volatiles.

2-Hydroxybenzaldehyde receptor. Another receptor in region VI of the capsule was strongly stimulated by 2-hydroxybenzaldehyde (Fig. 7A and C). Double successive stimulation with benzaldehyde and 2-hydroxybenzaldehyde permitted a clear distinction between the 2-hydroxybenzaldehyde receptor and the benzaldehyde receptor which were sometimes both captured in the same recordings (Fig. 7D–F). However, the 2-hydroxybenzaldehyde receptor also had the capacity to respond to very high doses of benzaldehyde (at least 10^{-8} moles in the stimulus cartridge) (Fig. 7B).

Three types of aliphatic aldehyde receptors. Three types of aliphatic aldehyde receptors with different specificity spectra were distinguished in region VI of the capsule (Figs. 8 and 9). One receptor (type 1) was particularly sensitive to saturated and unsaturated C_6 aliphatic aldehydes, but was only slightly sensitive to heptanal and hardly to nonanal (Fig. 10A and B). Another receptor (type 2) responded mainly to saturated aliphatic aldehydes such as hexanal and heptanal (Fig. 10C and D), whereas a third receptor (type 3) was excited by unsaturated aliphatic aldehydes such as (E)-2-hexenal and (E)-2-heptenal (Fig. 10E and F). These aliphatic aldehyde re-

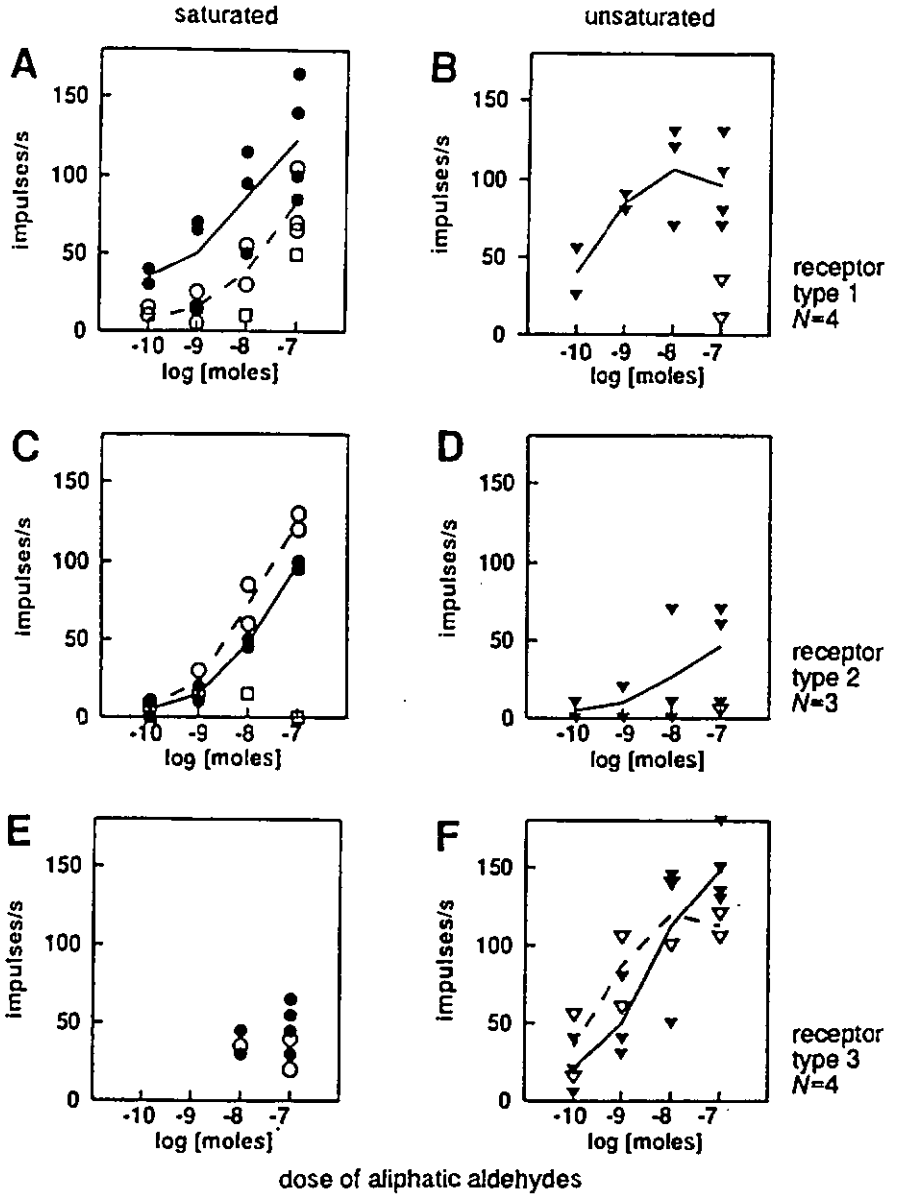

Fig. 10A–F. Representative dose response-curves of olfactory receptors located within the proximal region VI of the capsule of Haller's organ in male *A. variegatum* to saturated and unsaturated aliphatic aldehydes. *Solid circle* hexanal; *hollow circle* heptanal; *square* nonanal; *solid triangle* (E)-2-hexenal; *hollow triangle* (E)-2-heptenal. *Ordinates* in A–F are spike frequencies of the responding receptors calculated from the first 200 ms of the response, and *abscissas* dose of products tested (moles in stimulus cartridge). *Trend lines* connect mean values. In each case, the response of the receptors increased with increasing doses throughout the range tested. A and B Aldehyde receptor type 1 responded equally well to saturated and unsaturated C_6 aldehydes (A and B were established with the same receptors, n = 4); C and D aldehyde receptor type 2 was more sensitive to the saturated C_6 and C_7 aldehydes than to the corresponding unsaturated products (C and D were established with the same receptors, *n* = 3); E and F aldehyde receptor type 3 which was selectively stimulated by unsaturated aldehydes (E and F were established with the same receptors, n = 4) (see also Figs. 9 and 10)

ceptors seemed less sensitive than the benzaldehyde receptor since in GC-EL analyses an estimated concentration of ca. 3 × 10^{11} molecules of hexanal/cm^3 air arriving at the preparation was required to evoke responses of ca. 25 impulses/s from a base frequency of ca. 5 impulses/s in receptor type 1, and ca. 15 impulses/s from a base frequency of ca. 5 impulses/s in receptor type 2. In GC-EL analyses, responses to hexanal were due to either receptor type 1 or 2, or occasionally both (Fig. 5), heptanal and nonanal mostly excited receptor type 2, and (E)-2-heptenal stimulated receptor type 3 (Fig. 3). (E)-2-hexenal was not detected in any of the host-odour extracts analysed in this study. Responses to two unidentified components of bovine odour collected on Porapak (peaks 5 and 7 in Fig. 3B) always accompanied responses to (E)-2-heptenal, and spike shapes and amplitudes of the excited receptors were very similar. This suggests that components 5 and 7 may share common chemical properties with 2-heptenal and may likewise excite the aliphatic aldehyde receptor type 3. Nevertheless, we were unable to identify both peaks 5 and 7 by mass spectrometry.

Lactone receptor. Finally, when the glass electrode was introduced within the exterio-anterior part of the capsule (region I in Fig. 1), a receptor responsive to γ-valerolactone was systematically found among other sensory cells (Fig. 11). This receptor also responded weakly to γ-butyrolactone and 6-caprolactone, hardly at all to bovine odour extracts, and not at all to human breath and human axillary secretion. Nevertheless in GC-MS, a compound with three fragment ions (with mass to charge ratios of 56, 85, and 100) characteristic for γ-valerolactone and eluting at the same retention time as the synthetic analogue was detected in bovine and rabbit odour collected on Porapak. This suggested that these extracts may contain traces of γ-valerolactone but in insufficient quantity to clearly stimulate the lactone receptor in GC-EL.

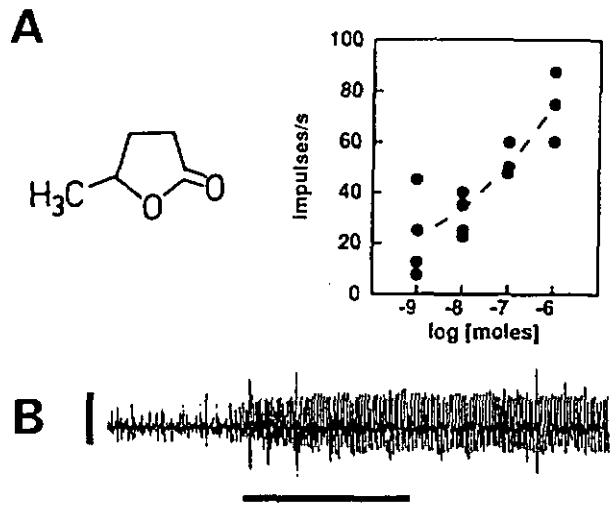

Fig. 11A, B. Response of an olfactory receptor within the exterio-anterior region I (Fig. 1) of the capsule of Haller's organ in male *A. variegatum* to γ-valerolactone. A Dose (moles in stimulus cartridge) of γ-valerolactone plotted against spike frequency of the responding receptor calculated from the first 200 ms of the response (n = 5 different ticks). *Trend line* connects mean values. B Representative response of the receptor to γ-valerolactone at 10^{-7} moles in the stimulus cartridge. *Horizontal bar* 1 s stimulation; *vertical bar* 1 mV

Discussion

The following components of rabbit and/or bovine odour stimulate olfactory receptors within chemosensilla in regions I and VI of the capsule of Haller's organ in *A. variegatum*: short-chain saturated and unsaturated aliphatic aldehydes, furfural, benzaldehyde, methylbenzaldehyde isomers, 2-hydroxybenzaldehyde, and γ-valerolactone. Except for saturated aliphatic aldehydes, these volatiles were remarkably minor components of the collected vertebrate odours.

Aliphatic and aromatic aldehydes as well as lactones are not known as kairomones for other haematophagous arthropods. Only propanal-sensitive neurons have been described in tsetse flies (Bogner 1992). These volatiles are furthermore not specific to vertebrate odours. Many phytophagous but also some scavenger arthropods are known to bear olfactory receptors sensitive to aliphatic aldehydes (i.e. constituents of the green leaf odour) or to benzaldehyde (Sass 1976; Seelinger 1977; Visser 1986). Benzaldehyde and 2-hydroxybenzaldehyde produced by different plants can act as repellents for several insects (Wallace and Mansell 1976). Finally, aliphatic aldehydes, benzaldehyde, 2-hydroxybenzaldehyde, and lactones also occur in the defensive secretions of various arthropods (Tschinkel 1975; Blum 1981).

In *A. variegatum*, benzaldehyde stimulates a receptor within region VI of the capsule. High amounts of this compound also excite another receptor most sensitive to 2-hydroxybenzaldehyde. Benzaldehyde has already been identified as a component of the aggregation-attachment pheromone of the related species *A. hebraeum* (Apps et al. 1988), but not in *A. variegatum* (Diehl et al. 1991; Diehl, unpublished). This aromatic aldehyde has been identified from a number of vertebrate sources: preorbital glands of the muskox (Flood et al. 1989), chin glands of the rabbit (Goodrich 1983), human vaginal secretion (Preti et al. 1977), and mouse urine (Andreolini et al. 1987). Benzaldehyde is also reported as a common volatile in air (Welsch and Watts 1990) and in this study was detected in the air of empty stalls (blanks), but at lower amounts than in rooms with steer or rabbits. The benzaldehyde receptor is also stimulated by furfural, a component of both bovine and rabbit odours collected on Porapak, bovine skin wash, and rabbit excrement, but also in human effluvia (Ellin et al. 1974), in human vaginal secretion (Preti et al. 1977) and in human urine (Zlatkis and Liebich 1971).

2-Hydroxybenzaldehyde strongly stimulates another receptor in the region VI of the capsule of Haller's organ. This product, present as a minor constituent of bovine and rabbit odours collected on Porapak and of the bovine skin wash, but absent in our blank odour, was once identified in extracts of replete female *A. variegatum* (Wood et al. 1975), but a physiological role was never proposed. Presence of 2-hydroxybenzaldehyde in vertebrate odours is nevertheless not well-documented, although it has been reported in the anal glands of the beaver (Lederer 1946).

Aliphatic aldehydes, found in our concentrates of vertebrate odour, are detected by three different types of receptors in region VI of the capsule of Haller's organ of *A. variegatum*. The type 1 responds best to both saturated and unsaturated C_6 aliphatic aldehydes, the type 2 to saturated C_6 and C_7 aliphatic aldehydes, and the type 3 to unsaturated C_6 and C_7 aliphatic aldehydes. The aliphatic aldehydes were generally present in our odour extracts at levels well above those of the aromatics. Saturated and unsaturated aliphatic aldehydes, as well as the corresponding ketones, alcohols, and fatty acids are well-documented commonly occurring volatiles in vertebrate odours. Some are also common air-pollutants (Welsh and Watts 1990), so it was not surprising to find traces of hexanal and nonanal in our blanks. Unsaturated aliphatic aldehydes are reported from goats (Smith et al. 1984), human breath (Krotoszynski et al. 1977), and the rabbit (Goodrich 1983), and saturated aliphatic aldehydes occur in the dog anal sac (Natynczuk et al. 1989), coyote urine (Schultz et al. 1988), skin glands of various bovidae (Burger et al. 1981), anal and chin glands of the rabbit (Goodrich 1983), human effluvia (Ellin et al. 1974; Goetz et al. 1988; Krotoszynski et al. 1977), and human axillary secretion (Labows et al. 1979b). The electrophysiological responses reported here for the human axillary secretion may be due to its aliphatic aldehyde content. Equipped with three aliphatic aldehyde receptor types, *A. variegatum* would be able to discriminate between volatile mixtures characteristic of different vertebrates. The ratio between responses of the different aldehyde receptors could thus serve as an odour-specific coding parameter as proposed by Tichy and Loftus (1983) for perception of alcohols by the stick insect, *Carausius morosus*.

γ-Valerolactone, present here in traces in bovine and rabbit odour, excites a receptor located in region I of the capsule of *A. variegatum*. Lactones have been reported from a variety of species within the Bovidae, Camelidae,

and Primates. Yeasts of the genus *Pityrosporum* are responsible for the production of different γ-lactones in areas rich in sebaceous glands of humans (Labows et al. 1979a). Several lactones are also reported in human scalp and urine (Goetz et al. 1988; Zlatkis and Liebich 1971), in occipital glands of *Camelus bactrianus* (Ayorinde et al. 1982), in preorbital glands of the muskox (Flood et al. 1989), blue and grey duikers (Burger and Pretorius 1987; Burger et al. 1990), and in pedal glands of bontebock (Burger et al. 1977). As adult *A. variegatum* mostly feed on Bovidae, lactones present in many of these vertebrates could possibly function as cues for host-finding and/or feeding-site selection.

In this study, receptor activity was recorded from six distinct regions within the capsule of Haller's organ of *A. variegatum*, corresponding to sensillum locations observed in microscopy. Recordings from five of these locations were highly reproducible in terms of the number of cells captured, spike shapes and amplitudes, spontaneous activity, and stimulus response, i.e. a situation typical for single sensillum recordings (Steullet and Guerin 1992a). By contrast, the recordings were not very reproducible in the posterio-interior region VI of the capsule. Here, the number of cells captured, and spike shapes and amplitudes varied from one preparation to another. We imagine that depending on the exact location of the tip of the glass recording electrode within region VI, different populations of receptors from one or both of the two closely associated sensilla in this region of the capsule were being sampled.

The seven wall-pore single-walled sensilla within the capsule carry between 21 and 35 receptor cells according to Hess and Vlimant (1982). About half of these cells have now been properly characterized: a CO_2-excited and a CO_2-inhibited receptor (Steullet and Guerin 1992a), two sulfide receptors (Steullet and Guerin 1992b), a methylsalicylate receptor (component of the aggregation-attachment pheromone, Hess and Vlimant 1986), and as described here benzaldehyde and 2-hydroxybenzaldehyde receptors, three aliphatic aldehyde receptors, and a lactone receptor. The capsule of the Haller's organ of *A. variegatum* is thus a very elaborate sense organ containing many olfactory receptors, each with a distinct sensitivity spectrum and capable of responding to various vertebrate odours such as breath, human axillary secretion, skin wash of steer, and to bovine and rabbit odour extracts.

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References

Andreolini F, Jemiolo B, Novotny M (1987) Dynamics of excretion of urinary chemosignals in the house mouse (*Mus musculus*) during the natural estrous cycle. Experientia 43:998–1002

Apps PJ, Viljoen HW, Pretorius V (1988) Aggregation pheromones of the bont tick *Amblyomma hebraeum*: identification of candidates for bioassay. Onderstepoort J Vet Res 55:135–137

Ayorinde F, Wheeler JW, Wemmer C, Murtaugh J (1982) Volatile components of the occipital gland secretion of the bactrian camel (*Camelus bactrianus*). J Chem Ecol 8:177–183

Barré N (1989) Biologie et écologie de la tique *Amblyomma variegatum* (Acarina: Ixodina) en Guadeloupe (Antilles Françaises). Thèse Université Paris-Sud, France

Barré N, Pavis C, Malosse C, Kermarrec A, Delot J, Booijol A, Aprelon R, Rousseau L (1991) Les phéromones d'agrégation-fixation de la tique *Amblyomma variegatum* en Guadeloupe. Utilisation possible en lutte intégrée. Rapport IEMVT-INRA, Guadeloupe

Blum MS (1981) Chemical defenses of artbropods. Academic Press, Orlando

Bogner F (1992) Response properties of CO_2-sensitive receptors in tsetse flies (Diptera: *Glossina palpalis*). Physiol Entomol 17:19–24

Burger BV, Pretorius PJ (1987) Mammalian pheromone studies, VI. Compounds from the preorbital gland of the blue duiker, *Cephalophus monticola*. Z Naturforsch 42c: 1355–1357

Burger BV, le Roux M, Garbers CF, Spies HSC, Bigalke RC, Pachler KGR, Vessels PL, Cbrist V, Maurer KH (1977) Further compounds from the pedal gland of the bontebok (*Damaliscus dorcas dorcas*). Z Naturforsch 32c: 49–56

Burger BV, le Roux M, Spies HSC, Truter V (1981) Mammalian pheromone studies, V. Compounds from the preorbital gland of the grysbok, *Raphicerus melanotis*. Z Naturforsch 36c: 344–346

Burger BV, Pretorius PJ, Spies HSC, Bigalke RC, Grierson GR (1990) Mammalian pheromones VIII. Chemical characterization of preorbital gland secretion of grey duiker, *Sylvicapra grimmia* (Artiodactyla: Bovidae). J Chem Ecol 16:397–416

Diehl PA, Guerin PM, Vlimant M, Steullet P (1991) Biosyothesis, production site, and emission rates of aggregation-attachment pheromone in males of two *Amblyomma* ticks. J Chem Ecol 17:833–847

Ellin RI, Farrand RL, Oberst FW, Crouse CL, Billups NB, Koon WS, Musselman NP, Sidell FR (1974) An apparatus for the detection and quantitation of volatile human effluents. J Chromatogr 100:137–152

Flood PF, Abrams SR, Muir GD, Rowell JE (1989) Odor of the muskox. A preliminary investigation. J Chem Ecol 15:2207–2217

Goetz N, Kaba G, Good D, Hussler G, Bore P (1988) Detection and identification of volatile compounds evolved from human hair and scalp using headspace gas chromatography. J Soc Cosmet Chem 39:1–13

Goodrich BS (1983) Studies of the chemical composition of secretion from skin glands of the rabbit *Oryctalagus cuniculus*. In: Müller-Schwarze D, Silverstein RM (eds) Chemical signals in vertebrates 3. Plenum Press, New York London, pp 275–290

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

Krotoszynski B, Gabriel G, O'Neill H, Claudio MPA (1977) Characterization of human expired air. A promising investigative and diagnostic technique. J Chromat Sci 15:239–244

Labows JN, McGinley KJ, Leyden JJ, Webster GF (1979a) Characteristic γ-lactone odor production of the genus *Pityrosporum*. Appl Environ Microbiol 38:412–415

Labows JN, Preti G, Hoelzle E, Leyden JJ, Kligman A (1979b) Analysis of human axillary volatiles: compounds of exogenous origin. J Chromatogr 163:294–299

Lederer E (1946) Chemistry and biochemistry of the scent glands of the beaver, *Castor fiber*. Nature 157:231–232

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

Natynczuk S, Bradsbaw JWS, Macdonald DW (1989) Chemical constituents of the anal sacs of domestic dogs. Biochem Syst Ecol 17:83–87

Norval RAI, Andrew HR, Yunker CE (1989) Pheromone-mediation of host-selection in bont ticks (*Amblyomma hebraeum* Koch). Science 243:364–365

Preti G, Smith III AB, Beauchamp GK (1977) Chemical and behavioral complexity in mammaliao chemical communicatioo systems: Guinea pigs (*Cavia porcellus*), Marmosets (*Saguinus fuscicollis*) and humans (*Homo sapiens*). In: Müller-Schwarze D, Mozell MM (eds) Chemical signals in vertebrates 1. Plenum Press, New York London, pp 95–114

Sass H (1976) Zur nervösen Codierung von Geruchsreizen bei *Periplaneta americana*. J Comp Physiol 107:49–65

Schoeni R (1987) Das wirtsgebundene Aggregationspheromon der tropischen Buntzecke *Amblyomma variegatum* (Acari: Ixodidae). Thèse Universitè Neuchâtel, Switzerland

Schoeni R, Hess E, Blum W, Ramstein K (1984) The aggregation-attachement pheromone of the tropical bont tick *Amblyamma variegatum* Fabricius (Acari: Ixodidae): isolation, identification and action of its components. J Insect Physiol 30:613–618

Schultz TH, Flath RA, Stern DJ, Mon TR, Teranishi R, McKenna Kruse S, Butler B, Howard WE (1988) Coyote estrous urine volatiles. J Chem Ecol 14:701–712

Seelinger G (1977) Der Antennenendzapfen der tunesischeo Wüstenassel *Hemilepistus reaumuri*, ein komplexes Sinnesorgan (Crustacea, Isopoda). J Comp Physiol 113:95–103

Sinitsina EE (1974) Electrophysiological reactions of Haller's organ neurons in the tick *Hyalomma asioticum* P.Sch. and E.Sch. to odor. Parazitologiya 8:223–226

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

Smith PW, Parks OW, Schwartz DP (1984) Characterization of male goat odors: 6-trans nonenal. J Dairy Sci 67:794–801

Steullet P, Guerin PM (1992a) Perception of breath componeots by the tropical bont tick, *Amblyomma variegatum* Fabricius (Ixodidae). I. CO_2-excited and CO_2-inhibited receptors. J Comp Physiol A 170:665–676

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

Tichy H, Loftus R (1983) Relative excitability of the anteooal olfactory receptors in the stick insect, *Carausius morosus* L.: in search of a simple, concentratioo-independent odor-coding parameter. J Comp Physiol 152:459–473

Tschinkel WR (1975) Uousual occurrence of aldehydes and ketones in the defensive secretion of the tenebrioaid beetle, *Eleodes beameri*. J Insect Physiol 21:659–671

Visser JH (1986) Host odour perception in phytophagous insects. Annu Rev Entomol 31:121–144

Wadhams LJ (1982) Coupled gas chromatography – siogle cell recordiog: a new technique for use in the analysis of insect pheromones. Z Naturforsch 37c: 947–952

Wallace JW, Mansell RL (1976) Biochemical interactioo between plants and insects. Plenum Press, New York

Welsh DJ, Watts CD (1990) Collection and identification of trace organic compounds in atmospheric depositioo from a semirural site in the U.K. Intern J Environ Anal Chem 38:185–198

Wood WF, Leahy MG, Galun R, Prestwich GD, Meinwald J, Purnell RE, Payne RC (1975) Phenols as pheromones of ixodid ticks: a general pheoomenon? J Chem Ecol 1:501–509

Zlatkis A, Liebich HM (1971) Profile of volatile metabolites in human urine. Clio Chem 17:592–594

J Comp Physiol A (1993) ■■:■■■-■■■

Journal of Comparative Physiology A

Identification of vertebrate volatiles stimulating olfactory receptors on tarsus I of the tick *Amblyomma variegatum* Fabricius (Ixodidae)

II. Receptors outside the Haller's organ capsule

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Abstract. Bovine odour excites olfactory receptor(s) in a wall-pore olfactory sensillum on the anterior pit of Haller's organ in *Amblyomma variegatum*. Gas chromatography-coupled electrophysiology recordings from this sensillum reveal the presence of 4 active compounds in bovine odour. The two strongest stimulants were identified as 2-nitrophenol and 4-methyl-2-nitrophenol by gas chromatography-coupled mass spectrometry, and by matching electrophysiological activity of synthetic analogues. Synthetic analogues of known vertebrate-associated volatiles also stimulate other olfactory receptors in sensilla on the surface of tarsus I: a lactone receptor responding to γ -valerolactone and 6-caprolactone; different fatty acid receptor types responding best to either pentanoic acid, 2-methylpropanoic acid or to butanoic acid; three receptors responding to NH_3; and one receptor responding to 3-pentanone. Gas chromatography-coupled mass spectrometry analysis of vertebrate volatiles revealed presence of a number of these olfactory stimulants in concentrates of rabbit and steer odour, i.e. 2-methylpropanoic acid, butanoic acid, 3-methylbutanoic acid, pentanoic acid, and γ-valerolactone.

Key words: Tick – Haller's organ – Olfactory receptors – Fatty acids – Ammonia – γ-Valerolactone – Nitrophenols

Introduction

While the previous paper (Steullet and Guerin 1993, companion paper) deals with the identification of vertebrate volatiles that stimulate olfactory receptors in the capsule of Haller's organ on leg pair I of *Amblyomma variegatum* (Acari, Ixodidae), this present study aims to identify host-odour volatiles that excite receptors housed in sensilla located outside the capsule. The 7 olfactory sensilla within the capsule of Haller's organ carry various host-odour receptors, i.e. CO_2-, sulfide-, aliphatic aldehyde-, benzaldehyde-, 2-hydroxybenzaldehyde-, and lactone-receptors (Steullet and Guerin 1992a, b, 1993): Adult *A. variegatum* also possess 12 other wall-pore sensilla on the surface of the tarsus of the first pair of legs (Hess and Vlimant 1982, 1983) (Fig. 1). The latter authors observed that the position of sensilla on the tarsus is conserved between tick species, and distinguished between different morphological types of wall-pore single-walled and wall-pore double-walled sensilla (according to Altner's classification, Altner et al. 1977). Based on detailed ultrastructural studies, Hess and Vlimant estimated that the 12 wall-pore sensilla on the surface of tarsus I in *A. variegatum* carry between 50 and 65 olfactory receptor cells. Only a few of them have been physiologically characterized in *A. variegatum*, or indeed in any other tick species. Receptors sensitive to 2,6-dichlorophenol (a tick pheromone component) were described in the DI.1 and DII.1 sensilla (Fig. 1) of *A. variegatum* (Waladde and Rice 1982; Schoeni 1987), and in a wall-pore sensillum distal to the Haller's organ in *Amblyamma americanum* and *Rhipicephalus appendiculatus* (Haggart and Davis 1981), and *Ixodes ricinus* (Thonney 1987). 2-Nitrophenol, a component of the aggregation-attachment pheromone of *A. variegatum*, excited receptor(s) in a wall-pore sensillum on the anterior pit of Haller's organ of this species (Schoeni 1987). Finally, NH_3-sensitive receptors were discovered in sensilla of the anterior pit, and also in more proximally placed sensilla on the tarsus of *Rhipicephalus sanguineus* (Haggart and Davis 1980).

Materials and methods

Animals. Male *A. variegatum* were reared and maintained in the conditions described previously (Steullet and Guerin 1993).

Electrophysiology. Tick was immobilized on a piece of perspex with double-sided sticky tape. The tip of the sensillum was cut with the

Abbreviations: GC-EL, gas chromatography-coupled electrophysiological recording; GC-MS, gas chromatography-coupled mass spectrometry

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broken tip of a heat-pulled glass rod (1.5 mm diameter) mounted on a micromanipulator and oscillating at ca. 120 MHz induced by a piezo electric transducer disk (n° 4322.020.177721, Philips) (Gödde 1989). A glass electrode (tip diameter: 10–20 µm) mounted on a micromanipulator and filled with 0.2 *M* KCl was brought into contact with the cut tip of the sensillum, whereas the reference glass electrode filled with 0.2 *M* NaCl was introduced into the coxa of the anterior leg. The coxa was previously pinched with blunt forceps to prevent muscle discharge during recordings. Experiments were achieved either under an inverted microscope (Nikon Diaphot TMD) for recording receptor activity in the DI.1, DII.1, DII.5, DII.6, DIII.1, and DIV.1–4 sensilla, or under a stereomicroscope (Olympus SZH) for recordings from the LAII.1, VII.1, and VII.4 sensilla (see Fig. 1). Sensilla are named after Hess and Vlimant (1982). Amplification, data acquisition and spike analysis were accomplished as described in Steullet and Guerin (1992a, b).

Stimulation. Stimulus delivery system as well as the synthetic chemicals used to study specificity of the olfactory receptors were as in Steullet and Guerin (1993). Human breath, human axillary secretion, and bovine and rabbit odours collected on Porapak Q were used as sources of natural stimuli (details in Steullet and Guerin 1992b and 1993).

Gas chromotography-coupled electrophysiology recordings (GC-EL) and gas chromatography-coupled mass spectrometry (GC-MS). GC-EL, with column effluent split, was used to locate active components of bovine and/or rabbit odour extracts. Two thirds of the effluent was sent to a flame ionisation (FID) or an electron capture detector (ECD), and one third to an electrophysiological preparation of an olfactory sensillum as biological detector. Compounds capable of stimulating olfactory receptors in sensilla on the surface of the tarsus were located due to a strong increase in spike frequency of the responding receptors. GC-MS was subsequently employed to identify these active chromatographic peaks. For further details of the GC-EL methodology and GC-MS, see Steullet and Guerin (1993). Identification of an electrophysiologically active peak in an extract was first based on a match between its mass spectrum and that of a known product stored in a computer-based library of the GC-MS. The mass spectrum and Kovat's index of the stimulant to be identified were then compared with those of the proposed synthetic analogue injected under the same conditions and on the same chromatographic phase (DBWAX). The biological activity of the synthetic product was subsequently confirmed by electrophysiology experiments on the olfactory receptor concerned. Thus, synthetic 2-nitrophenol and 4-methyl-2-nitrophenol (>98% purity) were tested on receptors to confirm their identification as olfactory stimulants in GC-EL and GC-MS analysis of odour extracts.

Concentrates of air from rooms without rabbits or steer (blanks) were also analysed by GC-MS to check for the occurrence of identified olfactory stimulants in ambient air, and the amount was compared with that found in bovine and rabbit odour extracts collected under the same conditions. For this purpose, 2-bromophenol (1.64 µg) was added as internal standard into 1.5 ml of rabbit, bovine, and blank extracts before concentration and analysis by GC-MS. Search for nitrophenols in each extract at the retention time of the corresponding synthetic analogues was made with their molecular ions whose mass to charge ratio (M/Z) was respectively 139 for 2-nitrophenol and 153 for 4-methyl-2-nitrophenol. Quantification was achieved by integrating the area under the peak of these characteristic ions. Abundance of each product in an extract was normalized with reference to 2-bromophenol using the peak area of one of its characteristic fragment ions (M/Z = 172).

Results

Human breath, human axillary secretion, and rabbit odour collected on Porapak failed to clearly stimulate olfactory receptors in any of the wall-pore sensilla located on the surface of the tarsus. Bovine odour collected on Porapak evoked a response of receptor(s) in the wall-pore DII.1 sensillum on the anterior pit of Haller' organ (Fig. 1).

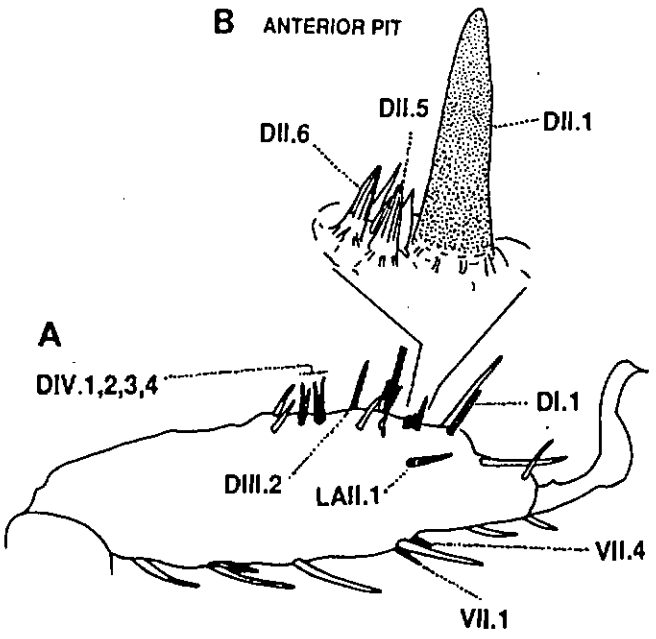

Fig. 1. A Tarsus of leg I of an adult *A. variegatum* indicating location and the name ascribed to each olfactory sensillum (*stippled*). *Arrow* indicates the slit-like opening to the capsule of Haller's organ which contains 7 additional olfactory sensilla. **B** Detailed view of the anterior pit sensilla of Haller's organ

Identification of bovine volatiles stimulating receptor(s) in the DII.1 sensillum

Gas chromatography-coupled electrophysiology analyses (GC-EL) with the DII.1 sensillum revealed 4 active compounds in bovine, but none in rabbit odour collected on Porapak (Table 1). Although two only occasional but weak stimulants were not identified, the other two were identified as 2-nitrophenol and 4-methyl-2-nitrophenol. Identification of peak 2 as 2-nitrophenol was based on 1) matching Kovat's index of the GC-EL active peak with the synthetic analogue (Table 1), 2) the presence of the molecular ion (M/Z = 139) of 2-nitrophenol in the unknown in GC-MS analyses at the same retention time as the synthetic analogue, and 3) matching electrophysiological activity of the synthetic analogue (Fig. 2). A full mass spectrum was not obtained because of the small amount of peak 2 in the extract and the presence of coeluting products. Peak 3 was identified as 4-methyl-2-nitrophenol on the basis of 1) matching mass-spectrum with the synthetic analogue, 2) correspondence of the Kovat's index of the unknown in GC-EL with the synthetic analogue (Table 1), 3) matching electrophysiological activity of the synthetic analogue (Fig. 2). Some 10–20 ng of 2-nitrophenol and 200–300 ng of 4-methyl-2-nitrophenol were found in 1.5 ml bovine odour extract after collecting 300 l of bovine odour-laden air on Porapak. Rabbit

Table 1. Identified constituents of bovine odour which stimulated olfactory receptors in the DII.1 sensillum of male *A. variegatum*

Peak number	Olfactory stimulant	a) Identification criteria	b) Odour source	c) Kovat's index in GC-EL	d) Kovat's index in GC-MS	e) Kovat's index of standards in GC-MS	f) Response occurrence
1	unidentified		steer	1803	–	–	1/8
			rabbit	–	–	–	0/5
2	2-nitrophenol	MKE	steer	1812±11	1818	1818	7/8
			rabbit	–	1814	1818	0/5
3	4-methyl-2-nitrophenol	MKE	steer*	1914±10	1922	1919	7/8
			rabbit	–	1919	1919	0/5
4	unidentified		steer	1992±15	–	–	1/8
			rabbit	–	–	–	0/5

This table is based on gas chromatography-coupled electrophysiology (GC-EL) and gas chromatography-mass spectrometry (GC-MS) analysis of bovine and rabbit odour collected on Porapak Q. Both types of analyses were made on the same gas chromatographic phase-DBWAX. a) Different criteria on which identification of a particular odour constituent was based, M – matching mass spectra, (in the case of 2-nitrophenol identification was based on presence of the molecular ion (M/Z=139) in peak 2 at the same retention time as the synthetic analogue), K – matching Kovat's index, and E – electrophysiological activity matching with that of the synthetic analogue. b) Analyses were made of bovine and rabbit odour as collected on Porapak and * indicates that the active compound was also detected by GC-MS in a bovine skin wash. c) Mean Kovat's index (±standard deviation) of the active peaks in GC-EL analyses. d) Kovat's index of the active peak located by GC-MS. e) Kovat's index of the synthetic product corresponding to that of the biologically active peak in GC-MS. f) Number of GC-EL analyses in which a response was observed/out of the total number of analyses with the DII.1 sensillum. 2-Nitrophenol and 4-methyl-2-nitrophenol were present at low amounts in rabbit odour extracts, but in insufficient quantity to elicit a response in GC-EL

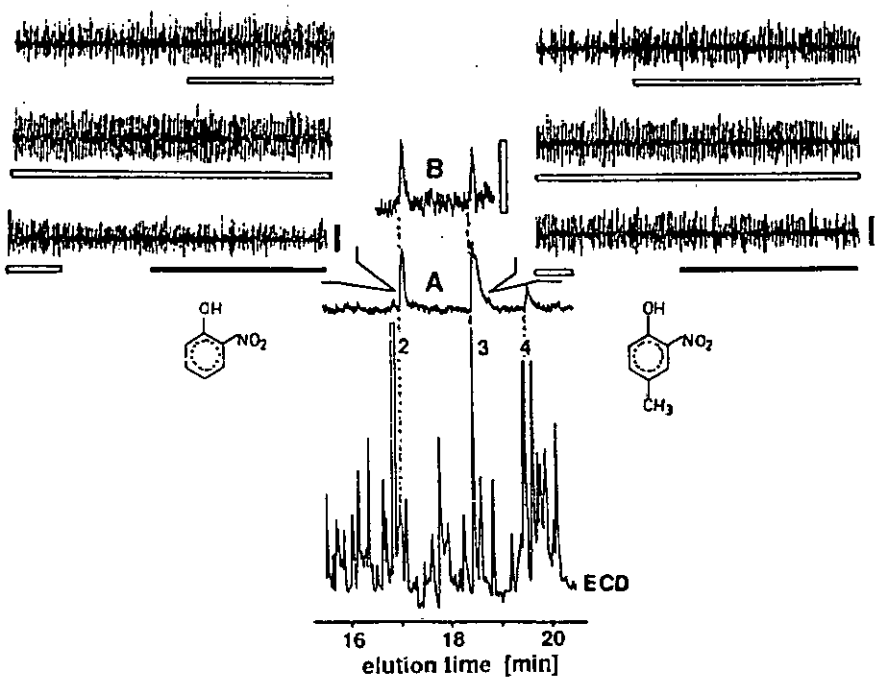

Fig. 2A, B. Section of a bovine odour extract analysis by gas chromatography-coupled electrophysiology of the DII.1 sensillum of a male *A. variegatum* (for details of technique, see text). The *lower trace* details part of the chromatogram of a bovine extract obtained with an ECD (electron capture detector); the *upper traces* (A and B) represent the summed frequencies of all cells recorded from the DII.1 sensillum (frequency to voltage converted signal) during elution (A) of components of the bovine odour extract and (B) during elution of 10 ng each 2-nitrophenol and 4-methyl-2-nitrophenol, respectively, at the same retention times as peak 2 and 3 of the extract. Peaks are numbered as in Table 1. Spike trains generated in A during elution of 2-nitrophenol (peak 2) and 4-methyl-2-nitrophenol (peak 3) are provided. *Hollow horizontal bars*: approximate elution time of the active compounds; *hollow verticol bor* 50 impulses/s; *solid horizontol bor* 1 s; *solid verticol bar* 0.3 mV. The great complexity of spike trains recorded from the DII.1 sensillum, which contain 14 receptor cells, prohibited us from properly analysing which and how many receptors responded to these nitrophenols

odour extract also contained small quantities of both compounds but in insufficient amount to evoke a response in GC-EL, i.e. 1–5 ng of both nitrophenols in 1.5 ml rabbit odour extract after collection of a similar volume of rabbit odour-laden air on Porapak. By contrast, extracts of room air without steer or rabbits contained no or hardly detectable amounts of either of these substances.

Receptors responding to vertebrate volatiles

Apart from receptor(s) in the DII.1 sensillum which responded to nitrophenols, receptors housed in this and other sensilla were excited by volatiles normally associated with vertebrate odours. The following types of receptors were found:

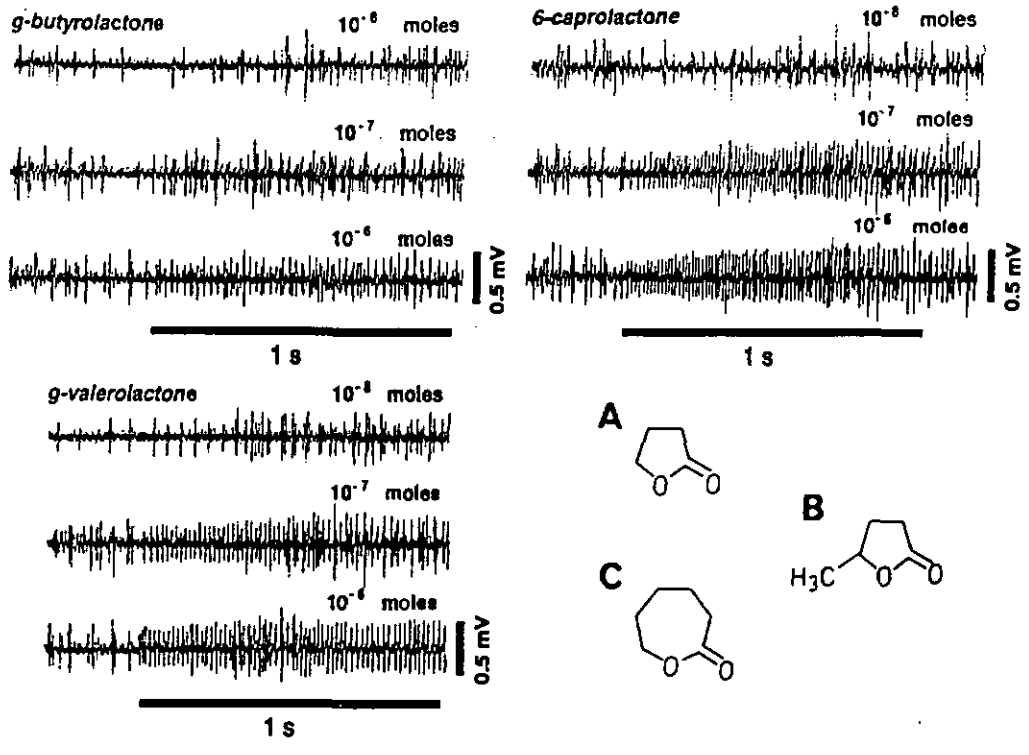

Fig. 3. Responses of an olfactory receptor of the DI.1 sensillum in male *A. variegatum* to γ-butyrolactone (*A*), γ-valerolactone (*B*), and 6-caprolactone (*C*). Stimulus intensity (number of moles in the stimulus cartridge) is indicated above each trace. Due to the multicellular responses, it was often not possible to clearly distinguish the spikes involved in responses to stimulation with low doses of these lactones (10^{-6} moles in the stimulus cartridge). *Horizontal bars* 1 s stimulation

Nitrophenol receptor(s). Both 2-nitrophenol and 4-methyl-2-nitrophenol stimulated receptor(s) in the DII.1 sensillum (Fig. 2). Both nitrophenols were equally active. Since an estimation of 10^9 molecules of 2-nitrophenol/cm^3 air in GC-EL experiments still strongly excited receptor(s) in the DII.1 sensillum the threshold can be considered at much below this level. However, recordings from the DII.1 sensillum, which contain 14 receptor cells, were extremely complex (Fig. 2). The great number of chemosensitive units firing did not permit either clear determination of the exact number of cells responding or if 2-nitrophenol and 4-methyl-2-nitrophenol stimulated the same receptor(s).

Lactone receptor. One receptor in the DI.1 sensillum was sensitive to γ-valerolactone, found only in traces in bovine and rabbit odour (Fig. 3). Double successive stimulations indicated that this receptor also responded to 6-caprolactone. γ-Butyrolactone was only a weak stimulant at a high concentration (10^{-6} moles in the stimulus cartridge). The difficulty of distinguishing the lactone receptor from the four other receptors housed in the same sensillum during weak stimulation prohibited the establishment of clear dose-response curves (Fig. 3).

Acid receptors. Short-chain fatty acid receptors in the DII.1 sensillum responded best to pentanoic acid (Fig. 4). 3-Methylbutanoic acid and butanoic acid were also active but only at relatively high concentration (Fig. 4). Nevertheless, the complexity of the multicellular recordings from the DII.1 sensillum (with 14 receptor cells) inhibited us from properly studying the responding receptor(s) and from establishing a dose response-curve. Two receptor cells of another sensillum (DII.5) on the anterior pit also responded to C_4 and C_5 fatty acids; hexanoic,

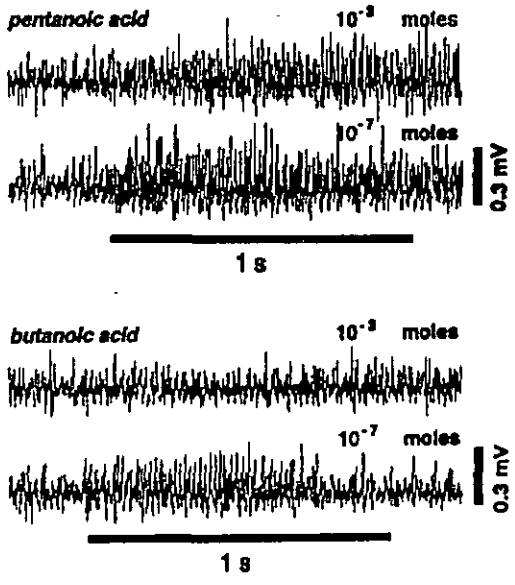

Fig. 4. Respooses of olfactory receptors of the DII.1 sensillum of male *A. variegotum* to butanoic acid and pentanoic acid. Stimulus intensity (number of moles in the stimulus cartridge) is indicated above each trace. *Horizontol bars* 1 s stimulation. The great complexity of spike trains recorded from the DII.1 sensillum, which contain 14 receptor cells, prohibited us from properly analysing the receptor(s) responding to these short-chain fatty acids

heptanoic and nonanoic acids only weakly excited these receptors. By contrast with the DII.1 sensillum, the DII.5 sensillum only contains three receptor cells. It was thus possible to differentiate the two fatty receptors of the latter sensillum according to spike shape and amplitude as well as by double successive stimulations (Fig. 5). One receptor in this sensillum (type 1 in Figs. 5 and 6) was most strongly stimulated by butanoic acid but also clear-

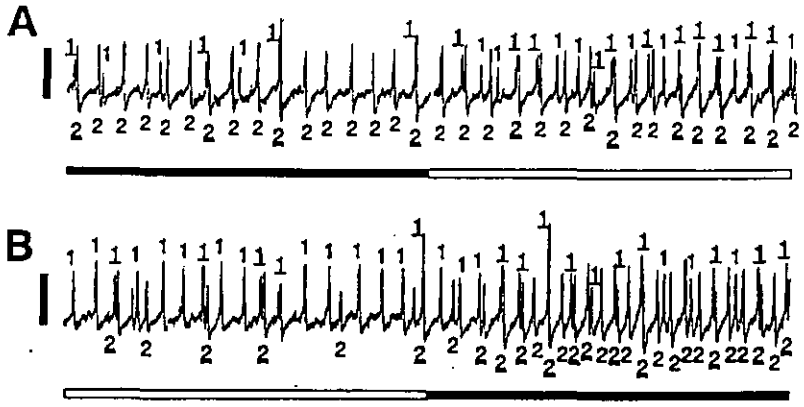

Fig. 5A, B. Double successive stimulations of fatty acid receptors in the DII.5 sensillum with 10^{-8} moles of 2-methylpropanoic acid (*solid horizontal bar*) and 10^{-6} moles of butanoic acid (*hollow horizontal bar*). Note that firing of receptor type 1 increased at the beginning of stimulation with butanoic acid (in A), whereas firing of receptor type 2 increased at the beginning of stimulation with 2-methylpropanoic acid (in B). Each trace (A and B) corresponds to a 700 ms spike train. Spikes with underlined numbering are near or overlapping events. *Vertical bar* 0.5 mV

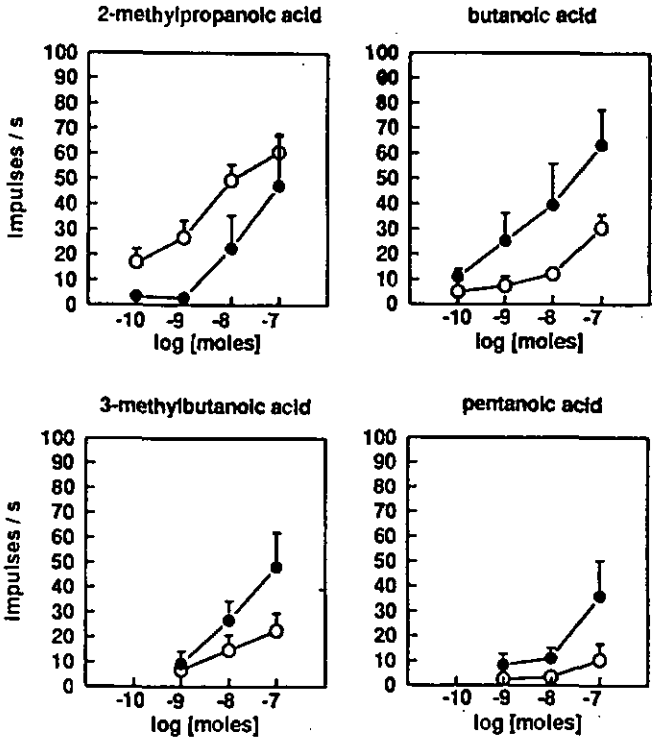

Fig. 6. Responses of two types of fatty acid receptors in the DII.5 sensillum located on the anterior pit of the Haller's organ in male *A. variegatum* to doses (moles in the stimulus cartridge) of 2-methylpropanoic, 3-methylbutanoic, butanoic, and pentanoic acid plotted against spike frequency calculated for the first 400 ms of the responses ($n = 8$ different ticks). *Trend lines* connect mean values; bars are standard deviations. *Solid circle*: acid receptor type 1 most sensitive to butanoic acid; *hollow circle*: acid receptor type 2 most sensitive to 2-methylpropanoic acid

ly by 2-methylpropanoic acid, 3-methylbutanoic acid and, to a lesser extent, by pentanoic acid. The second receptor was most sensitive to 2-methylpropanoic acid (type 2 in Figs. 5 and 6). To establish if the two fatty acid receptors of the DII.5 sensillum are capable of specifically coding for the different short-chain fatty acids, a firing ratio (Q) between the intensity of the response of receptor type 1 and type 2 to the different acids at various concentrations was calculated (Table 2). Q values for the dose series of 2-methylpropanoic acid were significantly different from those obtained with a similar dose series of 3-methylbutanoic acid ($P \leq 0.05$, ANOVA with the General Linear Model procedure of SAS). Both series of Q values were concentration-dependent ($P \leq 0.05$, Table 2). The butanoic acid and pentanoic dose-series did not elicit divergent Q values ($P > 0.05$), and these Q values were concentration-independent ($P > 0.05$, Table 2). However, Q values for these unbranched fatty acids differed significantly from those due to the branched fatty acids ($P \leq 0.05$, Table 2).

Butanoic and 2-methylpropanoic acids were the most prominent short-chain fatty acids in bovine and rabbit odour, respectively, as collected on Porapak (accounting for > 75% of the total amount of the C_4 and C_5 fatty acids present). However, no increase in spike frequency of the acid receptors was observed when fatty acids contained in bovine and rabbit odour eluted during GC-EL analysis of extracts, most probably because the receptors were not sensitive enough to detect the amounts present. Finally, despite its strong acidic odour, human axillary secretion did also not excite the acid receptors of either the DII.1 or DII.5 sensilla.

Table 2. Firing ratio Q (mean ± SD) of fatty acid receptor type 1 to receptor type 2 in the DII.5 sensillum of 8 different adult *A. variegatum* in response to stimulation with 4 short-chain fatty acids at 4 concentrations

	Number of moles in the stimulus cartridge				
	10^{-10}	10^{-9}	10^{-8}	10^{-7}	
2-methylpropanoic acid	0.7 ± 0.2	0.8 ± 0.3	0.9 ± 0.5	0.9 ± 0.4	§ a
3-methylbutanoic acid	1.4 ± 0.5	1.3 ± 0.6	1.8 ± 0.2	2.0 ± 0.7	§ b
Butanoic acid	2.3 ± 0.3	3.1 ± 1.0	2.9 ± 0.7	2.0 ± 0.5	c
Pentanoic acid	2.8 ± 1.1	3.2 ± 1.2	3.4 ± 0.9	2.5 ± 0.8	c

§ Signifies that Q is concentration-dependent for a defined stimulus, and Q for each stimulus is assigned a different letter when significantly different ($P \leq 0.05$ in both cases, ANOVA with the General Linear Model procedure on SAS)

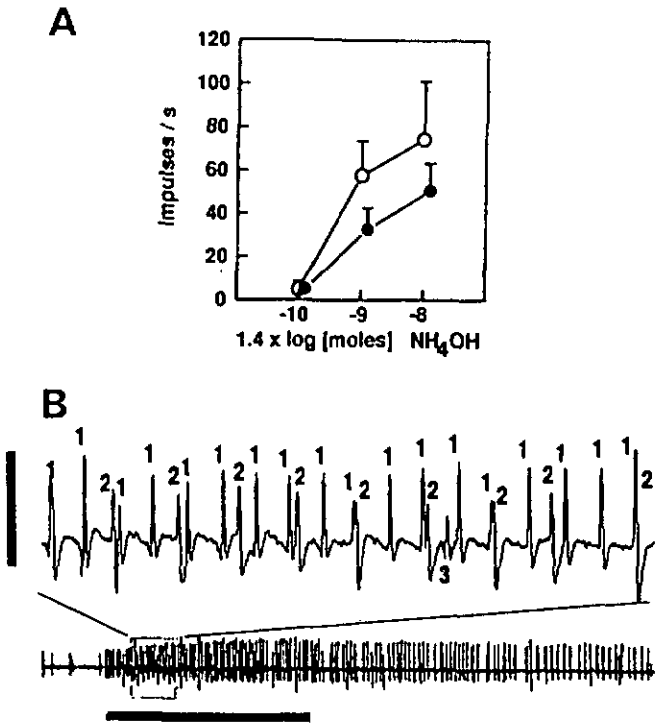

Fig. 7A, B. Responses of two NH_3 receptors of the DII.6 sensillum located on the anterior pit of Haller's organ in male *A. variegatum*. A Dose (moles in the stimulus cartridge) of NH_4OH plotted against spike frequency calculated for the first 400 ms of the responses ($n = 6$ different ticks). *Trend lines* connect mean values; *bars* are standard deviations. *Hollow circle*: NH_3 receptor type 1; *solid circle*: NH_3 receptor type 2. B Representative response of the NH_3 receptor types 1 and 2 to stimulation with 1.4×10^{-9} moles NH_4OH in the stimulus cartridge. The *upper trace* provides detail of 250 ms of the response. A spike of low amplitude from a third receptor also figures on this trace. *Horizontal bar* 1 s stimulation; *vertical bar* 0.5 mV

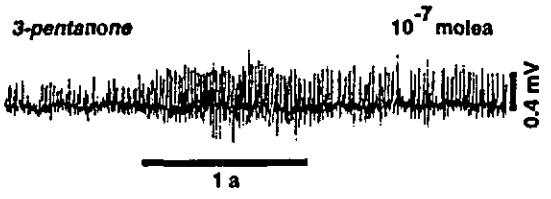

Fig. 8. Representative response of a receptor located in one of the DIV sensilla on the tarsus of adult *A. variegatum* to 10^{-7} moles 3-pentanone in the stimulus cartridge

NH_3 receptors and others. Two receptors responded strongly to the same range of NH_3 concentrations in the DII.6 sensillum such that between 10^{-10} and 10^{-9} moles of NH_4OH in the stimulus cartridge was sufficient to evoke a response (Fig. 7). However, the dose-response curve of the two receptors differed significantly ($P \leq 0.05$, ANOVA with the General Linear Model procedure of SAS). Another receptor in one of the DIV sensilla was also weakly stimulated by high, but not physiologically relevant concentrations of NH_3. This receptor was about 100 times less sensitive to NH_3 than either of the two receptors in the DII.6 sensillum. Except for a receptor in another of the DIV sensilla which responded to 3-pentanone (Fig. 8), no further receptors in the wall-pore sensilla located outside the capsule of Haller's organ on tarsus I of *A. variegatum* were characterized.

Discussion

Olfactory receptors in wall-pore sensilla on the surface of the tarsus of leg I in *A. variegatum* respond to constituents of vertebrate odours. GC-EL analyses of bovine odour presented here demonstrate the response of receptor(s) to nitrophenols in the large DII.1 olfactory sensillum on the anterior pit of Haller's organ. Other receptors, which were found in this and other sensilla, were sensitive to either lactones, short-chain fatty acids, NH_3, or 3-pentanone. However, no responses were obtained from receptors in any of these sensilla to vertebrate odours tested under the headings of human breath, human axillary secretion, or rabbit odour collected on Porapak. This contrasts with the responses of a range of olfactory receptors within the capsule of Haller's organ to different constituents of the same extracts (Table 3, Steullet and Guerin 1992a, b, 1993).

The largest wall-pore sensillum (DII.1) on the anterior pit of Haller's organ presumably bears several receptors for the aggregation-attachment pheromone component 2-nitrophenol as evidenced by the multiunit responses of Schoeni (1987). In the present study, GC-EL experiments employing this sensillum reveals the presence of four active components in odour of tick-naive steer collected on Porapak, i.e. 2-nitrophenol, 4-methyl-2-nitrophenol, and two unidentified volatiles. 4-Methyl-2-nitrophenol is the most abundant of the 4 stimulants in the odour and is also present in skin wash of steer. GC-MS analysis of air from an unoccupied stall (controls) indicates none, or at most traces of these nitrophenols, suggesting that these products are true vertebrate volatiles although neither of them has previously been reported to our knowledge from vertebrates. However, these compounds are known as air pollutants (Welsh and Watts 1990), but as such are likely to be less prevalent in the ambient air of the African habitat of *A. variegatum* than in the suburban environment in which controls were made for this study. There can be little doubt about the biogenic origin of nitrophenols, as 2-nitrophenol is also a major component of the aggregation-attachment pheromone of both *A. variegatum* and *A. hebraeum* (Schoeni et al. 1984; Apps et al. 1988). It is produced in high amounts in dermal glands (type 2) of males after successful attachment and feeding on a host (Diehl et al. 1991), thereby assuring attraction, aggregation, and attachment of conspecifics at the same feeding site (Schoeni et al. 1984; Norval et al. 1989; Delot 1990). The presence of nitrophenols in odours of even tick-naive steer could favour their infestation by pioneer males. *A. variegatum* does show a preference for parasitizing bulls over goats (Barré et al. 1991), and attach better on cattle than on sheep or rabbits (Norval et al. 1992). The present study shows that quantities of nitrophenols found in bovine odour were higher than in rabbit odour extracts, the latter containing 100 times less 4-methyl-2-nitrophenol. Could it be that *Amblyomma* has developed

P. Steullet, P.M. Guerin: Olfactory receptors of *Amblyomma*. II

Table 3. Responses and locations of characterized olfactory receptors on tarsus I of male *A. variegatum* to different vertebrate odours (human breath, human axillary secretion, and bovine and rabbit odour concentrates as collected on Porapak)

a) Receptor location	b) Sensillum type	Human breath	Human axillary secretion	Bovine odour	Rabbit odour	Characterized receptors	References
CI	wp-sw	–	–	(+)	(+)	lactone receptor	Steullet and Guerin (ibid.)
CII	wp-sw	–	–	–	–	methylsalicylate receptor	Hess and Vlimant (1984)
CIII	wp-sw	+ + +	–	–	–	CO_2-excited receptor	Steullet and Guerin (1992a)
CIII	wp-sw	+ + +	+ +	–	–	sulfide receptor type 2	Steullet and Guerin (1992b)
CIV	wp-sw	+ + +	–	–	–	CO_2-inhibited receptor	Steullet and Guerin (1992a)
CV	wp-sw	+ + +	+ +	–	–	sulfide receptor type 1	Steullet and Guerin (1992b)
CVI*	wp-sw	–	–	+ +	+ + +	benzaldehyde receptor	Steullet and Guerin (ibid.)
CVI*	wp-sw	–	–	+ +	+ + +	2-hydroxybenzaldehyde receptor	Steullet and Guerin (ibid.)
CVI*	wp-sw	–	+ +	+ +	+ + +	aliphatic aldehyde receptors	Steullet and Guerin (ibid.)
DI.1	wp-sw	–	–	(+)	(+)	lactone receptor	this paper
DI.1	wp-sw	–	–	–	–	2,6-dichloropbenol receptor	Waladde 1982, Schoeni 1987, this paper
DII.1	wp-sw	–	–	+ +	(+)	nitrophenol receptor(s)	Schoeni (1987), this paper
DII.1	wp-sw	–	–	(+)	(+)	pentanoic acid receptor(s)	this paper
DII.5	wp-dw	–	–	(+)	(+)	2-methylpropanoic acid receptor	this paper
DII.5	wp-dw	–	–	(+)	(+)	butanoic acid receptor	this paper
DII.6	wp-dw	–	–	–	–	NH_3 receptors §	this paper
DIV.group	wp-dw	–	–	–	–	receptor sensitive to high doses of NH_3 §	this paper
DIV.group	wp-dw	–	–	–	–	receptor sensitive to high doses of 3-pentanone	this paper

+ + + Strong response, + + medium response, (+) stimulant present in the extract but in insufficient quantity to elicit more than a slight response, – no response. a) Name assigned to the sensillum according to its location on the surface of the tarsus (DI-DIV of Fig. 1) or region within the capsule (CI-CVI of Fig. 1 in Steullet and Guerin 1993); * signifies presence of two sensilla in the region from which recordings were made within the capsule; b) morphological type of sensillum as described by Hess and Vlimant (1982): wall-pore single-walled sensillum (*wp-sw*), wall-pore double-walled sensillum (*wp-dw*). § NH_3 is a common vertebrate-associated volatile, e.g. in urine. No receptors from the DIII.2, LAII.1, VII.1, and VII.4 sensilla (Fig. 1) were characterized

a pheromone system which enhances attractivity of what proves to be a suitable host for pioneer males by secreting high amounts of a host-associated volatile? This would not constitute the first report on use of vertebrate-associated volatiles as components of an aggregation-attachment pheromone in the genus *Amblyomma*. 2-Methylpropanoic acid, nonanoic acid, 2-nitrophenol, and benzaldehyde are components of the aggregation-attachment pheromone of *A. variegatum* and/or *A. hebraeum*, but also vertebrate-associated volatiles to which some tick olfactory receptors are sensitive (Steullet and Guerin 1993).

A receptor in the DI.1 sensillum on the knoll distal to Haller's organ responds strongly to both γ-valerolactone and 6-caprolactone, as confirmed by double successive stimulations. The specificity of this receptor differs from that of another lactone-sensitive receptor found in the capsule of Haller's organ in *A. variegatum* which is more selectively sensitive to γ-valerolactone (Steullet and Guerin 1993). γ-Valerolactone is present in traces in bovine and rabbit odour collected on Porapak. But the quantity of γ-valerolactone in bovine odour extract was not sufficient to evoke a clear response of this receptor in GC-EL analyses employing the DI.1 sensillum, as was the case for the lactone receptor in the capsule of Haller's organ (Steullet and Guerin 1993). Lactones are reported from many vertebrate odours (Goetz et al. 1988; Burger et al. 1990), but are unknown as stimulants for other haematophagous arthropods.

Two wall-pore sensilla on the anterior pit of Haller's organ of *A. variegatum* possess fatty acid receptors: the wall-pore single-walled DII.1 sensillum with receptor(s) most sensitive to pentanoic acid, and the wall-pore double-walled DII.5 sensillum with one receptor most sensitive to butanoic acid and a second most responsive to 2-methylpropanoic acid. Comparison of the spike frequencies of these receptors in response to stimulation with the different acids indicates that the two acid receptors of the DII.5 sensillum can specifically code for 2-methylpropanoic acid and 3-methylbutanoic acid, but cannot discriminate between butanoic and pentanoic acid. However, discrimination between these straight-chain fatty acids may be possible with the acid receptor(s) of the DII.1 sensillum which are most sensitive to pentanoic acid. Equipped with these acid receptors, *A. variegatum* may be able to discriminate between mixtures of short-chain fatty acids which are widespread in vertebrate-associated volatiles (Müller-Schwarze et al. 1974; Ayorinde et al. 1982; Fox 1982; Albone 1984; Goetz et al. 1988; Kanda et al. 1990). GC-MS analysis of the odour extracts collected on Porapak indicated that butanoic was the most abundant fatty acid in bovine odour, whereas 2-methylpropanoic acid predominated in that of rabbit. However, the acid receptors of both the DII.1 and the DII.5 sensilla were not sensitive enough to respond to the fatty acid amounts present in extracts injected for GC-EL experiments. Furthermore, despite of its acidic odour, human axillary secretion did not clearly excite the

fatty acid receptors of *A. variegatum*. This may be due to the fact that human axillary secretion contains an abundance of different branched and unbranched, saturated and unsaturated C_6 to C_{11} fatty acids with (E)-3-methyl-2-hexenoic acid as the major component (Zeng et al. 1991, 1992), but lacks significant amounts of the shorter acids for which the tick possesses receptors. Short-chain fatty acids also act as olfactory stimulants for several blood-sucking insects, i.e. mosquitoes (Lacher 1967) and *Triatoma infestans* (Bernard 1974). Furthermore, these volatiles elicit probing behaviour in *Stomoxys calcitrans* (Hopkins 1964) and attraction in the sheep head fly *Hydrotaea irritans* (Thomas et al. 1985).

Two receptors sensitive to NH_3 are present in the DII.6 sensillum, each showing its own response profile. Another receptor in the DIV wall-pore sensilla group also responds to NH_3, but only at much higher doses. Similar NH_3 receptors exist in another tick species, *Rhipicephalus sanguineus* (Haggart and Davis 1980). NH_3 is known as a kairomone for some haematophagous arthropods, i.e. as a probing stimulus for *Stomoxys calcitrans* (Gatehouse 1970), and attractant for Tabanidae (Hribar et al. 1992). Although NH_3 is widely represented in vertebrate odours, none of the tick NH_3 receptors responded to the extracts tested here. One reason may be that the porous polymer (Porapak), which we used to collect vertebrate odours, is known to have a low affinity for polar low molecular weight volatiles such as NH_3 and amines (Sugisawa 1981).

Finally, as already described by Waladde (1982) and Schoeni (1987), receptors in the DI.1 and the DII.1 sensilla are highly sensitive to the common tick sex pheromone product, 2,6-dichlorophenol (Table 3). No clear responses were obtained from these receptors in single-unit recordings with whole odour extracts of vertebrates, or to individual components of these extracts in GC-EL analyses. These findings confirmed our GC-MS analyses which indicated that the halogenated phenol was not present in detectable amount in the vertebrate odours collected for this study.

Although Lees (1948) suggested that the olfactory receptors responsible for host-odour perception in ticks are mainly located within the capsule of Haller's organ, this study clearly demonstrates that sensilla located elsewhere on the tarsus also respond to some vertebrate-associated volatiles. However, these sensilla do not only contain olfactory receptors. Although human breath delivered directly to the tarsus activated receptors in both the DII.5 and DII.6 sensilla (Steullet, unpublished), the same stimulus delivered in a temperature- and humidity-controlled airflow to the preparation did not excite any receptor. This suggests that thermoreceptors might be involved in the response to breath. In some insects, grooved double-walled sensilla contain olfactory receptors together with a cold unit (Altner et al. 1981; Steinbrecht 1984). Breath components, essential for the arousal of resting *A. variegatum* to initiate host-finding, seem to be exclusively detected by olfactory receptors within the capsule of Haller's organ (Steullet and Guerin 1992a, b), while receptors sensitive to other vertebrate-associated volatiles and/or pheromone compounds are distributed among both the capsule sensilla and those situated on the surface of the tarsus I. Table 3 summarizes responses of all characterized olfactory receptors on tarsus I of *A. variegatum* to the different vertebrate odours tested in our studies (Steullet and Guerin 1992a and b, 1993). This array of characterized olfactory receptors certainly equips *A. variegatum* for a finely tuned image of its odorous environment, and opens a new avenue of research on the behavioural responses of this tick species to the identified stimulants and their role in host-finding.

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References

Alboae ES (1984) Mammalian semiochemistry. John Wiley & Sons, Chichester New York

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

Altner H, Routil Ch, Loftus R (1981) The structure of bimodal chemo-, thermo-, and hygroreceptive sensilla on the antenna of *Locusta migratoria*. Cell Tissue Res 215:289–308

Apps PJ, Viljoen HW, Pretorius V (1988) Aggregation pheromones of the bont tick *Amblyomma hebraeum*: identification of candidates for bioassay. Onderstepoort J Vet Res 55:135–137

Ayorinde F, Wheeler JW, Wemmer C, Murtaugh J (1982) Volatile components of the occipital gland secretion of the bactrian camel (*Camelus bactrianus*). J Chem Ecol 8:177–183

Barré N, Pavis C, Malosse C, Kermarrec A, Delot J, Bonijol A, Aprelon R, Rousseau L (1991) Les phéromones d'agrégation-fixation de la tique *Amblyomma variegatum* en Guadeloupe. Utilisation possible en lutte intégrée. Rapport IEMVT-INRA, Guadeloupe

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

Burger BV, Pretorius PJ, Spies HSC, Bigalke RC, Grierson GR (1990) Mammalian pheromones VIII. Chemical characterization of preorbital gland secretion of grey duiker, *Sylvicapra grimmia* (Artiodactyla: Bovidae). J Chem Ecol 16:397–416

Delot J (1990) Les phéromones d'agrégation-fixation chez la tique *Amblyomma variegatum* (Fabricius) (Ixodida): une étude en Guadeloupe. Thèse Ecole Nationale Vétérinaire d'Alfort, France

Diehl PA, Guerin PM, Vlimant M, Steullet P (1991) Biosynthesis, production site, and emission rates of aggregation-attachment pheromone in males of two *Amblyomma* ticks. J Chem Ecol 17:833–847

Fox GJ (1982) Potentials for pheromones in chimpanzee vaginal fatty acids. Folia Primatol 37:255–266

Gatehouse AG (1970) The probing response of *Stomoxys calcitrans* to certain physical and olfactory stimuli. J Insect Physiol 16:61–74

Gödde J (1989) Vibrating glass stylets: tools for precise microsurgery on cuticular structures. J Neurosci Methods 29:77–83

Goetz N, Kaba G, Good D, Hussler G, Bore P (1988) Detection and identification of volatile compounds evolved from human hair and scalp using headspace gas chromatography. J Soc Cosmet Chem 39:1–13

Haggart DA, Davis EE (1980) Ammonia-sensitive neurons on the first tarsi of the tick, *Rhipicephalus sanguineus*. J Insect Physiol 26:517–523

Haggart DA, Davis EE (1981) Neurons sensitive to 2,6-dichlorophenol on the tarsi of the tick *Amblyomma americanum* (Acari, Ixodidae). J Med Entomol 18:187–193

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

Hopkins BA (1964) The probing response of *Stomoxys calcitrans* (L.) (the stable fly) to vapours. Anim Behav 12:513–524

Hribar LJ, Leprince DJ, Foil LD (1992) Ammonia as an attractant for adult *Hybomitra lasiophthalma* (Diptera: Tabanidae). J Med Entomol 29:346–348

Kanda F, Yagi E, Fukuda M, Nakajima K, Ohta T, Nakata O (1990) Elucidation of chemical compounds responsible for foot malodour. Br J Dermatol 122:771–776

Lacher V (1967) Elektrophysiologische Untersuchungen an einzelnen Geruchsrezeptoren auf den Antennen weiblicher Moskitos (*Aedes aegypti* L.). J Insect Physiol 13:1461–1470

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

Müller-Schwarze D, Müller-Schwarze C, Singer AG, Silverstein RM (1974) Mammalian pheromone: identification of active component in the subauricular scent of the male pronghorn. Science 183:860–862

Norval RAI, Andrew HR, Yunker CE (1989) Pheromone-mediation of host-selection in bont ticks (*Amblyomma hebraeum* Koch). Science 243:364–365

Norval RAI, Peter T, Meltzer MI (1992) Comparison of the attachment rates of males of the ticks *Amblyomma hebraeum* and *A. variegatum* to cattle, sheep and rabbits in the absence of aggregation-attachment pheromone. Exp Appl Acarol 15:145–152

Schoeni R (1987) Das wirtsgebundene Aggregationspheromon der tropischen Buntzecke *Amblyomma variegatum* (Acari: Ixodidae). Thèse Université Neuchâtel, Switzerland

Schoeni R, Hess E, Blum W, Ramstein K (1984) The aggregation-attachment pheromone of the tropical bont tick *Amblyomma variegatum* Fabricius (Acari: Ixodidae): isolation, identification and action of its components. J Insect Physiol 30:613–618

Steinbrecht RA (1984) Arthropoda: chemo-, hygro-, and thermoreceptors. In: Bereiter-Hahn J, Matolsky AG, Richards KS (eds) Biology of the integument. Springer, Berlin, pp 523–553

Steullet P, Guerin PM (1992a) Perception of breath components by the tropical bont tick, *Amblyomma variegatum* Fabricius (Ixodidae). I. CO_2-excited and CO_2-inhibited receptors. J Comp Physiol A 170:665–676

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

Steullet P, Guerin PM (1993) Identification of vertebrate volatiles stimulating olfactory receptors on tarsus I of the tick *Amblyomma variegatum* Fabricius (Ixodidae) I. Receptors within the Haller's organ capsule. J Comp Physiol A 173: ■–■

Sugisawa H (1981) Sample preparation: isolation and concentration. In: Teranishi R, Flath RA, Sugisawa H (eds) Flavor research recent advances. Marcel Dekker Inc, New York Basel, pp 15–51

Thomas G, Schomaker CH, Been TH, van den Berg MJ, Prisjs HJ (1985) Host finding and feeding in *Hydrotaea irritans* (Diptera, Muscidae): the role of chemical senses. Vet Parasitol 18:209–221

Thonney F (1987) Etude morphologique et structurale des récepteurs sensoriels du tarse I de la tique *Ixodes ricinus* L. Thèse Université Neuchâtel, Switzerland

Waladde SM (1982) Tip-recording from ixodid tick olfactory sensilla: responses to tick related odours. J Comp Physiol 148:411–418

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

Welsh DI, Watts CD (1990) Collection and identification of trace organic compounds in atmospheric deposition from a semi-rural site in the U.K. Int J Environ Anal Chem 38:185–198

Zeng X-N, Leyden JJ, Lawley HJ, Sawano K, Nobara I, Preti G (1991) Analysis of characteristic odors from human male axillae. J Chem Ecol 17:1469–1492

Zeng X-N, Leyden JJ, Braod JG, Spielman AI, McGinley KJ, Preti G (1992) An investigation of human apocrine gland secretion for axillary odor precursors. J Chem Ecol 18:1039–1055

IV. GENERAL DISCUSSION

4.1. Location and number of the tick olfactory sense organs

Several behavioural studies have indicated that the site of odour perception in ticks is essentially located on the tarsus of the first leg pair (Hindle and Merriman 1912; Lees 1948) where typical olfactory sensilla are exclusively found in ticks (Hess and Vlimant 1986; Vlimant personal communication). Consequently, this study has only investigated the functionality of the tarsal wall-pore sensilla in *Amblyomma variegatum*, although some terminal pore sensilla on the tarsus or elsewhere on the tick (Hess and Vlimant 1982, 1983, 1986) might detect vapours at very close distance as Städler and Hanson (1975) demonstrated in tobacco hornworm larvae.

Compared to many adult insects, ticks possess relatively few olfactory sensilla and hence a low number of receptors (less than 100 per tarsus according to Hess and Vlimant 1986). In blood-sucking insects, McIver (1987) found a direct correlation between the approximate number of chemosensitive neurones per antenna and the distance travelled by the insect to the host. Thus, *Pediculus humanus* which lives on the host has only ca. 50 olfactory receptors, compared to the 12000 of *Stomoxys calcitrans.* On the other hand, *Cimex lectularius* which lives a short distance from its host, i.e. in bedsteads or under the wallpaper, possesses only some 200 chemosensitive neurones. Ticks, who do hardly respond over distances of 10-20 meters to a host, possess about the same number of olfactory receptors as *Cimex.* In ticks, the number of olfactory sensilla also tends to decrease in more advanced genera. Rather primitive ticks, such as *Ixodes ricinus*, carry 20 olfactory setae per tarsus whereas the highly specialized one-host tick, *Boophilus microplus*, has only 14 (Hess and Vlimant 1986; Thonney 1987). However, this reduction does not lead to a decrease in the olfactory capability (number of receptors), but to a concentration of the olfactory receptors within fused sensilla (Hess and Vlimant 1982). Most tarsal sensilla are grouped into two clusters, accounting for about half of all tick olfactory receptors, to form the Haller's organ on the dorsal side of the tarsus with an anterior pit and the posterior capsule.

4.2. The capsule of Haller's organ, a complex olfactory organ

Whereas the anterior pit of Haller's organ carries a group of structurally different sensilla (wall-pore single-walled, wall-pore double-walled, and no pore sensilla), the capsule consists of a cavity which encloses only wall-pore single-walled sensilla, i.e. primitively a total of 9 according to Leonovitch and Belozerov (1992), 7 in *A. variegatum*, *A. americanum* and *I. ricinus* (Foelix and Axtell 1972; Hess and Vlimant 1982; Thonney 1987), and only 4 in *Hyalomma asiaticum* and *B. microplus* (Leonovitch 1978; Waladde and Rice 1982). Furthermore, the degree of enclosure within a capsule varies from one tick species to another. In some *Ixodes* species, the capsule is completely open and forms a kind of pit (Homsher and Sonenshine 1979), whereas in most other Pro- and Metastriate ticks the capsule is a deep cavity with a small opening of variable shape, i.e. round in *I. ricinus* (Thonney 1987) and slit-like in *A. variegatum* (Hess and Vlimant 1982). The biological significance of this encapsulation is thought to protect some olfactory sensilla against mechanical damage (Foelix and Axtell 1972) and/or desiccation. Ticks do indeed lead a tough life with long non-parasitic phases surviving in sometimes quite hostile conditions and are exposed to violent grooming by the host during feeding-site selection, biting, and feeding phases. Interestingly, the wall-pore sensilla inside the capsule are more delicate than the wall-pore setae outside the capsule. Thus in *A. variegatum*, the capsule sensilla have ten times thinner walls (ca. 0.1 µm) and bigger pores than the wall-pore single-walled sensilla outside the capsule (Hess and Vlimant 1982). In *I. ricinus*, the most exposed capsule sensillum also possesses thicker walls than less exposed ones (Thonney 1987). Larger and numerous pores as well as remarkably thin walls favouring diffusion of odour molecules into the capsule sensilla essentially compensates for enclosure within a capsule. The present study has shown that the molecular diffusion and air turbulence around the tarsus permits access of vapours to the confined sensilla (chapter 3.1.). Therefore, encapsulation does not limit the spatial field of perception of the capsule sensilla, such that olfactory stimulants carried by wind from any direction are perceived (chapter 3.1.). Consequently, the capsule of Haller's organ is a sensory organ with a specialized architecture designed to protect olfactory sensilla without restricting vapour perception or sensitivity. The capsule contains olfactory receptors essential for successful completion of the life cycle in most tick species. Lees (1948) proposed that the capsule of *I. ricinus* comprises most of the olfactory functions whereas the anterior pit is essential for hygroreception. Such a functional distinction between the anterior pit and the capsule is however too simplistic, as we now

know from electrophysiological experiments in *A. variegatum* that many olfactory receptors are also located outside the capsule on the tarsus. Thus, nitrophenol, lactone, fatty acid, NH_3, and 2,6-dichlorophenol receptors are found in the anterior pit of Haller's organ (chapter 3.4.). However in *A. variegatum*, volatiles in vertebrate breath which are primordial tick arousers only stimulated receptors within the capsule , i.e. CO_2-receptors and sulfide-receptors (chapters 3.1. and 3.2.). This might also be the case for other tick species, although Waladde and Rice (1982) reported responses to human breath in receptors of the anterior pit of *B. microplus.* However, the latter finding might have been due to temperature changes associated with the stimulation method employed as was the case for receptors responding to human breath in the DII.5 and 6 sensilla of *A. variegatum* in this study (chapter 3.4.). Moreover, the capsule of Haller's organ of *A. variegatum* is a complex olfactory organ housing many receptors (21-35) of different specificities which respond to various host-associated volatiles (CO_2, H_2S, mercaptans, saturated and unsaturated aliphatic aldehydes, lactones, furfural, and benzaldehyde-related compounds), to products which can be considered as both host-odour volatiles and as tick pheromone components (benzaldehyde, 2-hydroxybenzaldehyde), and to the tick pheromone component, methylsalicylate (chapters 3.1., 3.2., and 3.3.). The peculiar life style of ticks and the low, albeit highly diversified, number of olfactory receptors per tick species concentrated in just a few wall-pore sensilla on the first legs (used both as "antennae" and for running) might in the end justify some special protection.

4.3. Specificity and sensitivity of the olfactory receptors

Ticks possess an outstanding variety of tarsal olfactory receptors inside but also outside the capsule. The present study reveals that *A. variegatum* can detect a vast range of volatiles such as CO_2, H_2S, mercaptans, NH_3, branched and unbranched short-chain fatty acids, saturated and unsaturated aliphatic aldehydes, lactones, furfural, benzaldehyde, 2-hydroxybenzaldehyde, other benzaldehyde-related compounds, methylsalicylate, and both nitro- and chlorophenols (Table 4.1. and chapters 3.1., 3.2., 3.3., and 3.4.). The diversity of olfactory receptors is even greater as several receptors were not successfully characterized. Some of them responded to unidentified components of human breath (chapters 3.1. and 3.2.), bovine and rabbit odours (chapters 3.3. and 3.4.), and others were not stimulated by any of the tested synthetic volatiles or host odours. Each characterized receptor of *A. variegatum* appears to have an unique response spectrum. Each has shown a response to

a limited range of compounds from the same chemical class, but a particular class of olfactory stimulants was in most cases monitored by at least two receptors with overlapping specificity spectra, i.e. acid receptors in the DII.1 and DII.5 sensilla (chapter 3.4.), aldehyde receptors 1, 2, 3 in the capsule (chapter 3.3.), sulfide receptors 1, 2 (chapter 3.2.), lactone receptors in both the capsule and DI.1 sensillum (chapter 3.3. and 3.4.), benzaldehyde and 2-hydroxybenzaldehyde receptors in the capsule (chapter 3.3.). Most of the olfactory receptors investigated in *A. variegatum* could not be considered as either "specialists" or "generalists", but rather of an intermediate form, the "specialized generalist" as proposed by Kaissling (1971). *A. variegatum* is thus equipped with paired or grouped receptors which allow odour quality coding as proposed by Tichy and Loftus (1983) and Visser (1986). This sensory organisation is widespread in insect olfaction, i.e. stick insects (Tichy and Loftus 1983) and cockroaches (Selzer 1984). The olfactory system of *A. variegatum* appears to be able to provide a maximum of information about quality of volatiles with a minimum number of receptors and seems to be, by and large, as sophisticated as that of most insects. With the different types of olfactory receptors, ticks seem to be capable to differentiate the complex of host odours from others in its environment. Furthermore, some special features also allow refined evaluation of volatile concentrations. Thus, CO_2 concentration is monitored by two antagonistic types of receptors which work in a different range. This consequently permits detection of a precise concentration over a wide range. The CO_2-inhibited receptor is sensitive to very small concentration changes between 0 and 0.2%, whereas the CO_2-excited receptor best monitors concentrations above 0.1% (chapter 3.1.). Systems with excited and inhibited receptors have already been described in insects, and have been ascribed different functions. Thus in mosquitoes, lactic acid respectively excites and inhibits two types of receptors (Davis and Sokolove 1976). The sensitivity of the "LA-excited" receptors, but not that of the "LA-inhibited" receptors, depends on the physiological state of mosquitoes. The inputs of both types of receptors are thought to interact via CNS integration to finally dictate the behavioural sensitivity to lactic acid (Davis et al. 1987).

Some of the volatiles detected by *A. variegatum* are commonly known as olfactory stimulants for other blood-sucking arthropods, whereas others are unusual. Tsetse flies possess receptors sensitive to CO_2 and propanal but also to acetone, 2-butanone, 1-octen-3-ol, and methylphenol isomers (Bogner 1992, Den Otter et al. 1992). Furthermore, indole-related compounds and carotenoid metabolites caused strong EAG responses in *Glossina* (Hall 1990). On the

other hand, mosquitoes bear chemosensitive neurones for CO_2, various fatty acids, and amines, but also for lactic acid, terpineol, and citral (Lacher 1967; Davis and Sokolove 1974; Geier, personal communication). Terpene receptors in mosquitoes are actually associated with plant-feeding behaviour (Bowen 1992). Finally in *Stomoxys calcitrans,* Lewis (1972) mentioned olfactory receptors responding to CO_2, fatty acids, NH_3, and amines, but also to esters and alcohols. Although haematophagous diptera possess a greater number of olfactory receptors than ticks (McIver 1987), the diversity of the chemosensitive neurones in both taxonomic groups seems to be about the same, but in contrast to ticks, several receptors of each type occur on the antennae of these insects. *Stomoxys, Glossina* and mosquitoes may then not necessarily detect more subtle differences in host-odour composition than ticks, but may have a higher sensitivity thus responding to host odours from longer distance. Such a high sensitivity might be obtained via a high spatial convergence of the numerous chemosensitive neurones in the brain. Amplification along the integration process is already well-established in several insects (c.f. reviews by Boeckh and Boeckh 1979; Light 1986; Boeckh and Ernst 1987; Visser and de Jong 1988)

Table 4.1. provides a list of the best characterized olfactory receptors in male *A. variegatum* along with an approximate estimation of the sensitivity threshold based on GC-EL experiments. The 2,6-dichlorophenol receptor in the DI.1 sensillum, the 2-nitrophenol receptor in the DII. 1 sensillum, and the methylsalicylate receptor, benzaldehyde receptor and 2-hydroxybenzaldehyde receptor within the capsule were the most sensitive with less than 10^8 molecules/cm^3 air of the stimulant already exciting these receptors (chapters 3.3. and 3.4.). The aliphatic aldehyde receptor (type 3) responding to (E)-2-heptenal and both sulfide receptors excited by H_2S were also highly sensitive with thresholds estimated at between 10^8 and 10^9 molecules/cm^3 air (chapters 3.2. and 3.3.). By contrast, CO_2 receptors responded only to much higher changes in stimulus intensity (chapter 3.1.) making the CO_2-excited receptor ca. 10^6 to 10^7 times less sensitive than for instance the 2-nitrophenol receptor. The relative lack of sensitivity of CO_2 receptors compared with that of other olfactory receptors has already been noted in insects (Boeckh et al. 1965). Presence of high ambient levels of CO_2 (0.04% or even more en the litter zone) might go some way to explain the low sensitivity of the CO_2 receptors. As defined by Weber's law, the resolving power of receptors should be better when the background level of the stimulant is low rather than high. On the whole, the estimated thresholds of the olfactory receptors of *A. variegatum* lie in

approximately the same range as most insect olfactory receptors, with the possible exception of some receptors for pheromone components, i.e. the bombycol receptor in *Bombyx* (Boeckh et al. 1965).

4.4. Correlation between receptor specificity and sensillum ultrastructure

Interestingly, most of the stimulants identified in this study were detected by receptors located in wall-pore single-walled sensilla (chapters 3.1., 3.2., and 3.3.; Table 4.1.). By contrast, only a few stimulants for receptors in wall-pore double-walled sensilla have been clearly identified, i.e. branched, unbranched short-chain fatty acids, and NH_3 excited separate receptors enclosed in wall-pore double-walled C sensilla (DII.1 and DII.5 sensilla) (chapter 3.4.); NH_3 and 3-pentanone weakly stimulated receptors within wall-pore double-walled A (DIV sensilla); but stimulants for the 4 to 7 receptors of wall-pore double-walled B sensilla (DIII.2, LAII.1, VII.1, and VII.4 sensilla) remain uncharacterized. However, the presence of a tubular body at the base of these wall-pore double-walled sensilla indicates that they have a mechanosensory modality. The ventral or lateral location of three of these sensilla may suggest a possible tactile function despite the fact that they appear to be typical wall-pore olfactory sensilla. But, further investigations on this type of sensillum are evidently needed. Correlations between receptor type and the sensillum ultrastructure have also been observed in insects: different classes of stimulants have been identified for receptors in wall-pore single-walled sensilla, i.e. fatty acids, aldehydes, alcohols, terpenes, esters, whereas wall-pore double-walled sensilla principally house fatty acid and amine receptors (Altner et al. 1977). In *A. variegatum*, pheromone and host-odour receptors are not enclosed in separate sensilla, i.e. 2,6-dichlorophenol and lactone receptors in the DI.1 sensillum (chapter 3.4.). Neither is the perception of pheromone products is confined to special hairs restricted to a particular site of the tarsus, i.e. the methylsalicylate receptor was located within the capsule, whereas nitro- and chlorophenol receptors are found on the anterior pit. This suggests that by contrast with many insects *A. variegatum* has not developed a highly specialized and distinct pheromone perception system separated from the host-odour receptors (for insects, see Visser 1986).

sensillum name	sensillum morphology	receptor type	best stimulant	a) threshold [molecules/cartridge]	b) estimated threshold [molecules/cm^3 air]
DI.1	wp-sw A	2,6-dichlorophenol	2,6-dichlorophenol	10^{12} - 10^{13}	10^7 - 10^8
DI.1	wp-sw A	lactone	γ-valerolactone	10^{16}	?
DII.1	wp-sw A	nitrophenol	2-nitrophenol	10^{12} - 10^{13}	10^7 - 10^8
DII.1	wp-sw A	2,6-dichlorophenol	2,6-dichlorophenol	10^{12} - 10^{13}	10^7 - 10^8
DII.1	wp-sw A	fatty acid	pentanoic acid	10^{14}	?
DII.6	wp-dw C	fatty acid (type 2)	butanoic acid	10^{13}	?
DII.6	wp-dw C	fatty acid (type 1)	2-methylpropanoic acid	10^{12} - 10^{13}	?
DII.6	wp-dw C	NH_3	NH_3 (NH_4OH)	10^{13} - 10^{14}	?
DIV	wp-dw A	NH_3	NH_3 (NH_4OH)	10^{16}	?
DIV	wp-dw A	3-pentanone	3-pentanone	10^{15} - 10^{16}	?
capsule	wp-sw B	methylsalicylate	methylsalicylate	10^{12} - 10^{13}	10^7 - 10^8
capsule	wp-sw B	lactone	γ-valerolactone	10^{13} - 10^{14}	?
capsule	wp-sw B	aldehyde (type 1)	hexanal	10^{12} - 10^{13}	10^9 - 10^{10}
capsule	wp-sw B	aldehyde (type 2)	heptanal	10^{13} - 10^{14}	10^9 - 10^{10}
capsule	wp-sw B	aldehyde (type 3)	(E)-2-heptenal	10^{12} - 10^{13}	10^8 - 10^9
capsule	wp-sw B	benzaldehyde	benzaldehyde	10^{12} - 10^{13}	10^7 - 10^8
capsule	wp-sw B	2-hydroxybenzaldehyde	2-hydroxybenzaldehyde	10^{12} - 10^{13}	10^7 - 10^8
capsule	wp-sw B	CO_2-inhibited	CO_2	10^{14} - 10^{15} g	10^{13} - 10^{14}
capsule	wp-sw B	CO_2-excited	CO_2	10^{16} g	10^{16}
capsule	wp-sw B	sulfide (type 1)	H_2S	10^9 - 10^{10} S	10^8 - 10^9
capsule	wp-sw B	sulfide (type 2)	H_2S	10^9 - 10^{10} S	10^8 - 10^9

Table 4.1. Characterized olfactory receptors of male *A. variegatum*. The estimated sensitivity for the best stimulant for each receptor is provided. a) minimum amount (in molecules) of stimulant in the stimulus cartridge which evoked a response; all the amount applied to the filter paper within the cartridge did not evaporate at once; g indicates that the stimulus within the cartridge was a gas; b) approximate concantration of the stimulant in air (molecules/cm^3 air) sufficient to elicit a response as estimated by gas chromatography-coupled electrophysiology. This estimation was based on tha minimum amount of stimulant injected onto the GC column necessary to elicit a response, the width of the eluting peak, the fraction of the column effluent directed to the preparation, and the dilution factor in the air stream carrying the product to the preparation. ? indicates that the threshold was not estimated with gas chromatography-coupled electrophysiology. Name as well as morphological type of sensillum housing the characterized receptor are also provided (nomenclature based on Hess and Vlimant 1982, 1986). No receptors within the wp-dw B sensilla (DIII.2, LAII.1, VII.1, and VII.4 sensilla) were characterized.

4.5. Conservatism in the evolution of the tick olfactory system

The results of the present work and of previous electrophysiological studies on tick olfactory receptors suggest a high ultrastructural and functional homology among Ixodidae (Table 4.2.). Thus, receptors sensitive to 2,6-dichlorophenol have been described in the anterior pit of *A. variegatum* (Waladde 1982; Schoeni 1987, chapter 3.4.), *A. americanum* (Haggart and Davis 1981), *Dermacentor variabilis* (Sonenshine 1991), *Rhipicephalus appendiculatus* (Waladde 1982), *I. ricinus* (Thonney 1987), and *B. microplus* (de Bruyne and Guerin 1993). The aggregation-attachment pheromone components of *A. variegatum*, 2-nitrophenol and methylsalicylate, stimulated, respectively, receptors in the DII.1 sensillum and in the capsule of *A. variegatum* (Hess and Vlimant 1986; Schoeni 1987; chapter 3.4.), but also of *B. microplus* (de Bruyne and Guerin 1993). Methylsalicylate also excited a receptor in the capsule of *I. ricinus* (Guerin, unpublished). NH_3 receptors have been found in the anterior pit of *A. variegatum* (Guerin et al. 1992 and chapter 3.4.), and in *Rhipicephalus sangineus* (Haggart and Davis 1980). The DI.1 sensillum bears a lactone receptor in *A. variegatum* (chapter 3.4.), *A. hebraeum* (Steullet, unpublished) and also in *B. microplus* (de Bruyne, unpublished). Finally in the capsule, sulfide receptors were discovered in both *A. variegatum* and *Hyalomma dromedarii* (chapter 3.2.). Nevertheless, whereas fatty acids stimulated only receptors outside the capsule in *A. variegatum* (Hess and Vlimant 1980 and chapter 3.4.), pentanoic acid was reported by Sinitsina (1974) to excite receptors in the capsule of *H. asiaticum*.

From the above, the specificity of olfactory receptors in ticks would appear very homogeneous. Although a systematic comparative electrophysiological study on chemosensitive neurones of different tick species would be imperative for any firm conclusions, the tick olfactory system seems to have only weakly evolved. Based on very fragmentary knowledge, mites also seem to possess, at least partially, a closely-related olfactory system. A methylsalicylate receptor has been found on the dorsal side of the tarsus of the first leg pair of the mite, *Phytoseiulus persimilis* (de Bruyne et al. 1991). Moreover, many pheromone components of mites are furfural-related compounds or hydroxybenzaldehyde-related compounds (Kuwahara 1991). These are all chemically close to some stimulants for receptors of *A. variegatum* (chapter 3.3.). Most of the known tick stimulants also constitute commonly occurring semiochemicals for many phytophagous insects, i.e. CO_2 (Bogner et al. 1986), fatty acids (Visser 1986), aldehydes (Visser 1986), benzaldehyde (Visser 1986; Hansson et al. 1989), 2-hydroxybenzaldehyde

(Wallace and Mansell 1976), or for scavenger insects, i.e. aldehydes, NH_3, fatty acids, H_2S, mercaptans (Waldow 1973). This and the fact that tick ancestors were presumably scavenger or phytophagous Acari suggests that the tick olfactory system has not significantly evolved. Tick ancestors were more likely generalist and opportunistic species with morphological and physiological potential to explore new environments and occupy new ecological niches. Parasitism is indeed thought to be an innovation of such "chance feeder" species (Kim 1985). The olfactory system of tick ancestors was probably generalist enough to efficiently detect and recognize novel environments and novel diets without significant modifications. The lack of physiologically relevant differences in the peripheral olfactory system among ticks suggests that the different life cycles, host-finding strategies and host specificities has mostly evolved at the level of the integration of the sensory information rather than at the level of the sensory inputs. Such an hypothesis was already proposed by Hess and Vlimant (1986). Consequently, further studies on behaviour and on the CNS integration process seem to be highly essential to really understand host specificity in ticks. Furthermore, commonly occurring constituents in the pheromone communication system of ticks also suggests that intraspecific communication systems in ticks have only partially evolved without significant evolutionary specialization of the peripheral sensory system in contrast to many insects (Hansson et al. 1989). Vogt et al. (1991) proposed that the pheromone sensory system of insects had evolved from a non-specialized olfactory system via modifications of odour-binding-proteins. However, such an evolution has not apparently occurred systematically in ticks. According to our current knowledge, only the tick pheromone component 2,6-dichlorophenol presumably involves a significant evolutionary specialization of the peripheral olfactory system in these organisms. By contrast, the aggregation-attachment pheromone components emitted by fed male *Amblyomma* to mark the infested host and make it more attractive for conspecifics do not constitute of highly specific or autonomous compounds. Except for methylsalicylate, the other components of the aggregation-attachment pheromone of *A. variegatum* (2-nitrophenol, nonanoic acid) and *A. hebraeum* (2-nitrophenol, nonanoic acid, 2-methylpropanoic acid, and benzaldehyde) have also been found in bovine and/or rabbit odour collected on Porapak (chapters 3.3., 3.4., and appendix). These compounds have also been reported in the literature as vertebrate-associated volatiles, i.e. benzaldehyde from muskox, rabbit, human, and mouse (Flood et al. 1989; Goodrich 1983; Preti et al. 1977; Andreolini et al. 1987), and nonanoic and 2-methylpropanoic

acid from chimpanzee, grysbok, rabbit and human (Fox 1982; Burger et al. 1981; Goodrich 1983; Kanda et al. 1990). This suggests that, to favour feeding and meeting of the sexes on an adequate host, *A. variegatum* and *A. hebraeum* have developed a pheromone communication system using volatiles associated with uninfested hosts and perceived by what were presumably pre-existing host-odour receptors. A methylsalicylate receptor has been also found in phylogenetically different tick species and even in mites, suggesting that this receptor was already present before *A. variegatum* incorporated the volatile in its aggregation-attachment pheromone. Though absent in the aggregation-attachment pheromone of *A. variegatum*, benzaldehyde and 2-methylpropanoic acid (present in that of *A. hebraeum*) were also detected by receptors of *A. variegatum*. From a fitness viewpoint, it would appear more astute to emit an aggregation-attachment pheromone composed of substances autonomous of the odour of uninfested hosts. However from an evolutionary viewpoint, the chance of developing an aggregation-attachment pheromone composed of host-associated volatiles which are perceived by the olfactory receptors necessary for the survival of the parasite was certainly higher. Indeed, it only involves an adjustment of the host-finding behaviour of male and female *A. variegatum* to respond to the increased amounts of these compounds released by mature males (see also chapter 4.6.) and not a significant modification or specialization of the peripheral olfactory system, confined as it is to a relatively few sensilla on the first leg pair.

4.6. Perception of vertebrate volatiles and host-finding

Hidden in the litter zone, unfed resting adult *A. variegatum* await for a host (mostly large ruminants) passing nearby to evoke a succession of behavioural responses, i.e. arousal, initiation of locomotion, orientation and attraction, contact with the host, feeding-site selection, and finally attachment to the host. The establishment of a host-parasite relationship in adult *A. variegatum* involves two phases: some pioneer males first parasitize a large ruminant (primary host infestation) and if these males succeed in establishing a feeding site they become sexually mature and emit an aggregation-attachment pheromone to attract conspecific females and males (secondary host infestation). Whereas males can colonize and select an adequate uninfested host, females only parasitize an already infested one, where a blood-meal can be guaranteed and meeting of the sexes is assured. Various olfactory cues intervene along the successive behavioural events of host-finding in male and female *A. variegatum*, although host-location is likely to depend also on other

senses as suggested by studies on vision (Kaltenrieder et al. 1989; Kaltenrieder 1990) and on IR detection (Poffet 1988). However in this chapter, only the role in host-finding of the olfactory stimulants identified in the present study will be discussed here.

Receptor characterized	Location (sensillum)	Tick species	Reference
2,6-dichlorophenol	DI.1 / DII.1	*Amblyomma variegatum*	Waladde 1982; Schoeni 1987
	anterior pit *	*Amblyomma americanum*	Haggart and Davis 1981
	DI.1 / DII.1	*Rhipicephalus appendiculatus*	Waladde 1982
	anterior pit *	*Dermacentor variabilis*	Sonenshine 1991
	DI.1 / DII.1	*Boophilus microplus*	de Bruyne and Guerin 1993
	DI.1	*Ixodes ricinus*	Thonney 1987
2-nitrophenol	DII.1	*Amblyomma variegatum*	Schoeni 1987; Steullet and Guerin 1993b
	DII.1	*Boophilus microplus*	de Bruyne and Guerin 1993
γ-valerolactone	DI.1 / capsule	*Amblyomma variegatum*	Steullet and Guerin 1993a, b
	DI.1	*Amblyomma hebraeum*	Steullet, unpublished
	DI.1	*Boophilus microplus*	de Bruyne, unpublished
ammonia	DII.6 / DIV group	*Amblyomma variegatum*	Steullet and Guerin 1993b
	anterior pit */ DIV group	*Rhipicephalus sanguineus*	Haggart and Davis 1981
fatty acids	DII.1 / DII.5	*Amblyomma variegatum*	Steullet and Guerin 1993b
	capsule	*Hyalomma asiaticum*	Sinitsina 1974
methylsalicylate	capsule	*Amblyomma variegatum*	Hess and Vlimant 1986
	capsule	*Ixodes ricinus*	Guerin, unpublished
	capsule	*Boophilus microplus*	de Bruyne, unpublished
sulfide	capsule	*Amblyomma variegatum*	Steullet and Guerin 1992b
	capsule	*Hyalomma dromedarii*	Steullet and Guerin 1992b

Table 4.2. Characterized olfactory receptors of *A. variegatum* also reported in other tick species. * Indicates that the receptor could not be surely ascribed to a definite wall-pore sensillum of the anterior pit (DII.I, or DII.5, or DII.6) because of use of tungsten electrodes.

Most of the volatiles detected by *A. variegatum* are widespread in nature and rather unspecific to vertebrates. Resting adult *A. variegatum* waiting in the litter zone are confronted by very large amounts of gases exhaled or released by eructation at the level of the ground by large ungulates. Therefore, breath components constitute the most abundant vertebrate-associated cues for the unfed resting ticks. The breath component CO_2 is an essential cue during host-finding for many blood-sucking insects as well as for ticks (i.e. Garcia 1962; Nevill 1964; Guglielmone et al. 1985; Beelitz and Gothe 1991; Gillies and Wilkes 1968, Turner 1971, Warnes and Finlayson 1985, French and Kline 1989). This product arouses adult *A. variegatum* and induces them to move as demonstrated in activation bioassays (chapter 3.2.), in the wind tunnel (chapter 3.1.) and in the field (Yunker and Norval 1991). CO_2 also evoked upwind orientation in the wind tunnel (chapter 3.1.), but did not efficiently attract adult *A. variegatum* from more than 1-2 meters in the field (Barré 1989; Barré et al. 1991; Norval et al. 1992a). Despite its intrinsic unspecificity, CO_2 might provide specific information to *A. variegatum*. Since the amount of vertebrate-emitted CO_2 is directly correlated with animal size, a common host such as a buffalo represents a stronger source of CO_2 than a small mammal. Furthermore, CO_2 exhaled at ground level by a grazing herbivore is more easily detectable by a waiting adult *A. variegatum* in the litter zone than CO_2 emitted from an upright human some 1.5 meters above the ground. Thus, the host-finding strategy of the parasite and the behaviour of its usual host can serve to determine the effective specificity of a kairomone such CO_2. The CO_2-excited receptor of *A. variegatum* resembles CO_2-excited receptors described for tsetse flies (Bogner 1992) and *Lucilia cuprina* (Stange 1974). On the other hand, the CO_2-inhibited receptor of *A. variegatum*, which at least equals the high sensitivity of the CO_2-excited receptor of *Aedes aegypti* (Kellogg 1970), is unknown to-date for other blood-sucking arthropods. Such a CO_2-inhibited receptor nevertheless exists in the temporal organ of the Japanese house-centipede (Yamana et al. 1986, Yamana and Toh 1987) and in wall-pore single-walled sensilla of the termite *Schedorhinothermes lamanianus* (Ziesmann et al. 1992). Interestingly, *A. variegatum*, the Japanese house-centipede, and *Schedorhinothermes lamanianus* spend a part of their life time in microhabitats (litter, soil, and nest, respectively) where CO_2 concentration is higher than normal ambient values (0.03-0.04%). Such CO_2-inhibited receptors might monitor CO_2 changes around relatively high ambient levels better than classical CO_2-excited receptors. The ambient CO_2 level to which each of these organisms is confronted in its microhabitat falls into the working range of the CO_2-inhibited

receptor (0-0.2% for *Amblyomma,* 0-1% for the centipede, and 0-5 % for the termite). Considering the working range of the CO_2-inhibited receptors, *A. variegatum* appears to be equipped better for detecting minute CO_2 changes than either the centipede or the termite. The CO_2-inhibited receptor of *A. variegatum* is extremely sensitive to CO_2 shifts, i.e. 0.001-0.002% (chapter 3.1.) and could serve to first alert resting ticks of the presence of a vertebrate. In the wind tunnel, concentrations of 0.1% to 0.2% were the most effective levels to strongly arouse resting ticks and initiate locomotion (chapter 3.1.). Adult *A. variegatum* may consequently detect shifts in CO_2 levels long before eliciting any active search. Thus, active search is only initiated when the potential host is not too far away and when CO_2 levels reach a certain threshold, i.e. 0.1%. The CO_2-excited receptor best monitors concentrations above 0.1% (chapter 3.1.), and should therefore intervene during initiation and maintenance of the active search. Perritt et al. (1993) recently found that even 9 ppm (0.0009%), corresponding to the sensitivity threshold of the CO_2-inhibited in *A. variegatum*, elicited questing in *D. variabilis* and movement in *A. americanum.*

The breath product, H_2S, which stimulated two types of sulfide receptors within the capsule of Haller's organ of *A. variegatum*, also alerts resting individuals but without strongly inducing them to walk (chapter 3.2.). This compound is unknown to-date as a kairomone for other haematophagous arthropods, but another sulfur-containing product, dimethyldisulfide, is an attractant for the New World screwworm *Cochliomyia hominivorax* in the field. Omitting dimethyldisulfide from an attractant odour mixture (swormlure-4) significantly reduced catches of *Cochliomyia hominivorax* in wind-orientated traps (Green et al. 1993). H_2S is generally present at low concentrations in vertebrate breath (i.e. 0.007 to ca. 0.7 ppm in human, Tonzetich 1977) but is very abundant in gases released by ruminants during eructation (ca. 100 ppm in the rumen, Hungate 1966). Considering the sensitivity thresholds of the CO_2-inhibited receptor (0.001-0.002%) and the sulfide receptors (0.0001 ppm) in *A. variegatum* (chapters 3.1. and 3.2.) as well as the concentrations of CO_2 (25%) and H_2S (100 ppm) in the rumen (Hungate 1966), resting *A. variegatum* may detect an eructation from a host from a longer distance with the sulfide receptors than with the CO_2-receptors alone. In this context, H_2S might be, long before CO_2, the first alerting signal for the presence of a suitable host such as a ruminant in the vicinity of the waiting tick. Whereas H_2S alone activated resting adult ticks but without often inducing them to move, CO_2 and H_2S do not act synergistically as an arousal signal (chapter 3.2.). However, in

preliminary bioassays on the locomotion compensator, mixtures of CO_2 and H_2S significantly altered the behaviour of excited walking male *A. variegatum* by frequently eliciting reorientating behaviour (stopping, changing direction, and moving for a short distance upwind). This behaviour was totally different from that induced by CO_2 or H_2S alone (Table 4.3. and Fig. 4.1.). Further investigations on host-finding behaviour of *A. variegatum* in relation with the eructation cycles of the host might be worthwhile to better understand the exact role of H_2S and CO_2 in host selection.

	a) Proportion time spent in reorientating (mean)			b) number of reorientating behaviours / 30 s (mean)			c) reorientating angle γ [°] (mean)		
	H_2S	CO_2	CO_2/H_2S	H_2S	CO_2	CO_2/H_2S	H_2S	CO_2	CO_2/H_2S
prestimulation	0.05	0.07	0.03	0.81	0.38	0.44	61.3	81.3	61.3
atimulation	0.06	0.15 *	0.18 *	1	0.94	2.06 *	65.9	84.6	110.3 *

Table 4.3. Reorientating behaviour of excited male *A. variegatum* walking on a locomotion compensator when stimulated with either H_2S, CO_2, or mixtures of both (for mathod, see chapter 2.6.). Ticks were considered to evoke reorientating behaviours if they stopped or moved slowly (< 0.5 cm/s). Mean walking speed of *A. variegatum* on the locomotion compensator was about 2.7 cm/s. During reorientating behaviour, ticks often raised the first pair of legs in the air, initiated large turns, and even moved backward briefly. Such behaviour could not be satisfactorily described by the locomotion compensator. Consequently, reorientating was analysed from the video tapes. a) The proportion time spent by each tick in reorientating during the prestimulation and stimulation period was compared pairewise with a Wilcoxon signed rank test. b) The frequency of reorientating behaviour recorded in the prestimulation and stimulation periods was compared pairewise with a sign test for each tick. c) The reorientating angles γ, expressing the amplitude of the change in orientation between the beginning and end of the reorientating behaviour elicited during the prestimulation and stimulation periods were compared with a Wilcoxon-Mann-Whitney test. Tracks are more tortuous when ticks are submitted to stimulation with a mixture of CO_2 and H_2S than tracks elicited during stimulation with either H_2S or CO_2 alone (see Fig. 4.1.). * Indicates that behaviour during stimulation was significantly different from that elicited during prestimulation periods. For each stimulus, 16 ticks were observed.

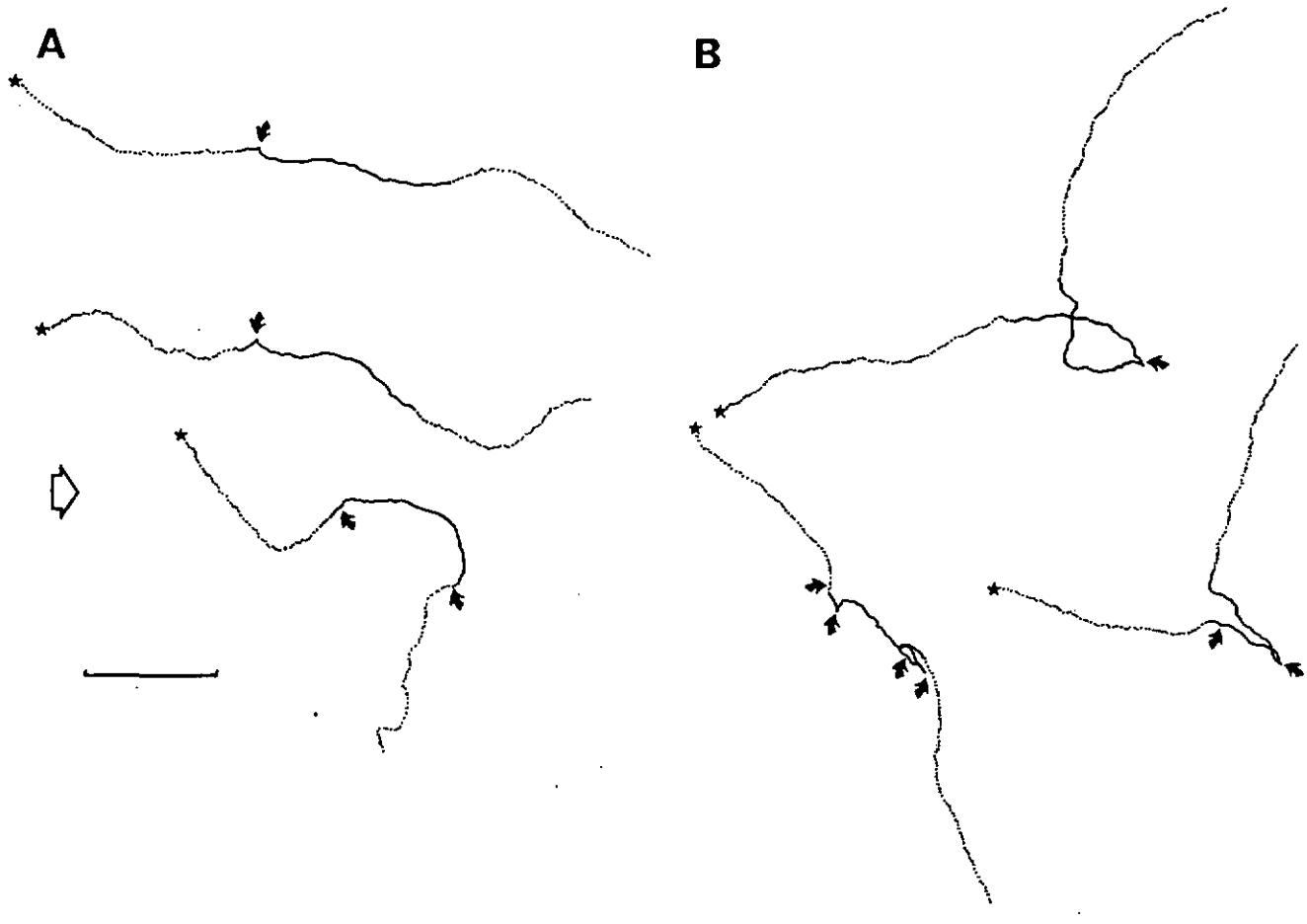

Fig. 4.1. Representative tracks of three different males of *A. variegatum* submitted to **A** 0.15% CO_2, and **B** 0.15% CO_2 and H_2S (aqueous solution of 1 mg Na_2S/ml at the source). *White arrow* indicates wind direction (0.1 m/s); *star* represents the beginning of track; *dashed lines* are tracks made during prestimulation and poststimulation (30 s each), whereas *continuous lines* are tracks during stimulation (30 s); small *bold arrow* indicates a reorientating behaviour (walking speed < 0.5 cm/s); *horizontal bar* 50 cm.

A host infested by attached male *A. variegatum* arouses and attracts many more resting conspecific ticks than an uninfested host. Thus, 20% of unfed males released within a few meters of uninfested bulls had fixed on these animals after 2 days, against hardly 2% on uninfested goats (Barré et al. 1991), indicating a preference for bovidae. However, 30% of released unfed males had attached after 2 days on bulls previously infested by pioneer male *A. variegatum*, and about 10% on infested goats (Barré et al. 1991). By contrast,

no unfed females were observed on uninfested bulls or goats 2 days after release, whereas about 10% of females had attached after the same period of time on infested bulls and goats (Barré et al. 1991). This behaviour was mediated by three volatiles emitted in large amounts by fed attached males: 2-nitrophenol, nonanoic acid and methylsalicylate (Schoeni et al. 1984; Diehl et al. 1991). 2-Nitrophenol which stimulated very sensitive receptor(s) in the anterior pit of Haller's organ of *A. variegatum* has also been found in bovine odour (chapter 3.4.). This product greatly improved the stimulant effect of CO_2 on locomotion, but only weakly excited resting ticks when presented in absence of CO_2 in a wind tunnel (Steullet 1987). However, 2-nitrophenol on its own clearly attracted excited adults in the field (Hess and De Castro 1986; Norval et al. 1991a), and in the wind tunnel (Steullet 1987). 2-Nitrophenol is evidently for long-range attraction the most important component of the aggregation-attachment pheromone. Field experiments demonstrated that low doses of the components of the aggregation-attachment pheromone added together to a CO_2-baited trap improved capture of unfed males but not of females. Much higher doses were needed to attract females (Barré et al. 1991). Thus, it may be considered that males are behaviourally sensitive to even low amounts of 2-nitrophenol, levels encountered in uninfested hosts such as steers (chapter 3.4.) or in weakly infested ones. By contrast, females are more likely to respond only to higher levels which can be found in vertebrates well infested with male ticks. Indeed, the production rate of the aggregation-attachment pheromone by feeding males, frequently found in aggregations of tens or even hundreds of individuals on the same host, is high (ca. 1.8 µg of 2-nitrophenol/hour male, Diehl et al. 1991). This shows how finely host-finding in *A. variegatum* may be tuned to amounts of compounds such as 2-nitrophenol parsimoniously serving as a host-odour kairomone and component of the aggregation-attachment pheromone in this tick species. Furthermore, females only attach on hosts infested by fed males releasing the aggregation-attachment pheromone (Schoeni et al. 1984; Schoeni 1987; Barré 1989). Two components of the pheromone were found to evoke attachment in females, i.e. 2-nitrophenol and methylsalicylate (Schoeni et al. 1984; Schoeni 1987; Norval et al. 1991b). By contrast, males can also attach on hosts in the absence of the aggregation-attachment pheromone, but the rate of attachment greatly depends on host type, i.e. 98% on cattle against 37% on rabbits and only 19% on sheep (Norval et al. 1992b). Feeding-site selection and attachment in males might be mediated by olfaction in conjunction with contact and heat perception. Nevertheless, the role in host-finding of vertebrate volatiles found to stimulate

receptors of male *A. variegatum* in this study (chapters 3.3. and 3.4.) remains largely unknown.

NH_3, lactones, aliphatic and aromatic aldehydes, furfural, and short-chain fatty acids have not yet been properly investigated in behaviour tests. NH_3, which is found in vertebrate urine but also in sweat (Spector 1956), has nevertheless been reported as an olfactory stimulant for haematophagous insects and tick species other than *A. variegatum*. NH_3 is an arousal signal for *R. sanguineus* (Haggart and Davis 1980). The soft tick *Ornithodoros erraticus* exhibited a marked preference for ammonia vapours than for acetic acid (El-Ziady 1958). Furthermore, NH_3 attracts *Hydrotaea irritans* (Thomas et al. 1985) and *Tabanidae* (Hribar et al. 1992). NH_3 also evoked probing in *Stomoxys calcitrans* according to Hopkins (1964) but not to Gatehouse (1970).

Lactones, reported from many bovidae and primates (i.e. Burger et al. 1987; Goetz et al. 1988; Flood et al. 1989), and in urinary volatiles of the pine vole (Boyer et al. 1989), remain unknown as stimuli for haematophagous arthropods. Benzaldehyde and 2-hydroxybenzaldehyde which are reported to be emitted by some tick species (Wood et al. 1975; Apps et al. 1988) but also to be associated with vertebrate odours (chapter 3.3.; appendix; Lederer 1946; Preti et al. 1977; Andreolini et al. 1987; Goodrich 1983; Flood et al. 1989), stimulated some very sensitive capsule receptors of *A. variegatum* (chapter 3.3.). These aromatic aldehydes are nevertheless unknown to-date in the literature as olfactory stimulants for other blood-sucking arthropods, but some electrophysiological experiments undertaken in this laboratory have revealed that sandflies also carry receptors sensitive to benzaldehyde (Dougherty and Guerin, personal communication).

Saturated and unsaturated aliphatic aldehydes, which stimulated 3 receptors of differing but overlapping specificity in the capsule of Haller's organ of *A. variegatum* (chapter 3.3.), are common vertebrate-associated volatiles (i.e. Ellin et al. 1974; Burger et al. 1981; Schultz et al. 1988; Goetz et al. 1988; Natynczuk et al. 1989). These products are also widespread in many other natural sources (i.e. green leaf odour). Except for tsetse flies which carry propanal-sensitive neurones (Bogner 1992), no other blood-sucking arthropod is known to hold aliphatic aldehyde receptors or to behaviourally respond to such products.

Short-chain fatty acids excited several different olfactory receptors with overlapping specificity in *A. variegatum* (chapter 3.4.). These volatiles, like aliphatic aldehydes, are often associated with vertebrate odours (i.e. Ayorinde et al. 1982: Fox 1982: Goetz et al. 1988; Goodrich 1983). Fatty acids also

constitute well-known olfactory stimulants for some other blood-sucking invertebrates. Mosquitoes and the bug *Triatoma infestans* possess acid receptors (Lacher 1967; Bernard 1974), and various short-chain fatty acids also evoke probing in *Stomoxys calcitrans* (Hopkins 1964) or attraction in *Hydrotaea irritans* and *Cochlyomyia hominivorax* (Thomas et al. 1985; Green et al. 1993). The relative abundance of short-chain fatty acids varies significantly between animal type. In the present study, extracts of bovine odour collected on Porapak contained butanoic acid as the major fatty acid but 2-methylpropanoic acid predominated in rabbit odour (chapter 3.4., appendix). *A. variegatum* could presumably differentiate with its olfactory receptors between various acid and aldehyde mixtures, and thus between potential hosts. Subtle variations in amounts of these fatty acids and in the relative abundance of each acid in a complex odour might evoke different behaviours in ticks and be partially responsible for host specificity. At such it is not surprising that most results reported in the literature are often contradictory. Butanoic acid, for example, is reported as both an attractant and repellent for *I. ricinus* (Totze 1933, Lees 1948). Future behavioural studies on attraction, induction of attachment and host specificity as related to short-chain fatty acids and the other olfactory stimulants identified in the present study are clearly necessary and will have to carefully take into account concentration effects as well as mixtures. Our current knowledge would nevertheless suggest that host-finding in adult *A. variegatum* is mediated by fine tuning of its olfactory system to host-related volatiles.

V. SUMMARY

1. Several olfactory receptors within wall-pore single-walled sensilla (type A and B) and wall-pore double-walled sensilla (type C) on tarsus I of the tick *Amblyomma variegatum* respond to various host-associated volatiles. By contrast, receptors in wall-pore double-walled sensilla (type A and B) located outside the Haller's organ are stimulated by neither host-odours (bovine and rabbit odours, human breath, human axillary secretion), nor by synthetic compounds associated with vertebrate odours or tick pheromones. The function of the latter sensilla thus remains unknown.

2. Neither the anterior pit, nor the capsule of Haller's organ is exclusively dedicated to the perception of host odours or pheromones. Pheromone receptors are also not restricted within specialized sensilla, but are generally confined to the same setae as host-odour receptors. However, receptors to breath components are only present in the capsule. The capsule of Haller's organ is consequently an essential sense organ for host-finding since breath is the main cue in initiating this behaviour. CO_2 which is perceived by capsule receptors is indeed a strong locomotor stimulant, whereas H_2S, a breath constituent stimulating receptors in the capsule, also arouses resting adult *A. variegatum*. On the other hand, the anterior pit of Haller's organ may also play a role in host-finding as it bears for instance receptors for 2-nitrophenol, a volatile used by *A. variegatum* in selecting adequate hosts and a suitable feeding-site.

3. Enclosure of wall-pore single-walled sensilla (type B) within a capsule which protects them against mechanical damage and desiccation does not restrict vapour perception. Molecular diffusion in air and air turbulence around the tarsus are likely to permit access of odours to the confined sensilla and permit perception of olfactory stimulants independent of wind direction.

4. About half of the receptors within the capsule have been characterized:

- a CO_2-excited receptor best monitoring changes in CO_2 concentration above 0.1% (1000 ppm)
- a CO_2-inhibited receptor highly sensitive to minute changes in CO_2 levels (0.001 to 0.002% or 10 to 20 ppm) around ambient

- two sulfide receptors highly sensitive to H_2S (estimated threshold at ca. 0.1 ppb) but showing a dissimilar overall response to small sulfides and mercaptans
- three aliphatic aldehyde receptors (bovine and rabbit odour): one highly sensitive to C_6 saturated and unsaturated aldehydes; another best responding to C_6 and C_7 saturated aldehydes; and a third stimulated by C_6 and C_7 unsaturated aldehydes
- a benzaldehyde receptor (bovine and rabbit odour) also responding but to a lesser extent to furfural (bovine and rabbit odour)
- a 2-hydroxybenzaldehyde receptor (bovine and rabbit odour) also weakly responding to benzaldehyde
- a receptor sensitive to γ-valerolactone (bovine and rabbit odour)
- a methylsalicylate receptor (component of the aggregation-attachment pheromone of *A. variegatum*)
- several other receptors stimulated by either breath, bovine and/or rabbit odour but whose adequate stimuli have not been identified.

5. About half of receptors in the two wall-pore single-walled sensilla (type A) and in the two wall-pore double-walled sensilla (type C) have also been characterized as responding to host-associated volatiles and/or pheromones:

- short-chain fatty acid receptors (bovine and rabbit odour) responding best either to pentanoic acid, butanoic acid, or 2-methylpropanoic acid
- two NH_3 receptors (vertebrate odours) which differ from each other by their response intensity
- a receptor sensitive to γ-valerolactone (bovine and rabbit odour) and to 6-caprolactone
- receptor(s) to 2-nitrophenol (a component of the *A. variegatum* aggregation-attachment pheromone and of bovine and rabbit odour) also sensitive to 4-methyl-2-nitrophenol (bovine and rabbit odour)
- a 2,6-dichlorophenol receptor (tick sex pheromone component) also weakly responding to 2-nitrophenol.

6. Receptors of *A. variegatum,* which have been characterized, can not be considered "generalist" but show responses to a limited range of components from the same chemical class. Sulfides and mercaptans, short-chain fatty acids, aliphatic aldehydes, aromatic aldehydes, and lactones are all detected by at least two receptors with overlapping specificity spectra. All receptors on the tarsus are unique in terms of response characteristics. Thus, *A. variegatum* possesses an olfactory system which provides a maximum of information about host-odour quality with a quite few number of olfactory receptors.

7. This work also suggests that development of the aggregation-attachment pheromone of the genus *Amblyomma* did not require a high specialization of the olfactory system. Two of the three components of the pheromone of *A. variegatum* (2-nitrophenol and nonanoic acid) and all components of the pheromone of *Amblyomma hebraeum* (2-nitrophenol, benzaldehyde, 2-methylpropanoic acid, and nonanoic acid) have also been identified in bovine and rabbit odour. Though both benzaldehyde and 2-methylpropanoic acid do not seem to be constituents of the aggregation-attachment pheromone of *A. variegatum*, the latter species does possess receptors for these volatiles. It seems that male *A. variegatum* and *A. hebraeum* have developed a pheromone system by producing higher amounts of compounds associated with odour of vertebrates in order to reinforce attractivity of colonized hosts for conspecifics. Mimicry of a part of the host bouquet in the aggregation-attachment pheromone to favour meeting of the sexes on adequate hosts would thus not have involved a profound modification of the olfactory system used for host finding as would have been the case with the use of a more autonomous pheromone system.

RESUME

1. Ce travail a permis de mettre en évidence différents récepteurs olfactifs des sensilles à pores (type A et B) ou à fentes (type C) du tarse de la patte I de la tique *Amblyomma variegatum* répondant à diverses classes de volatiles associés à l'odeur des vertébrés. Par contre, aucun récepteur des sensilles à fentes (type B) situées en dehors de l'organe de Haller n'a montré de réponses évidentes aux odeurs de vertébrés (odeur de lapin, de bovin, haleine et sueur humaine) et à différents analogues synthétiques de composés de l'odeur des vertébrés ou des phéromones de tiques. La fonction de ces dernières sensilles reste donc énigmatique.

2. Aucun des deux groupes de sensilles formant l'organe de Haller (capsule et "anterior pit") est dédié spécifiquement à la perception des odeurs d'hôte ou des phéromones. Seule la détection des volatiles de l'haleine semble être l'apanage exclusif de récepteurs olfactifs de la capsule. La capsule représente donc un organe sensoriel essentiel pour la recherche de l'hôte chez *A. variegatum* étant donné que l'haleine constitue le déclencheur principal de ce comportement. Le CO_2, perçu par des récepteurs de la capsule, est en effet un puissant déclencheur de la recherche active alors que l'H_2S, autre composé de l'haleine détecté par des récepteurs de la capsule, éveille les tiques en repos. Cependant, "l'anterior pit" joue aussi un rôle important dans la recherche d'un hôte puisqu'il est notamment le siège de la perception du 2-nitrophénol, volatile essentiel dans la sélection d'un hôte adéquat.

3. L'entrée des volatiles dans la capsule renfermant sept sensilles à pores (type B) n'est pas entravée. La diffusion des molécules dans l'air et les turbulences crées autour du tarse suffisent en effet pour que les volatiles pénètrent dans la capsule indépendamment de la direction du vent.

4. La moitié environ des récepteurs contenus dans la capsule ont pu être caractérisés. Il s'agit de:

- un récepteur excité par le CO_2 répondant particulièrement à des changements de concentration supérieurs ou égaux à 0.1% (1000 ppm)

- un récepteur inhibé par le CO_2 extrèmement sensible pour des très petits changements de concentration (0.001 à 0.002% ou 10 à 20 ppm) autour des valeurs ambiantes de CO_2
- deux récepteurs à H_2S au seuil de sensibilité estimé à environ 0.1 ppb, mais dont un seul récepteur répond bien à des stimulations de petits mercaptans (éthyl mercaptan) et de diméthyl sulfide
- trois récepteurs aux aldéhydes aliphatiques (odeur de bovin et de lapin): l'un particulièrement sensible aux aldéhydes saturés et insaturés à 6 carbones, un deuxième aux aldéhydes saturés à 6 et 7 carbones, et un troisième aux aldéhydes insaturés à 6 et 7 carbones
- un récepteur au benzaldéhyde (odeur de bovin et de lapin) répondant également mais moins intensément au furfural (odeur de bovin et de lapin)
- un récepteur au 2-hydroxybenzaldéhyde (odeur de bovin et de lapin) répondant aussi faiblement au benzaldéhyde
- un récepteur sensible au γ-valérolactone (odeur de bovin et de lapin)
- un récepteur au méthylsalicylate (composant de la phéromone d'agrégation et d'attachement de *A. variegatum*)
- plusieurs autres récepteurs répondant soit à l'haleine, soit aux odeurs de bovin et/ou de lapin mais dont les stimuli n'ont pas été identifiés.

5. On a pu également mettre en évidence plusieurs récepteurs olfactifs stimulés par des volatiles associés aux vertébrés et/ou aux phéromones de tiques dans deux sensilles à pores (type A) et deux sensilles à fentes (type C) de "l'anterior pit" de l'organe de Haller:

- des récepteurs aux petits acides gras (odeur de bovin et de lapin) répondant particulièrement bien soit à l'acide pentanoique, soit à l'acide butanoique, ou à l'acide 2-méthylpropanoique.
- deux récepteurs au NH_3 (odeur de vertébrés) différant par l'intensité à laquelle ils répondent
- un récepteur sensible au γ-valérolactone (odeur de bovin et de lapin) et au 6-caprolactone

- au moins un récepteur au 2-nitrophénol (composé de la phéromone d'agrégation et d'attachement de *A. variegatum* mais aussi composé mineur de l'odeur de bovin et de lapin) et sensible au 4-méthyl-2-nitrophénol (odeur de lapin et de bovin)

-un récepteur au 2,6-dichlorophénol (phéromone sexuelle des tiques) répondant faiblement au 2-nitrophénol.

6. Parmi tous les récepteurs caractérisés, aucun n'est un véritable "généraliste" répondant à un large spectre de volatiles. Ils sont plutôt sensibles à un groupe de substances de parenté chimique étroite. Une catégorie de stimuli (ex.: sulfides et mercaptans, acides gras, aldéhydes aliphatiques, aldéhydes aromatiques, lactones) est généralement détectée par au moins deux récepteurs au spectre sensiblement différent. Parmi tous les récepteurs caractérisés, il n'existe pas deux récepteurs olfactifs exactement identiques sur le même tarse. Armée d'un nombre limité de récepteurs olfactifs (moins de 100) comparativement à la majorité des insectes adultes, *A. variegatum* possède un système sensoriel lui permettant de détecter un maximum de volatiles différents.

7. Ce travail suggère également que la phéromone d'agrégation et d'attachement des tiques du genre *Amblyomma* ne résulte pas d'une forte spécialisation du système olfactif. Deux des trois composés de la phéromone de *A. variegatum* (2-nitrophénol et acide nonanoique) et tous les composés de la phéromone de *Amblyomma hebraeum* (2-nitrophénol, benzaldéhyde, acide 2-méthylpropanoique et acide nonanoique) ont en effet été clairement identifiés dans l'odeur de bovin et de lapin. Bien que n'étant pas des composés de la phéromone de *A. variegatum*, le benzaldéhyde et l'acide 2-méthylpropanoique possèdent leur propre récepteur chez cette dernière espèce. Il semble donc que les mâles de *A. variegatum* et *A. hebraeum* ont développé un système phéromonal d'agrégation et d'attachement en produisant en grande quantité des substances déjà associées à l'odeur des vertébrés qui renforcent ainsi l'attractivité des hôtes déjà infestés pour leurs conspécifiques.

VI. REFERENCES

Aeschlimann A (1967) Biologie et écologie des tiques (Ixodoidea) de Côte d'Ivoire. Acta Trop 24:281-405

Aeschlimann A (1976) Les tiques, leur biologie et les maladies qu'elles transmettent. Annales Université de Neuchâtel 1975-1976:1-27

Albone ES (1984) Mammalian Semiochemistry. John Wiley & Sons Ltd, Chichester New York

Al Roux H, Thibout E (1988) Analyse en olfactomètre de l'attraction des larves d'*Acrolepiopsis assectella* par des substances allélochimiques. Entomologia Exp Appl 47:231-237

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

Altner H, Prillinger L (1980) Ultrastructure of invertebrate chemo-, thermo- and hygroreceptors and its functional significance. Int Rev Cytol 67:69-139

Altner H, Routil Ch, Loftus R (1981) The structure of bimodal chemo-, thermo-, and hygroreceptive sensilla on the antenna of *Locusta migratoria*. Cell Tissue Res 215:289-308

Andreolini F, Jemiolo B, Novotny M (1987) Dynamics of excretion of urinary chemosignals in the house mouse (*Mus musculus*) during the natural estrous cycle. Experientia 43:998-1002

Apps PJ, Viljoen HW, Pretorius V (1988) Aggregation pheromones of the bont tick *Amblyomma hebraeum*: identification of candidates for bioassay. Onderstepoort J Vet Res 55:135-137

Auger J, Lecomte C, Paris J, Thibout E (1989a) Identification of leek-moth and diamondback-moth frass volatiles that stimulate parasitoid, *Diadromus pulchellus*. J Chem Ecol 15:1391-1398

Auger J, Lecomte C, Thibout E (1989b) Leek odor analysis by gas chromatography and identification of the most active substance for the leek moth, *Acrolepiopsis assectella*. J Chem Ecol 15:1847-1854

Ayorinde F, Wheeler JW, Wemmer C, Murtaugh J (1982) Volatile components of the occipital gland secretion of the bactrian camel (*Camelus bactrianus*). J Chem Ecol 8:177-183

Barré N (1989) Biologie et écologie de la tique *Amblyomma variegatum* (Acarina: Ixodina) en Guadeloupe (Antilles Françaises). Thèse, Université Paris-Sud (France)

Barré N, Pavis C, Malosse C, Kermarrec A, Delot J, Bonijol A, Aprelon R, Rousseau L (1991) Les phéromones d'agrégation-fixation de la tique *Amblyomma variegatum* en Guadeloupe. Utilisation possible en lutte intégréé. Rapport IEMVT-INRA (Guadeloupe)

Batschelet E (1981) Circular statistics in biology. Academic press, London New York

Beelitz P, Gothe R (1991) Investigations on the host seeking and finding of *Argas (Persicargas) walkerae* (Ixodoidea: Argasidae). Parasitol Res 77:622-628

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 Rennes (France)

Blum MS (1981) Chemical defenses of arthropods. Academic Press, Orlando

Boeckh J, Boeckh V (1979) Threshold and odor specificity of pheromone-sensitive neurons in the deutocerebrum of *Antheraea pernyi* and *A. polyphemus* (Saturnidae). J Comp Physiol 132:235-242

Boeckh J, Ernst K-D (1987) Contribution of single unit analysis in insects to an understanding of olfactory function. J Comp Physiol A 161:549-565

Boeckh J, Kaissling K-E, Schneider D (1965) Insect olfactory receptors. Cold Spring Harbor Symp Quant Biol 30:263-280

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

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 (1992) Response properties of CO_2-sensitive receptors in tsetse flies (Diptera: *Glossina palpalis*). Physiol Entomol 17:19-24

Bowen MF (1992) Terpene-sensitive receptors in female *Culex pipiens* mosquitoes: electrophysiology and behaviour. J Insect Physiol 38:759-764

Boyer ML, Jemiolo B, Andreolini F, Wiesler D, Novotny M (1989) Urinary volatile profiles of pine vole, *Microtus pinetorum*, and their endocrine dependency. J Chem Ecol 15:649-662

Burger BV, le Roux M, Garbers CF, Spies HSC, Bigalke RC, Pachler KGR, Vessels PL, Christ V, Maurer KH (1977) Further compounds from the pedal gland of the bontebok (*Damaliscus dorcas dorcas*). Z Naturforsh 32c:49-56

Burger BV, le Roux M, Spies HSC, Truter V (1981) Mammalian pheromone studies, V. Compounds from the preorbital gland of the grysbok, *Raphicerus melanotis*. Z Naturforsh 36c:344-346

Burger BV, Pretorius PJ (1987) Mammalian pheromone studies, VI. Compounds from the preorbital gland of the blue duiker, *Cephalophus monticola*. Z Naturforsh 42c:1355-1357

Burger BV, Pretorius PJ, Spies HSC, Bigalke RC, Gnerson GR (1990) Mammalian pheromones VIII. Chemical characterization of preorbital gland secretion of grey duiker, *Sylvicapra grimmia* (Artiodactyla: Bovidae). J Chem Ecol 16:397-416

Davis EE, Haggart DA, Bowen MF (1987) Receptors mediating host-seeking behaviour in mosquitoes and their regulation by endogenous hormones. Insect Sci Appl 8:637-641

Davis EE, Sokolove PG (1976) Lactic acid-sensitive receptors on the antennae of the mosquito, *Aedes aegypti*. J Comp Physiol 105:43-54

de Bruyne M, Dicke M, Tjallingii WF (1991) Receptor cell responses in the anterior tarsi of *Phytoseiulus persimilis A.-H.* to volatile kairomone components. Exp Appl Acarol 13:53-58

de Kramer JJ (1985) The electrical circuitry of an olfactory sensillum in *Antheraea polyphemus*. J Neurosci 5:2484-2493

de Kramer JJ, Hemberger J (1987) The neurobiology of pheromone perception. In: Prestwich GD, Blomquist GJ (eds) Pheromone biochemistry. Academic Press, Orlando, pp 433-472

Delot J (1990) Les phéromones d'agrégation-fixation chez la tique *Amblyomma variegatum* (Fabricius) (Ixodida): une étude en Guadeloupe.Thèse, Ecole Nationale Vétérinaire d'Alfort (France)

Den Otter CJ, Van der Goes van Naters WM (1992) Single cell recordings from tsetse (*Glossina m.morsitans*) antennae reveal olfactory, mechano- and cold receptors. Physiol Entomol 17:33-42

Diehl PA, Guerin PM, Vlimant M, Steullet P (1991) Biosynthesis, production site, and emission rates of aggregation-attachment pheromone in males of two *Amblyomma* ticks. J Chem Ecol 17:833-847

Ellin RI, Farrand RL, Oberst FW, Crouse CL, Billups NB, Koon WS, Musselman NP, Sidell FR (1974) An apparatus for the detection and quantitation of volatile human effluents. J Chromatogr 100:137-152

El Ziady S (1958) The behaviour of *Omithodoros erraticus* (Lucas, 1849), small form (Ixodoidae, Argasidae, towards certain environmental factors. Ann Ent Soc Am 51:317-336

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

Flood PF, Abrams SR, Muir GD, Rowell JE (1989) Odor of the muskox. A preliminary investigation. J Chem Ecol 15:2207-2217

Foelix RF, Axtell RC (1971) Fine structure of tarsal sensilla in the tick *Amblyomma americanum*. Z Zellforsch 114:22-37

Foelix RF, Axtell RC (1972) Ultrastructure of Haller's organ in the tick *Amblyomma americanum* L. Z Zellforsch 124:275-292

Fox GJ (1982) Potentials for pheromones in chimpanzee vaginal fatty acids. Folia primatol 37:255-266

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 Ent Soc Am 55:605-606

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

Gatehouse AG (1970) The probing response of *Stomoxys calcitrans* to certain physical and olfactory stimuli. J Insect Physiol 16:61-74

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

Gladney WJ, Grabbe RR, Ernst SE, Oehler DD (1974) The gulf coast tick: Evidence of a pheromone produced by males. J Med Entomol 11:303-306

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 29:77-83

Goetz N, Kaba G, Good D, Hussler G, Bore P (1988) Detection and identification of volatile compounds evolved from human hair and scalp using headspace gas chromatography. J Soc Cosmet Chem 39:1-13

Goodrich BS (1983) Studies of the chemical composition of secretions from skin glands of the rabbit *Oryctolagus cuniculus*. In: Müller-Schwarze D, Silverstein RM (eds) Chemical signals in vertebrates 3. Plenum Press, New York London, pp 275-290

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

Green CH, Hall MJR, Fergiani M, Chirico J (1993) Attracting adult New World screwworm, *Cochliomyia hominivorax*, to odour-baited targets in the field. Med Vet Entomol 7:59-65

Guerin PM, Städler E, Buser HR (1983) Identification of host plant attractants for the carrot fly, *Psilae rosae*. J Chem Ecol 9:843-861

Guerin PM, Steullet P, Kröber T, Diehl PA, Vlimant M, de Bruyne M, Cordas T, Falk-Vairant J, Kuhnert, Lösel PM (1992) The chemical ecology of ticks at the host vector interface. In: Munderloh, UG Kurtii TJ (eds) Proceedings of the 1st International conference on tick-borne pathogens at the host-vector interface: an agenda for research. Minnesota University Saint Paul, Minnesota, pp 314-323

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

Hafez M, Bishara SI (1982) Reactions ot three species of *Rhipicephalus* ticks to host odour and light (Ixodidea:Ixodidae). J Egypt Soc Parasitol 12:309-318

Haggart DA, Davis EE (1980) Ammonia-sensitive neurons on the first tarsi of the tick, *Rhipicephalus sanguineus*. J Insect Physiol 26:517-523

Haggart DA, Davis EE (1981) Neurons sensitive to 2,6-dichlorophenol on the tarsi of the tick *Amblyomma americanum* (Acari,Ixodidae). J Med Entomol 18:187-193

Hall DR, Beevor PS, Cork A, Nesbitt BF, Vale GA (1984) 1-octen-3-ol, a potent olfactory stimulant and attractant for tsetse isolated from cattle odours. Insect Sci Appl 5:335-339

Hall DR (1990) Use of host odor attractants for monitoring and control of tsetse flies. In: Ridgway RL, Silverstein RM, Inscoe MN (eds) Behavior-modifying chemicals for insect management. Applications of pheromones and other attractants. Marcel Dekker, New York Basel, pp 517-530

Haller G (1881) Vorläufige Bemerkungen über das Gehörorgan der Ixodiden. Zool Anz 4:165-167

Hansson BS, Van der Pers JNC, Löfqvist J (1989) Comparison of male and female olfactory cell response to pheromone compounds and plant volatiles in the turnip moth, *Agrostis segetum*. Physiol Entomol 14:147-155

Hess E, Vlimant M (1980) Morphology and fine structure of tarsal chemosensory hairs of the tick *Amblyomma variegatum* (Acarina, Ixodidae, Metastriata), including preliminary electrophysiological results. In: van der Starre (ed) Olfaction and Taste VII. IRC, London Washington DC, p 190

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, 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, De Castro JJ (1986) Field tests of the response of female *Amblyomma variegatum* (Acari:Ixodidae) to the synthetic aggregation-attachment pheromone and its components. Exp Appl Acarol 2:249-255

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 Chichester, pp 361-390

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

Holscher KH, Gearhardt HL, Barker RW (1980) Electrophysiological responses of three tick species to carbon dioxide in the laboratory and field. Ann Ent Soc Am 73:288-292

Homsher PJ, Sonenshine DE (1979) Scanning electron microscopy of ticks for systematic studies: 3. Structure of Haller's organ in five species of the subgenus *Multidentatus* of the genus *Ixodes*. Recent Advances in Acarology 2:485-490

Hoogstraal H (1956) Ticks of Sudan. US Naval Medical Research Unit n° 3, Cairo (Egypt)

Hopkins BA (1964) The probing response of *Stomoxys calcitrans* (L.) (the Stable Fly) to vapours. Anim Behav 12:513-524

Hungate RE (1966) The rumen and its microbes. Academic Press, New York

Hribar LJ, Leprince DJ, Foil LD (1992) Ammonia as an attractant for adult *Hybomitra lasiophthalma* (Diptera: Tabanidae). J Med Entomol 29:346-348

Kaissling K-E (1971) Insect olfaction. In Beichler LM (ed) Handbook of sensory physiology vol IV/1. Springer, Berlin Heidelberg New York, pp 351-431

Kaissling K-E (1986) Chemo-electrical transduction in insect olfactory receptors. Annu Rev Neurosci 9:121-145

Kaissling K-E, Strausfeld CZ, Rumbo ER (1987) Adaptation processes in insect olfactory receptors. Mechanisms and behavioral significance. In: Roper SD, Atema J (eds) Olfaction and taste IX. Annals New York of Sciences, New York, pp 104-112

Kaltenrieder M, Labhart T, Hess E (1989) Spectral sensitivity and visual field of two tick species (*Hyalomma dromedarii* and *Amblyomma variegatum*) (Acari,Ixodidae). J Comp Physiol A 165:155-164

Kaltenrieder M (1990) Scototaxis and target perception in the camel tick *Hyalomma dromedarii.* Exp Appl Acarol 9:267-278

Kanda F, Yagi E, Fukuda M, Nakajima K, Ohta T, Nakata O (1990) Elucidation of chemical compounds responsible for foot malodour. Br J Dermatol 122:771-776

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

Kim KC (1985) Evolutionnary relationship of parasitic arthropods and mammals. In: Kim KC (ed) Coevolution of parasitic arthropods and mammals. John Wiley & Sons Ltd, Chichester New York, pp 3-82

Kline DL, Takken W, Wood JR, Carlson DA (1990) Field studies on the potential of butanone, carbon dioxide, honey extract, 1-octen-3-ol, L-lactic acid and phenols as attractants for mosquitoes. Med Vet Entomol 4:383-391

Kneidel KA (1984) Carrion as an attractant to the American dog tick, *Dermacentor variabilis* (Say). J NY Entomol Soc 92:405-406

Kramer E (1976) The orientation of walking honeybees in odour fields with small concentration gradients. Physiol Entomol 1:27-37

Krotoszynski B, Gabriel G, O'Neill J, Claudio MPA (1977) Characterization of human expired air: A promising investigative and diagnostic technique. J Chromatogr Sci 15:239-244

Kuwahara Y (1991) Pheromone studies on astigmatid mites-alarm aggregation and sex. In: Dusbábek F, Bukva V (eds) Modern acarology. Academia Prague, pp 43-52

Lacher V (1967) Elektrophysiologische Untersuchungen an einzelnen Geruchsrezeptoren auf den Antennen weiblicher Moskitos (*Aedes aegypti L.*). J Insect Physiol 13:1461-1470

Labows JN, McGinley KJ, Leyden JJ, Webster GF (1979a) Characteristic γ-lactone odor production of the genus *Pityrosporum*. Appl Environ Microbiol 38:412-415

Labows JN, Preti G, Hoelzle E, Leyden JJ, Kligman A (1979b) Analysis of human axillary volatiles: compounds of exogenous origin. J Chromatogr 163:294-299

Lederer E (1946) Chemistry and biochemistry of the scent glands of the beaver, *Castor fiber*. Nature 157:231-232

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

Leonovich SA (1978) Fine structure of Haller's organ in the tick *Hyalomma asiaticum* P.Sch.et E.Schl. (Parasitiformes, Ixodidae, Amblyomminae). Entomol Obodzr 57:221-225

Leonovich SA (1979) Ultrastructural investigations of Haller's organ in Argasid ticks, *Argas tridentatus* (Argasinae). Parasitologiye (Leningrad) 13:483-488

Leonovich SA (1980) Fine structural investigation of Haller's organ in argasid ticks *Ornithodoros Moubata* and *Alveonasus lahorensis*. Parasitologiye (Leningrad) 14:376-385

Leonovich SA, Belozerov VN (1992) Regeneration of Haller's sensory organ in the tick *Ixodes ricinus* L. Exp Appl Acarol 15:59-79

Lerner MR, Gyorgyi TK, Reagan J, Roby-Shemkovitz A, Rybczynski R, Vogt RG (1990) Peripheral events in moth olfaction. Chem Senses 15:191-198

Light DM (1986) Central integration of sensory signals: an exploration of processing of pheromonal and multimodal information in lepidopterian brains. In: Payne TL, Birch MC, Kennedy CEJ (eds) Mechanisms in insect olfaction. Clarendon Press, Oxford, pp 287-302

Lindvall T (1977) Perception of composite odorous air polluants. In: Le Magnen J, Macleod P (eds) Olfaction and Taste VI. Information Retrievial, London Washington, pp 449-458

Matthysse JG, Colbo MH (1987) The Ixodid ticks of Uganda. Entomol Soc Am, College Park, Maryland

McIver SB (1987) Sensilla of haematophagous insects sensitive to vertebrate host-associated stimuli. Insect Sci Appl 8:627-635

Morel PC (1969) Contribution à la connaissance de la distribution des tiques (Acariens, Ixodidae et Amblyommidae) en Afrique éthiopéenne continentale. Thèse, Faculté des Sciences d'Orsay, Université Paris (France)

Morita H, Yamashita S (1961) Receptor potential recorded from sensilla basiconica on the antenna of the silkworm larvae, *Bombyx mori.* J Exp Biol 38: 851-861

Mount GA, Dunn JE (1983) Economic thresholds for Lone Star Ticks (Acari: Ixodidae) in recreational areas based on a relationship between CO_2 and human subject sampling. J Econ Ent 76:327-329

Müller-Schwarze D, Müller-Schwarze C, Singer AG, Silverstein RM (1974) Mammalian pheromone: identification of active component in the subauricular scent of the male pronghorn. Science 183:860-862

Natynczuk S, Bradshaw JWS, Macdonald DW (1989) Chemical constituents of the anal sacs of domestic dogs. Biochem Syst Ecol 17:83-87

Nevill,E.M. (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, Rechav YH (1979) An assembly pheromone and its perception in the tick *Amblyomma variegatum* (Acarina, Ixodidae). J Med Entomol 16:507-511

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

Norval RAI, Andrew HR, Yunker CE (1989a) Pheromone-mediation of host-selection in bont ticks (*Amblyomma hebraeum* Koch). Science 243:364-365

Norval RAI, Butler JF, Yunker CE (1989b) Use of carbon dioxide and natural or synthetic aggregation-attachment pheromone of the bont tick, *Amblyomma hebraeum*, to attract and trap unfed adults in the field. Exp Appl Acarol 7:171-180

Norval RAI, Peter T, Yunker CE, Sonenshine DE, Burridge MJ (1991a) Responses of the ticks *Amblyomma hebraeum* and *A. variegatum* to known or potential components of the aggregation-attachment pheromone. I. Long-range attraction. Exp Appl Acarol 13:11-18

Norval RAI, Peter T, Yunker CE, Sonenshine DE, Burridge MJ (1991b) Responses of the ticks *Amblyomma hebraeum* and *A. variegatum* to known or potential components of the aggregation-attachment pheromone. II. Attachment stimulation. Exp Appl Acarol 13:19-26

Norval RAI, Peter T, Meltzer MI (1992a) A comparison of the attraction of nymphs and adults of the ticks *Amblyomma hebraeum* and *A.variegatum* to carbon dioxide and the male-produced aggregation-attachment pheromone. Exp Appl Acarol 13:179-186

Norval RAI, Peter T, Meltzer MI (1992b) Comparison of the attachment rates of males of the ticks *Amblyomma hebraeum* and *A. variegatum* to cattle, sheep and rabbits in the absence of aggregation-attachment pheromone. Exp Appl Acarol 15:145-152

Norval RAI, Peter T, Sonenshine DE, Burridge MJ (1992c) Responses of the ticks *Amblyomma hebraeum* and *A. variegatum* to known or potential components of the aggregation-attachment pheromone. III. Aggregation. Exp Appl Acarol 16:237-245

O'Connell RJ, Singer AG, Pfaffmann C, Agosta WC (1979) Pheromones of hamster vaginal discharge. Attraction to femtogram amounts of dimethyl disulfide and to mixtures of volatile components. J Chem Ecol 5:575-585

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

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

Perritt DW, Couger G, Barker RW (1993) Computer-controlled olfactometer system for studying behavioural responses of ticks to carbon dioxide. J Med Entomol 30:571-578

Poffet JM (1988) L'utilisation des infrarouges dans l'orientation de la tique d'*Amblyomma variegatum* (Fabricius 1794). Travail de licence, Université Neuchâtel (Suisse)

Preti G, Smith III AB, Beauchamp GK (1977) Chemical and behavioral complexity in mammalian chemical communication systems: Guinea pigs (*Cavia porcellus*), Marmosets (*Saguinus fuscicollis*) and humans (*Homo sapiens*). In: Müller-Schwarze D, Mozell MM (eds) Chemical signals in vertebrates 1. Plenum Press, New York London, pp 95-114

Rechav Y, Parolis H, Whitehead GB, Knight AG (1977) Evidence for an assembly pheromone(s) produced by males of the bont tick, *Amblyomma hebraeum* (Acarina: Ixodidae). J Med Entomol 14:71-78

Rickli M., Guerin PM, Diehl PA (1992) Palmitic acid released from honeybee worker larvae attracts the parasitic mite *Varroa jacobsoni* on a servosphere. Naturwissenschaften 79:320-322.

Roshdy MA Axtell RC (1972) Ultrastructure of arthropod sensory structure: scanning electron microscopy of *Haemaphysalis* ticks. Technical report No.3; Office of Naval Research, Naval Biology program. Raleigh, North Carolina

Roshdy MA, Foelix RF, Axtell RC (1972) The subgenus *Persicargas* (Ixodoidea: Argasidae: *Argas*). 16. Fine structure of Haller's organ and associated tarsal setae of adult *A.(P.) arboreus* Kaiser, Hoogstraal, and Kohls. J Parasitol 58:805-816

Rumbo ER (1989) The shape of extracellularly recorded nerve impulses from pheromone receptors. Chem Senses 14:361-369

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

Sass H (1976) Zur nervösen Codierung von Geruchsreizen bei *Periplaneta americana*. J Comp Physiol 107:49-65

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 Ent Soc Am 67:150-152

Schöni R, Hess E, Blum W, Ramstein K (1984) The aggregation-attachement pheromone of the tropical bont tick *Amblyomma variegatum* Fabricius (Acari:Ixodidae): isolation, identification and action of its components. J Insect Physiol 30:613-618

Schöni R (1987) Das wirtsgebundene Aggregationspheromon der tropischen Buntzecke *Amblyomma variegatum* (Acari: Ixodidae). Thèse, Université Neuchâtel (Suisse)

Schultz TH, Flath RA, Stem DJ, Mon TR, Teranishi R, McKenna Kruse S, Butler B, Howard WE (1988) Coyote estrous urine volatiles. J Chem Ecol 14:701-712

Schulze P (1941) Das Geruchsorgan der Zecken. Untersuchungen über die Abwandlungen eines Sinnesorgans und seine Stammegeschichtliche Bedeutung. Z Morphol Oekol Tiere 37:491-546

Seelinger G (1977) Der Antennendzapfen der tunesischen Wüstenassel Hemilepistus reaumuri, ein komplexes Sinnesorgan (Crustacea, Isopoda). J Comp Physiol 113:95-103

Selzer R (1984) On the specificities of the antennal olfactory receptor cells of *Periplaneta americana*. Chem Senses 8:375-395

Sinitsina EE (1974) Electrophysiological reactions of Haller's organ neurons in the tick *Hyalomma asiaticum P.Sch.* and *E.Sch.* to odor. Parasitologiye (Leningrad) 8:223-226

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

Smith PW, Parks OW, Schwartz DP (1984) Characterization of male goat odors: 6-trans-nonenal. J Dairy Sci 67:794-801

Sonenshine DE (1991) Sense organs and sensory physiology. In: Sonenshine DE (ed) Biology of ticks I. Oxford University Press, New York Oxford, pp 249-279

Spector W (1956) Handbook of biological data. Saunders, London

Städler E, Hanson FE (1975) Olfactory capabilities of the gustatory chemoreceptors of the Tobacco Hornworm larvae. J Comp Physiol 104:97-102

Stange G (1975) Linear relation between stimulus concentration and primary transduction process in insect CO_2-receptors. In: Denton DA, Coghan JP (eds) Olfaction and Taste V. Academic Press, New York San Francisco London, pp 207-211

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 Neuchâtel (Suisse)

Steinbrecht RA (1984) Arthropoda: chemo-, hygro-, and thermoreceptors. In: Bereiter-Hahn J, Matolsky AG, Richards KS (eds) Biology of the integument. Springer-Verlag, Berlin, pp 523-553

Stengl M, Hatt H, Breer H (1992) Peripheral processes in insect olfaction. Annu Rev Physiol 54:665-681

Steullet P (1987) Importance des stimuli olfactifs lors de la recherche de l'hôte par les adultes d'*Amblyomma variegatum* (Acarina: Ixodidae): approche éthologique à l'aide d'un wind-tunnel. Travail de licence, Université Neuchâtel (Suisse)

Steullet P, Guerin PM (1992a) Perception of breath components by the tropical bont tick, *Amblyomma variegatum* Fabricius (Ixodidae). I. CO_2-excited and CO_2-inhibited receptors. J Comp Physiol A 170:665-676

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

Steullet P, Guerin PM (1993a) Identification of vertebrate volatiles stimulating olfactory receptors on tarsus of the tick *Amblyomma variegatum* Fabricius (Ixodidae). I Receptors within the Haller's organ capsule.In J Comp Physiol A

Steullet p, Guerin PM (1993b) Identification of vertebrate volatiles stimulating olfactory receptors on tarsus of the tick *Amblyomma variegatum* Fabricius (Ixodidae). II Receptors outside the Haller's organ capsule. In J Comp Physiol A

Sugisawa H (1981) Sample preparation: isolation and concentration. In: Teranishi R, Flath RA, Sugisawa H (eds) Flavor research recent advances. Marcel Dekker, New York Basel, pp 15-51

Thomas G, Schomaker CH, Been TH, van den Berg MJ, Prijs HJ (1985) Host finding and feeding in *Hydrotaea irritans* (Diptera, Muscidae): the role of chemical senses. Vet Parasitol 18:209-221

Thonney F (1987) Etude morphologique et structurale des récepteurs sensoriels du tarse I de la tique *Ixodes ricinus L.* Thèse, Université Neuchâtel (Suisse)

Tichy H, Loftus R (1983) Relative excitability of the antennal olfactory receptors in the stick insect, *Carausius morosus L.*: in search of a simple, concentration-independant odor-coding parameter. J Comp Physiol 152:459-473

Tonzetich J (1977) Production and origin of oral malodor: a review of mechanisms and methods of analysis. J Periodontol 48:13-20

Totze (1933) Beitrage zur Sinnesphysiologie der Zecken. Z vergl Physiol 19:110-161

Tschinkel WR (1975) Unusual occurrence of aldehydes and ketones in the defensive secretion of the tenebrionid beetle, *Eleodes beameri*. J Insect Physiol 21:659-671

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

Vale GA, Hall DR (1985) The role of 1-octen-3-ol, acetone and carbon dioxide in the attraction of tsetse flies, *Glossina* spp. (Diptera: Glossinidae) to ox odour. Bull Ent Res 75:209-217

Visser JH (1986) Host odour perception in phytophagous insects. Annu Rev Entomol 31:121-144

Visser JH, de Jong R (1988) Olfactory coding in the perception of semiochemicals. J Chem Ecol 14:2005-2018

Vogt RG (1987a) The molecular basis of pheromone reception: its influence on behavior. In: Prestwich GD, Blomquist GJ (eds) Pheromone biochemistry. Academic Press, Orlando, pp 385-432

Vogt RG, Prestwich GD (1987b) Variation in olfactory proteins. Evolvable elements encoding insect behaviour. In: Roper SD, Atema J (eds) Olfaction and taste IX. Annals New York Academy of Sciences, New York, pp 689-691

Vogt RG, Prestwich GD, Lerner MR (1991) Odorant-binding-protein subfamilies associate with distinct classes of olfactory receptor neurons in insects. J Neurobiol 22:74-84

Wadhams LJ (1982) Coupled gas chromatography - single cell recording: a new technique for use in the analysis of insect pheromones. Z Naturforsh 37c:947-952

Wallace JW, Mansell RL (1976) Biochemical interaction between plants and insects. Plenum Press, New York

Waladde SM (1976) The sensory nervous system of the adult cattle tick *Boophilus microplus* (Canestrini) Ixodidae. Part I. Light microscopy. J Aust ent soc 15:379-387

Waladde SM (1977) The sensory nervous system of the adult cattle tick *Boophilus microplus*. II. Scanning electron microscopy. J Aust ent soc 16:73-79

Waladde SM (1982) Tip-recording from ixodid tick olfactory sensilla: responses to tick related odours. J Comp Physiol 148:411-418

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

Waldow U (1973) Elektrophysiologie eines neuen Aasgeruchrezeptors und seine Bedeutung für das Verhalten des Totengräbers (*Necrophorus*). J Comp Physiol 83:415-424

Warnes ML, Finlayson LH (1985a) Responses of the stable fly, *Stomoxys calcitrans* (L) (Diptera: Muscidae) to carbon dioxide and host odours. 1. Activation. Bull Ent Res 75:519-527

Warnes ML, Finlayson LH (1985b) Responses of the stable fly, *Stomoxys calcitrans* (L) (Diptera: Muscidae) to cardon dioxide and host odours. 2. Orientation. Bull Ent 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

Welsh DI, Watts CD (1990) Collection and identification of trace organic compounds in atmospheric deposition from a semi-rural site in the U.K.. Intern J Environ Anal Chem 38:185-198

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

Wood WF, Leahy MG, Galun R, Prestwich GD, Meinwald J, Purnell RE, Payne RC (1975) Phenols as pheromones of ixodid ticks: a general phenomenon? J Chem Ecol 1:501-509

Yamana K, Toh Y, Tateda H (1986) Electrophysiological studies on the temporal organ of the japenese house centipede, *Thereuonepa hilgendorfi*. J Exp Biol 126:297-314

Yamana K, Toh Y (1987) Intracellular recording from receptor cells of the temporal organ of the japenese house centipede, *Thereuonema hilgendorfi*: receptor potential and conductance changes. J Exp Biol 131:205-213

Yunker CE, Norval RAI (1991) Field studies on the aggregation-attachment pheromones of *Amblyomma* spp., vectors of human and animal rickettsioses, in Zimbabwe. In: Dusbábek F, Bukva V (eds) Modern acarology. Proceedings of the VIII International Congress of Acarology, Prague, pp 79-82

Yunker CE, Peter T, Norval RAI, Sonenshine DE, Burridge MJ, Butler JF (1992) Olfactory responses of adult *Amblyomma hebraeum* and *A.variegatum* (Acari: Ixodidae) to attractant chemicals in laboratory tests. Exp Appl Acarol 13:295-301

Zacharuk RY (1985) Antennae and sensilla. In: Kerkut GA, Gilbert LI (eds) Comprehensive insect physiology, biochemistry, and pharmacology. Vol 6, Pergamon Press, Oxford, pp 1-69

Zeng X-N, Leyden JJ, Lawley HJ, Sawano K, Nohara I, Preti G (1991) Analysis of characteristic odors from human male axillae. J Chem Ecol 17:1469-1492

Zeng X-N, Leyden JJ, Brand JG, Spielman AI, McGinley KJ, Preti G (1992) An investigation of human apocrine gland secretion for axillary odor precursors. J Chem Ecol 18:1039-1055

Zlatkis A, Liebich HM (1971) Profile of volatile metabolites in human urine. Clin Chem 17:592-594

Ziesmann J, Kaib M, Wolfrum U (1992) CO_2-dependent response of an olfactory sensillum in the termite *Schedorhinothermes lamanianus*. Proceedings of ECRO, München, p 171

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VIII. APPENDIX

stimuli	rabbit 1 *	rabbit 2	rabbit 3	steer 1 *	steer 2	skin of steer
hexanal	+	+	+	+	+	+
heptanal	--	--	+	--	+	--
(E)-2-heptenal	?	?	+	?	?	+
nonanal	+	+	+	+	+	+
1-octen-3-ol	+	+	+	+	+	+
furfural	+	+	+	+	+	+
benzaldehyde	+	+	+	+	+	+
γ-valerolactone	+	--	--	--	+	--
2-mathylpropanoic acid	+	+	+	+	+	+
butanoic acid	+	+	+	+	+	+
3-methylbutanoic acid	+	+	+	+	+	+
pentanoic acid	(+)	+	+	+	+	+
nonanoic acid	(+)	+	+	+	+	+
2-hydroxybenzaldehyde	+	+	+	+	+	+
2-methylbenzaldehyde	+	+	+	--	+	--
3-methylbenzaldehyde	+	+	+	--	+	--
4-methylbenzaldehyde	+	+	+	--	+	--
2-nitrophenol	+	+	--	+	+	--
4-methyl-2-nitrophenol	+	+	--	+	+	+

Table 8.1. Olfactory stimuli for *Amblyomma variegatum* in bovine and rabbit odour as collected on Porapak and in skin wash of steer. * Signifies that the extract has been collected simultaneously from air in an adjacent room without rabbit or an animal stall without steer (blanks). Presence and abundance of stimuli were compared between rabbit or bovine odour and their respective blanks. (+) Indicates that the stimulus was also present in the blank but at a level < 2 times lower, + that the stimulus was also present in the blank but at a level > 2 timas lower., and + that the stimulus was not detected in the blank.

Table A

acetonitrile	p, w	dimethyl sulfoxide	p
4-methyl-2-pentanone	w	2-(2-butoxyethoxy)ethanol	p, w
2-methyl-3-buten-2-ol	w	dimethyl sulfone	p
2-ethoxyethanol	p	benzothiazole	p
styrene	w	phenol	p, w
2-propanol	p, w	γ-nonanolactone	p, w
2-butoxybutanol	p, w	4-methylphenol	p, w
nonenal	w	2-ethylphenol	p
2-ethylhexanol	p, w	3-ethylphenol	p

Table B

α-pinene	2-ethylhexanol
camphene	pentadecane
undecane	camphor
β-pinene	jupinene
2-heptanone	indene
L-limonene	1-phenylethanone
dodecane	α-terpineol
3-methylbuten-1-ol	2-(2-butoxyethoxy)ethanol
styrene	dimethyl sulfone
2-nonanone	phenol
tetradecane	4-methylphenol
	4-ethylphenol

Table 8.2. Tentative gas chromatography-coupled mass spectrometry identification of some components of bovine (A) and rabbit odour (B) but not found to stimulate olfactory receptors of *A. variegatum* during gas chromatography-coupled electrophysiology analyses. Identification was based on comparison of mass spectra of unknowns with those of standards in a computer-based library (percent match > 90% in each case). p presence in bovine odour as collected on Porapak Q; w presence in the bovine skin wash extract.

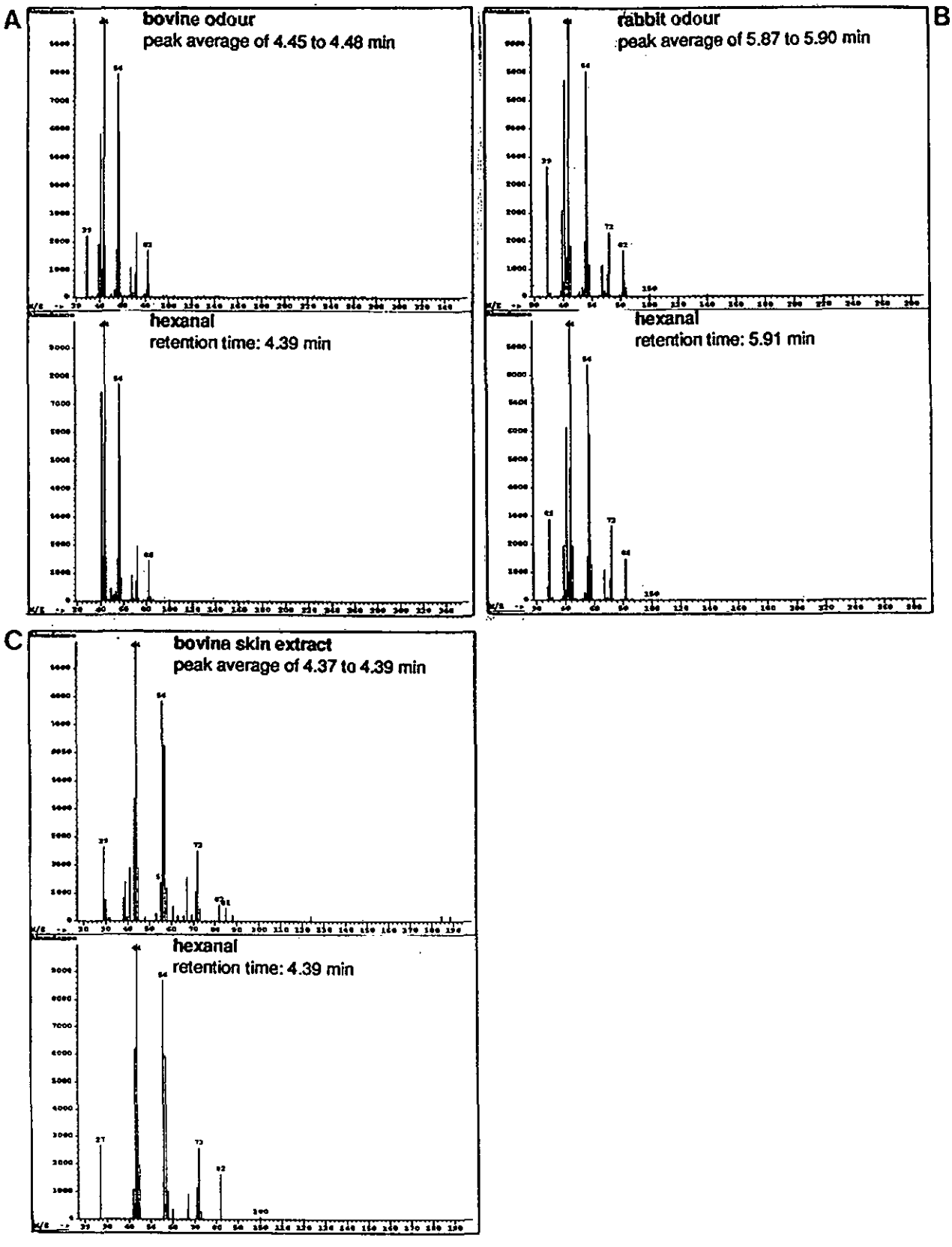


Fig. 8.1. Gas chromatography-coupled mass spectrometry analyses. Comparison of the mass spectrum and retention time of an unknown stimulant found in bovine odour (A), rabbit odour (B) and in the bovine skin extract (C) with that of synthetic hexanal injected under the same conditions as for the natural odour in each case.

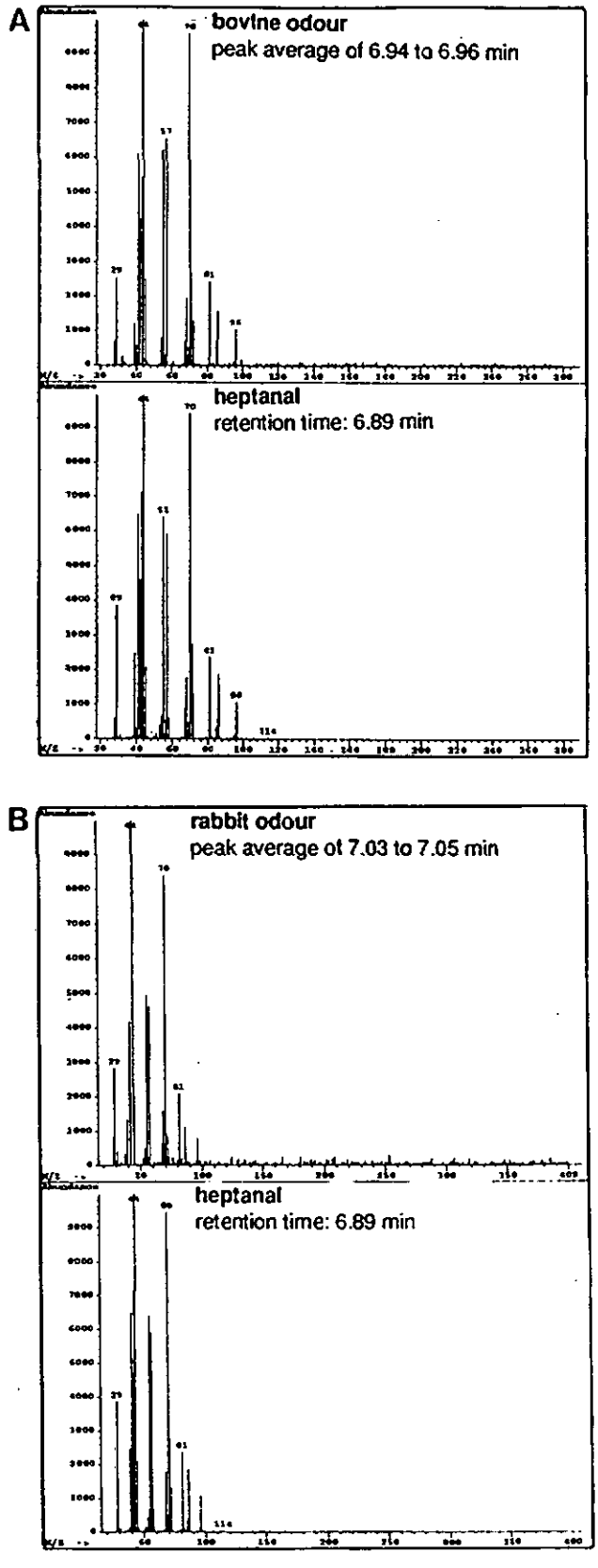


Fig. 8.2. Gas chromatography-coupled mass spectrometry analyses. Comparison of the mass spectrum and retention time of an unknown stimulant found in bovine odour (A) and rabbit odour (B) with that of synthetic heptanal injected under the same conditions as for the natural odour in each case.

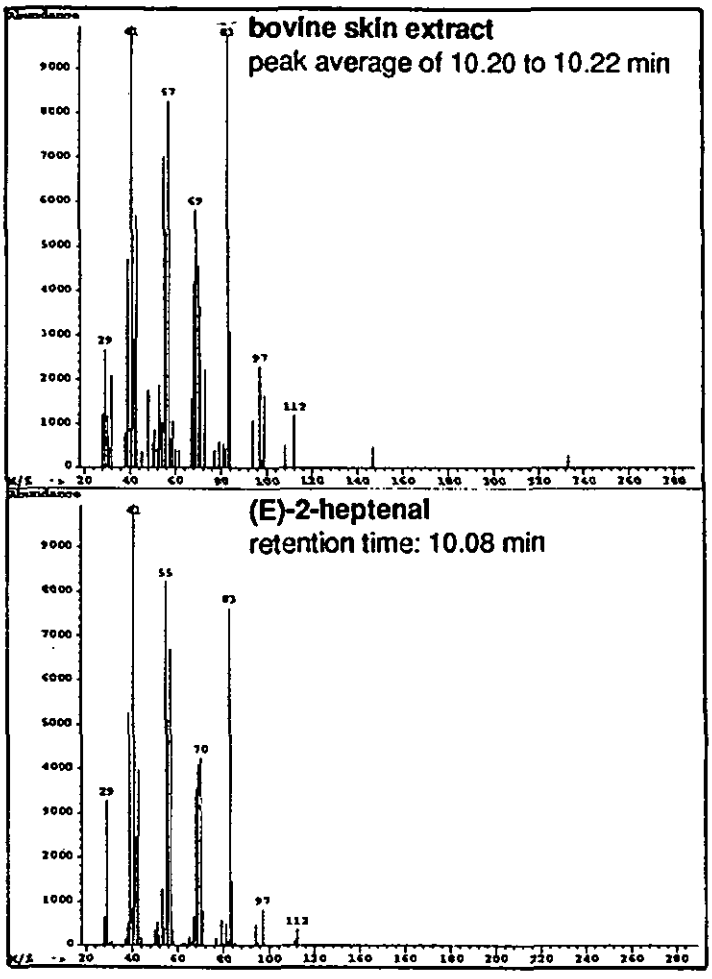


Fig. 8.3. Gas chromatography-coupled mass spectrometry analyses. Comparison of the mass spectrum and retention time of an unknown stimulant found in the bovine skin extract with that of synthetic (E)-2-heptenal injected under the same conditions as for the extract. Full identification of (E)-2-heptenal in bovine and rabbit odour as collected on Porapak, based on a full mass spectrum, was not feasible because of coeluting products which obscured the spectrum. Presence of the molecular ion of heptenal (M^+=112) was however detected in a peak in bovine and rabbit odour at the same retention as the synthetic analogue.

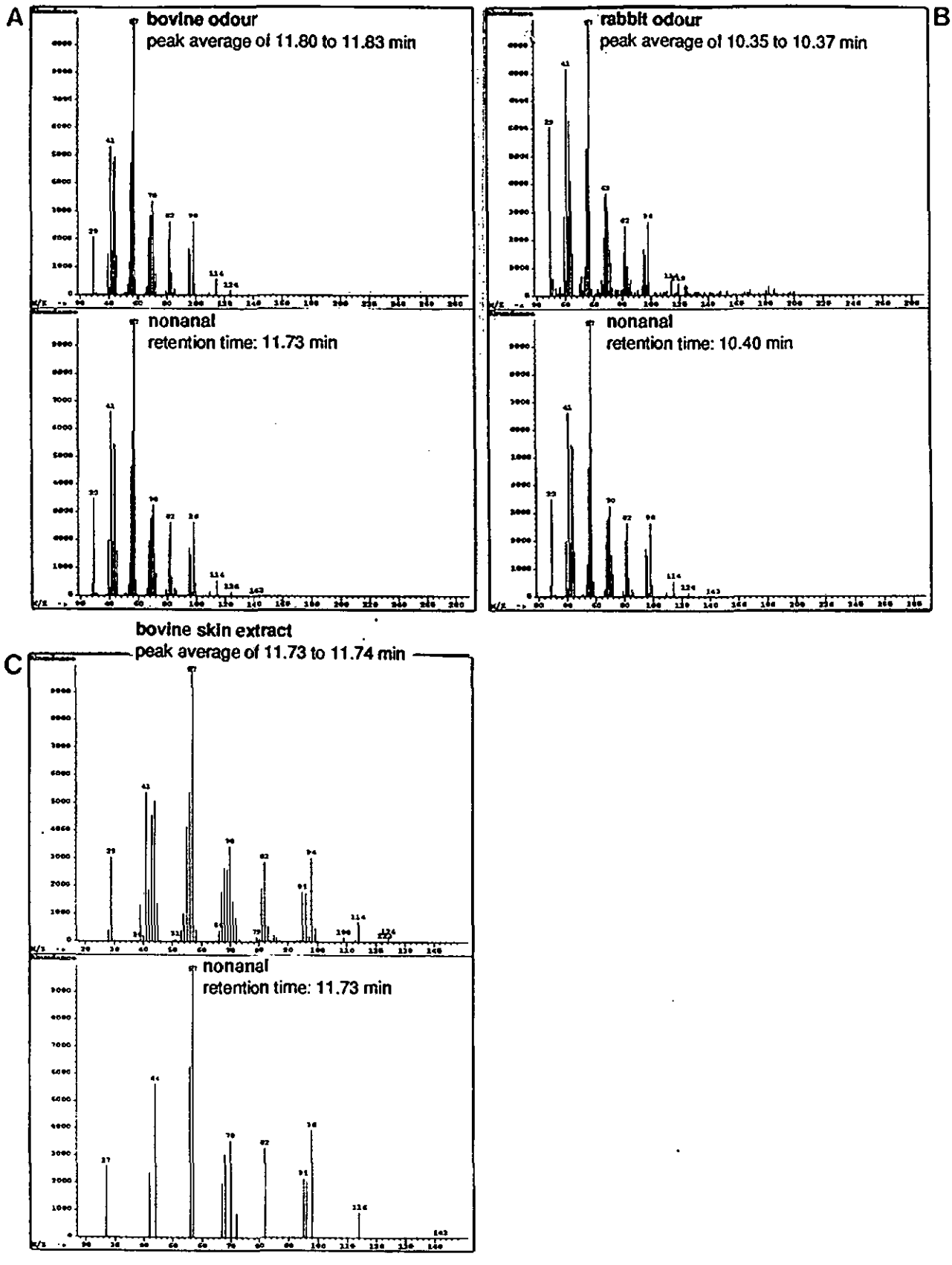


Fig. 8.4. Gas chromatography-coupled mass spectrometry analyses. Comparison of the mass spectrum and retention time of an unknown stimulant found in bovine odour (**A**), rabbit odour (**B**) and in the bovine skin extract (**C**) with that of synthetic nonanal injected under the same conditions as for the natural odour in each case.

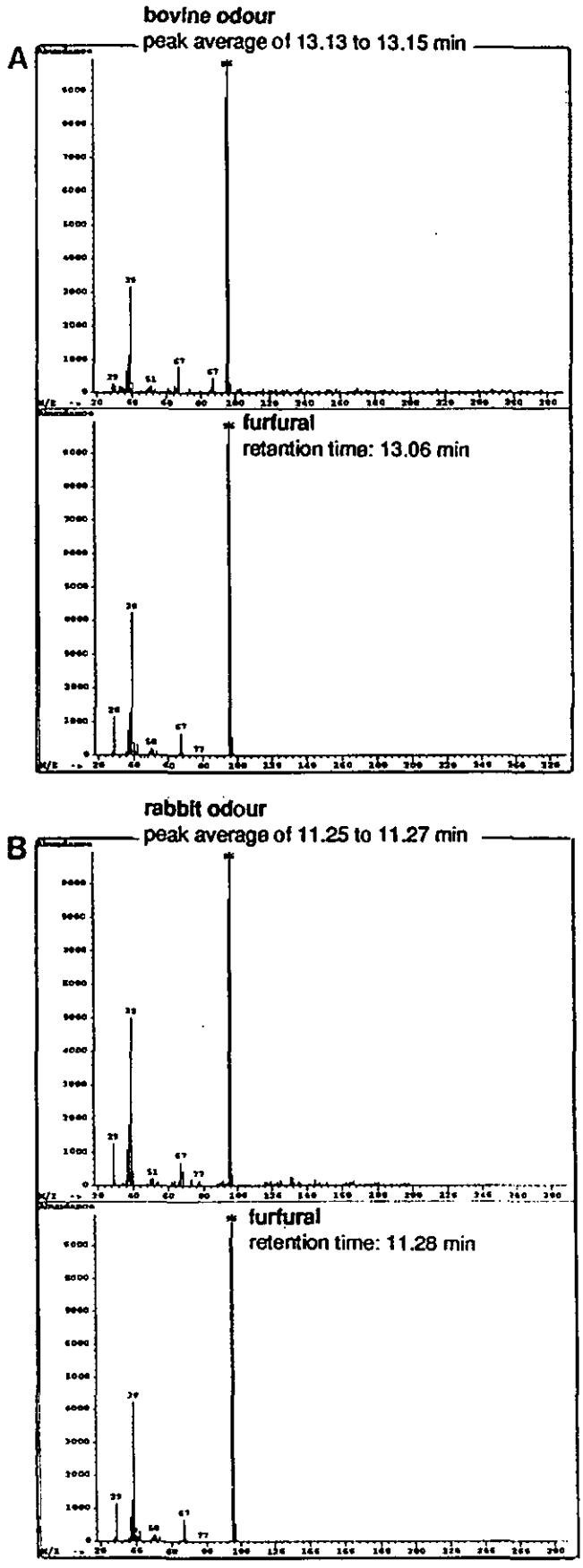


Fig. 8.5. Gas chromatography-coupled mass spectrometry analyses. Comparison of the mass spectrum and retention time of an unknown stimulant found in bovine odour (A) and rabbit odour (B) with that of synthetic furfural injected under the same conditions as for the natural odour in each case. Presence of furfural was indicated in the bovine skin extract by its molecular ion (M^+=96) in a peak eluting at the same retention time as the synthetic analogue.

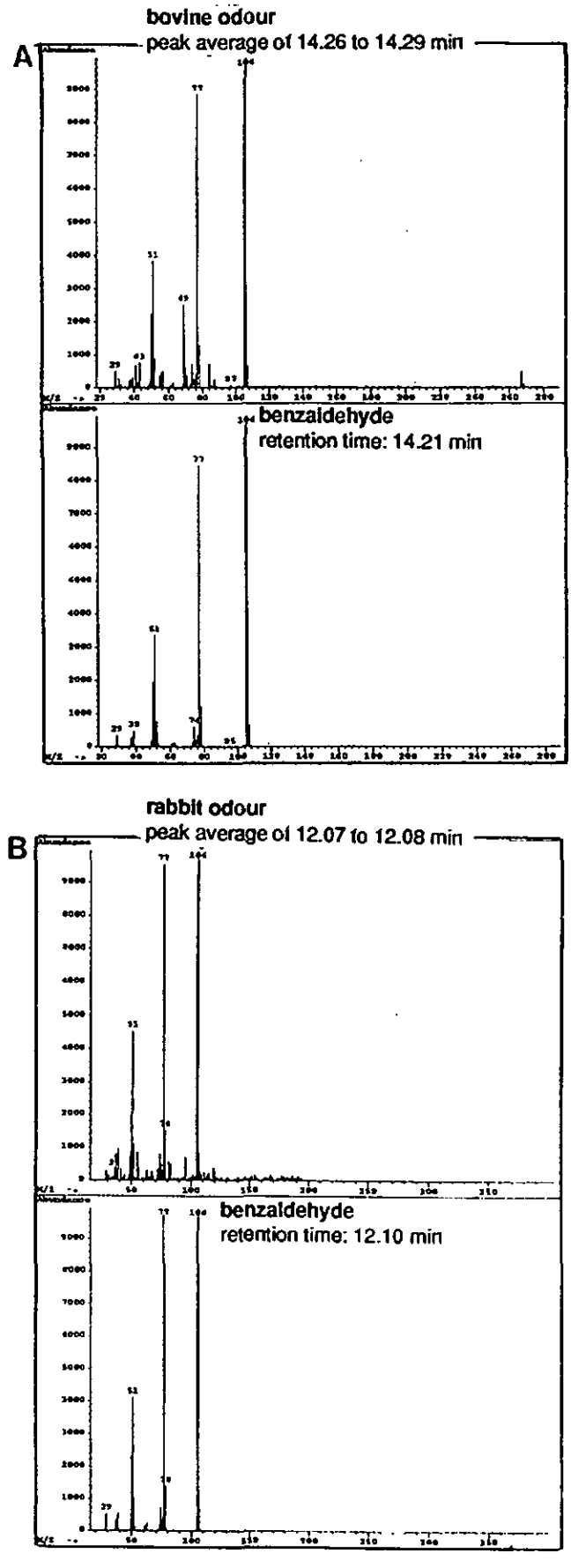


Fig. 8.6. Gas chromatography-coupled mass spectrometry analyses. Comparison of the mass spectrum and retention time of an unknown stimulant found in bovine odour (A) and rabbit odour (B) with that of synthetic benzaldehyde injected under the same conditions as for the natural odour in each case. Presence of benzaldehyde was indicated in the bovine skin extract by its molecular ion (M^+=106) in a peak eluting at the same retention time as the synthetic analogue.

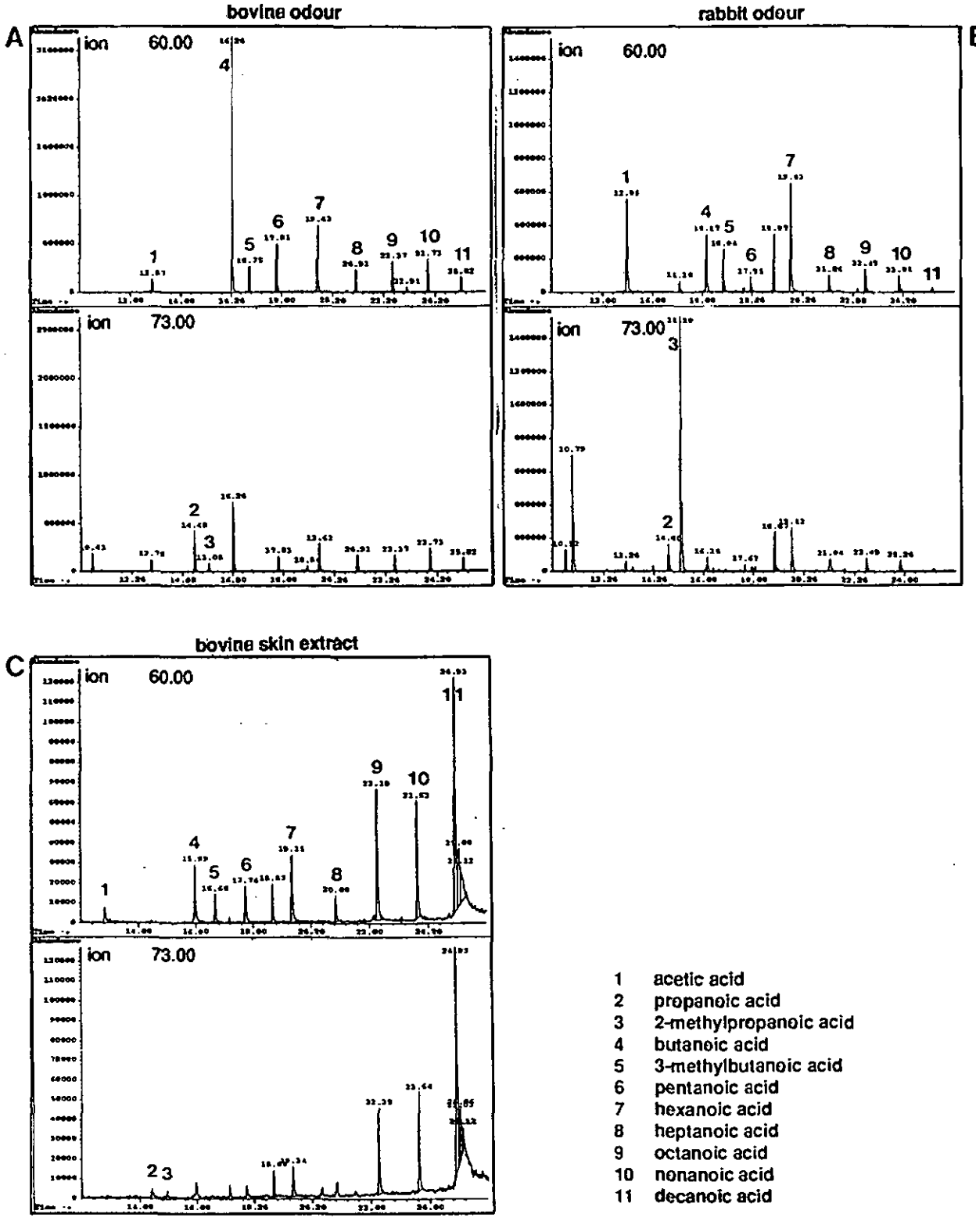


Fig. 8.7. Gas chromatography-coupled mass spectrometry analyses. Full identification of short-chain fatty acids (C_2 to C_{10}) in bovine and rabbit odour, based on full mass spectra, was often not feasible because of coeluting products which obscured the spectra. Presence of these short-chain fatty acids in bovine odour (A), rabbit odour (B), and in the bovine skin extract (C) was however indicated using 2 characteristic ions (M/Z=60; and M/Z=73) for single ion monitoring. Peaks eluting at the same retention time as the synthetic analogues contained these ions.

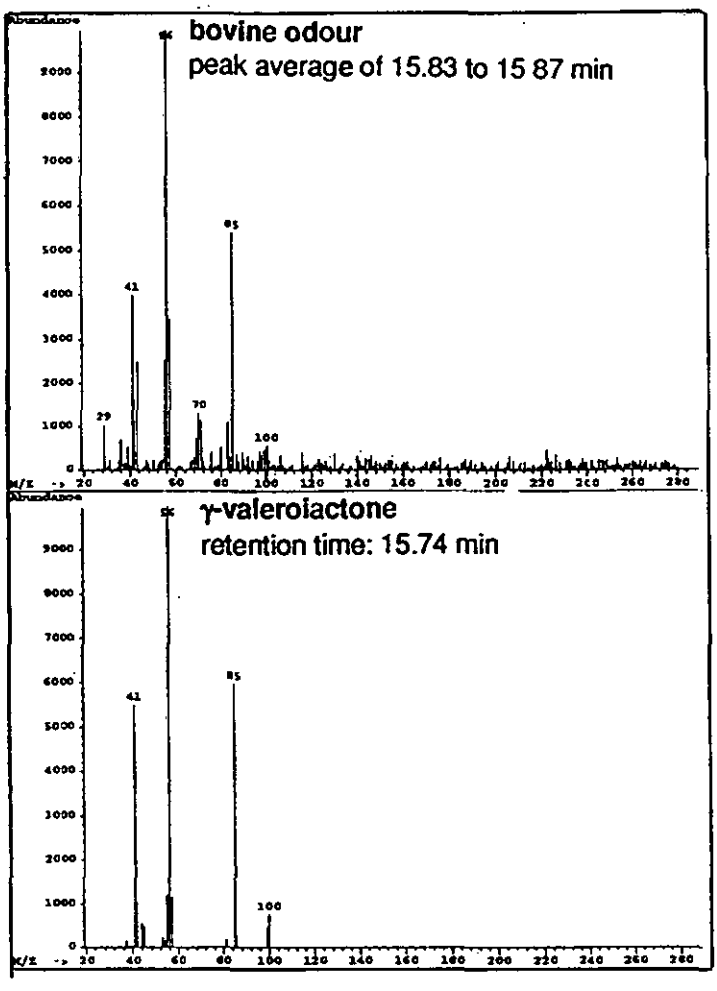
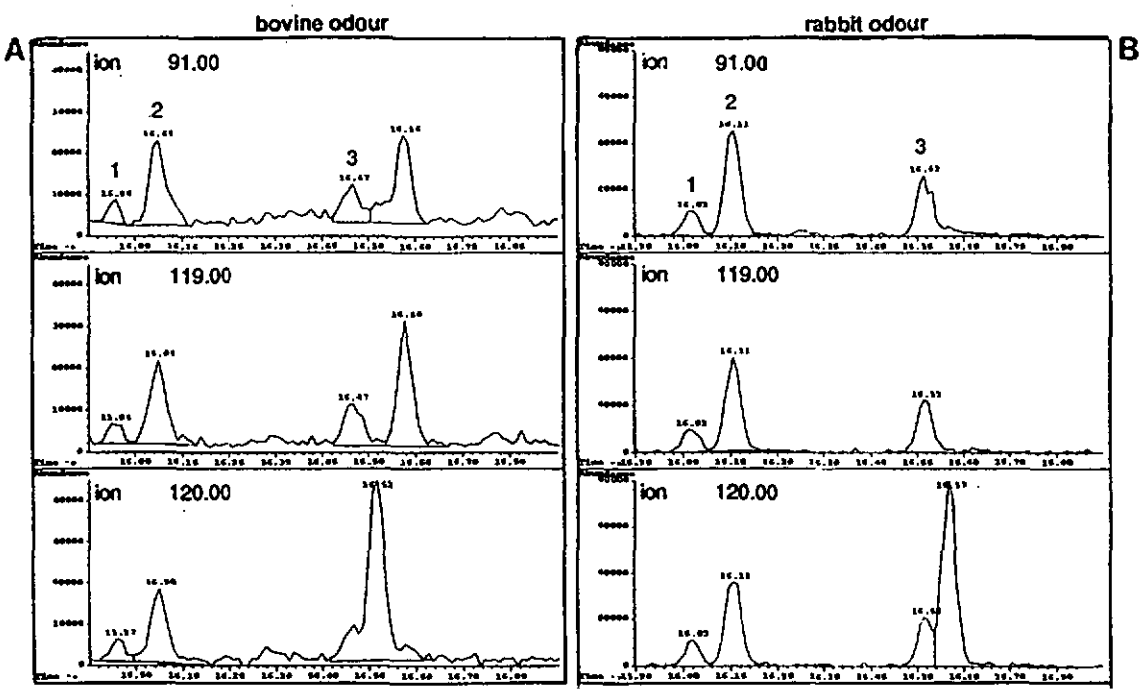


Fig. 8.8. Gas chromatography-coupled mass spectrometry analyses. Comparison of the mass spectrum and retention time of an unknown stimulant found in bovine odour with that of synthetic γ-valerolactone injected under the same conditions as for the natural odour. γ-Valerolactone was detected in an extact of rabbit odour with 3 characteristic ions (M/Z=56, 85, and 100, respectively) in a peak eluting at the same retention time as the synthetic analogue.



1 2-methylbenzaldehyde (15.88 min)

2 3-methylbenzaldehyde (15.96 min)

3 4-methylbenzaldehyde (16.38 min)

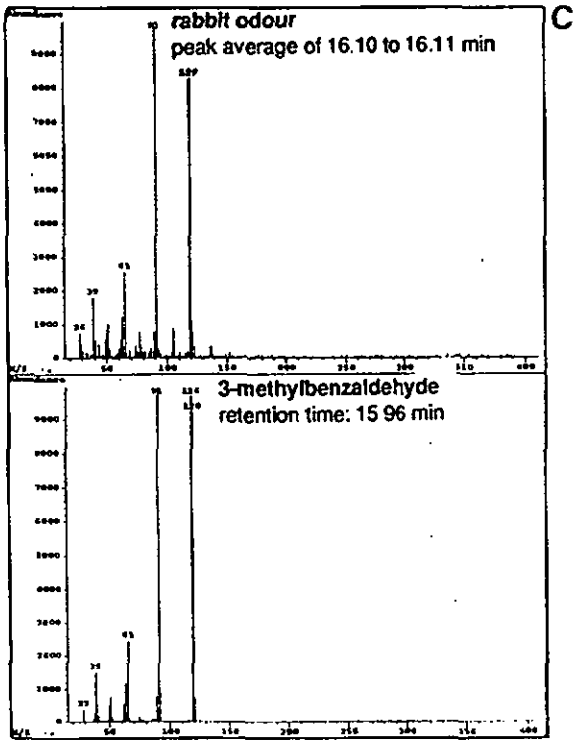


Fig. 8.9. Gas chromatography-coupled mass spectrometry analyses. Full identification of methylbenzaldehyde isomers, in bovine (A) and rabbit odour (B), based on full mass spectra, was often not feasible because of numerous coeluting products which obscured the spectra. Presence of methylbenzaldehyde isomers in bovine and rabbit odour was indicated using 3 characteristic ions (M/Z=91, 119, and 120, respectively) in peaks eluting at the same retention time as the synthetic analogues. C Comparison of the mass spectrum and retention time of an unknown stimulant found in bovine odour with that of synthetic 3-methylbenzaldehyde injected under the same conditions as for the natural odour.

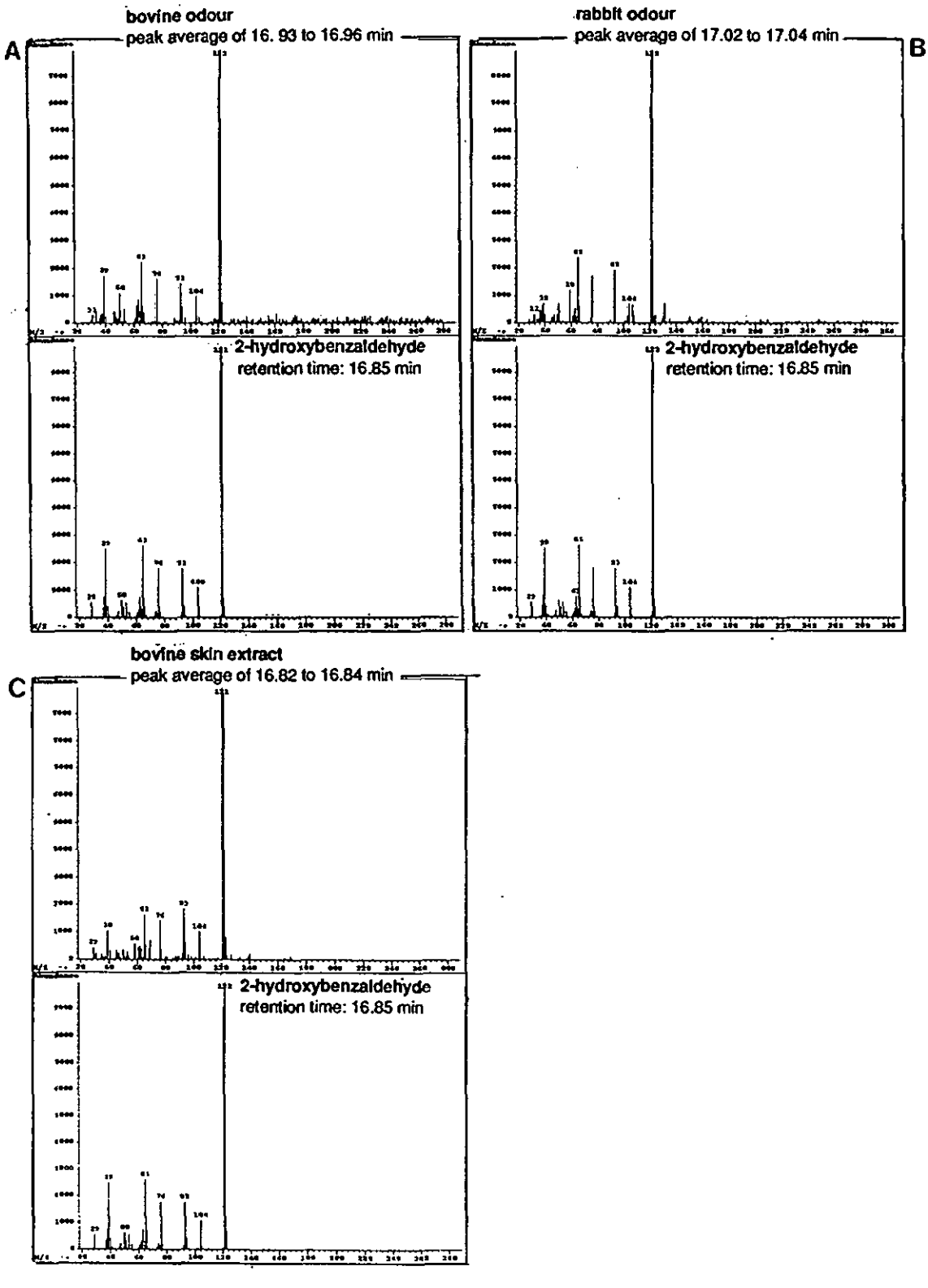


Fig. 8.10. Gas chromatography-coupled mass spectrometry analyses. Comparison of the mass spectrum and retention time of an unknown stimulant found in bovine odour (A), rabbit odour (B) and in the bovine skin extract (C) with that of synthetic 2-hydroxybenzaldehyde injected under the same conditions as for the natural odour in each case.

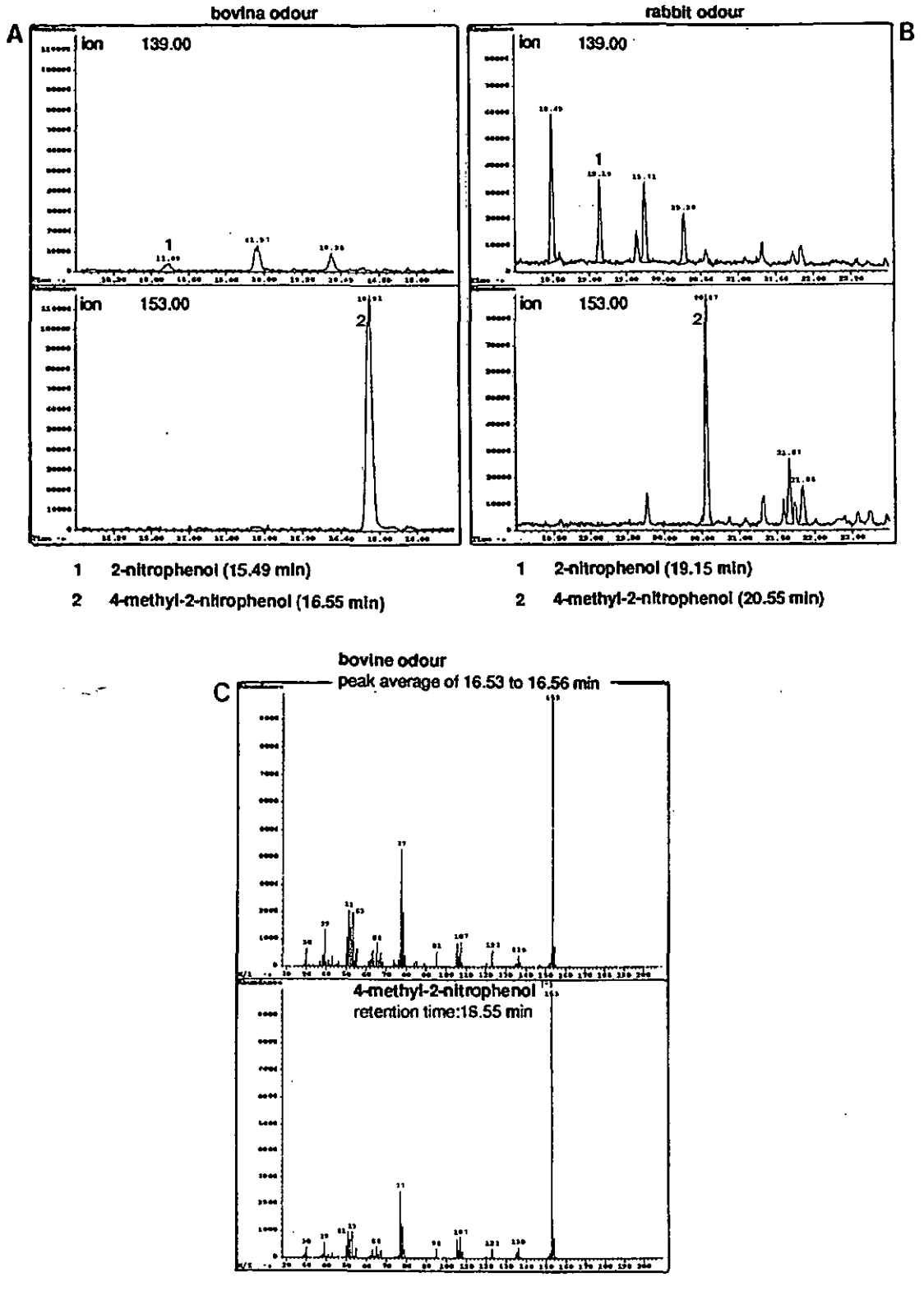


Fig. 8.11. Gas chromatography-coupled mass spectrometry analyses. Presence of 2-nitrophenol and 4-methyl-2-nitrophenol in bovine (A) and rabbit odour (B) was indicated by their respective molecular ions (M^+=139 for 2-nitrophenol and M^+=153 for 4-methyl-2-nitrophenol) in peaks eluting at the same retention time as the synthetic analogues. Retention times of the synthetic analogues are provided under figures A and B. C Comparison of the mass spectrum and retention time of an unknown stimulant found in bovine odour with that of synthetic 4-methyl-2-nitrophenol injected under the same conditions as for the natural odour. Identification of 2-nitrophenol based on a fulll mass spectrum was not feasible.