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Ultrastructure and receptor cell responses of the antennal grooved peg sensilla of *Triatoma infestans* (Hemiptera: Reduviidae)

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Abstract

Ultrastructural examination of grooved-peg (GP) sensilla on the antenna of fifth instar *Triatoma infestans* nymphs by scanning electron microscopy and transmission electron microscopy reveal that they are 8–18 μm long with a diameter of about 2–2.8 μm at the non-articulated base. Some pegs have a terminal pore. These double-walled wall-pore (dw-wp) sensilla have an outer cuticular wall with 13–18 longitudinal grooves at the distal part of the peg. Groove channels are present at the bottom of the grooves from which radial spoke channels lead into the inner sensillum-lymph cavity. A dendrite sheath connects the tip of the thecogen cell to the inner cuticular wall thus forming separated outer and inner sensillum-lymph cavities. Four or five bipolar receptor cells are ensheathed successively within the GP sensilla by the thecogen cell, trichogen and tormogen cells. The inner dendritic segments of each sensory cell give rise at the ciliary constriction to an unbranched outer dendritic segment which can reach the tip of the sensillum.

Electrophysiological recordings from the GP sensilla indicate that they house NH_3, short-chain carboxylic acid and short-chain aliphatic amine receptor cells and can be divided into three functional sub-types (GP 1–3). All GP sensilla carry a receptor cell excited by aliphatic amines, such as isobutylamine, a compound associated with vertebrate odour. GP type 1 and 2 sensilla house, in addition, an NH_3-excited cell whereas the type 2 sensilla also contains a short-chain carboxylic acid receptor. No cell particularly sensitive to either NH_3 or carboxylic acids was found in the grooved-peg type 3 sensilla. GP types 1, 2 and 3 represent ca. 36, 10 and 43% of the GP sensilla, respectively, whereas the remaining 11% contain receptor cells that manifest normal spontaneous activity but do not respond to any of the afore mentioned stimuli.

Keywords: Sensillum; Olfaction; Sensory receptor cells; *Triatoma infestans*; Triatomine bugs; Ultrastructure; Electrophysiology

1. Introduction

Triatomines such as *Triatoma infestans* (Klug, Hemiptera: Reduviidae) are vectors of American trypanosomiasis or Chagas' disease in Central and South America. They are night active, occupying sylvatic, peridomestic and domestic niches. They seek out birds and mammals, man and his domestic animals, for a blood-meal from nearby refuges. *T. infestans* use several sensory cues for host location including host volatiles such as NH_3, isobutyric acid and nonanal which are detected by olfactory sensilla on the antennae (Taneja and Guerin, 1995, 1997; Guerenstein and Guerin, 2001).

To date, the structure of three morphologically distinguishable types of olfactory sensilla have been studied on the antennae of triatomines. Using electron microscopy, Bernard (1974) reported 35 μm long single-(thick)walled wall-pore 'D' type and single-(thin)walled wall-pore 'E' type sensilla in adult *T. infestans*. The latter consists of non-articulated, 26 μm long sensilla with a diameter of 2 μm at the base and 1 μm at the tip; the cuticle is pierced by numerous wall-pores which are linked to pore tubules. In a later study, these single-walled E type sensilla were named basiconic by Guerenstein and Guerin (2001). The other morphologically distinguishable third type, the double-walled wall-pore 'F' type sensilla consists of non-articulated 6–10 μm long pegs with a diameter of about 1 μm (Bernard, 1974). These double-walled sensilla were named the grooved-peg (GP) type by Taneja and Guerin (1997) who reported two functional GP sub-types (below). They possess longitudinal grooves with wall pores that communicate with the central lumen of the sensillum via spoke channels that cross the double cuticular wall; three

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dendrites, but four electrophysiologically distinguishable units were reported by Bernard (1974).

A recent electrophysiological study by Guerenstein and Guerin (2001) indicates that receptor cells in basiconic sensilla on the antenna of fifth instar *T. infestans* nymphs respond to aldehydes such as nonanal, a constituent of both sheep wool and chicken feather odour. In addition, the authors confirmed the presence of two functionally different GP sensilla sub-types first suggested by Taneja and Guerin (1997): type 1 housing an NH_3-excited receptor neurone and type 2 housing an NH_3-excited cell in addition to a neurone responding to short-chain carboxylic acids such as isobutyric acid, present in host odour (Guerenstein, 1999).

Here we report on the ultrastructure of the GP sensilla from fifth instar nymphs of *T. infestans* using classical chemical fixation and cryofixation. Additional functions for some of the olfactory receptor cells (ORCs) in the GP sensilla have been found using single sensillum electrophysiological recordings. The number of adequate stimuli identified is in good agreement with the number of receptor cells in these sensilla and, in addition, support the hypothesis of Altner et al. (1977) that receptor cells in the double-walled olfactory sensilla respond preferentially to more polar stimuli.

2. Material and methods

2.1. Animals

Fifth instar *T. infestans* nymphs were from our laboratory culture (Taneja and Guerin, 1997). After moulting, individuals were placed in a climate chamber at 19 ± 0.5 °C, 80 ± 5% RH and 12:12 h L/D cycle.

2.2. Electron microscopy

Antennae were prepared by cryosubstitution and chemical fixation for transmission electron microscopy (TEM). Cryosubstitution was made at the Max Planck Institute, Seewiesen, Germany (Steinbrecht, 1980, 1993). Antennae of *T. infestans* were excised, mounted on a tungsten wire and plunged into supercooled propane. Frozen specimens were stored in liquid nitrogen and transferred into the substitution medium (acetone containing 2% OsO_4) cooled by dry ice. After several days the specimens were warmed to room temperature and embedded in Epon.

For chemical fixation, antennae were cut at the base of the flagellum (antennal terminal segment) and fixed in 2.5% glutaraldehyde and 1.5% paraformaldehyde in a cacodylate buffer (0.1 M; pH 7.4) for 2 h at room temperature. They were then washed by gentle immersion in cacodylate buffer (0.2 M; pH 7.4) with 5% sucrose, postfixed with 1% OsO_4 in the same buffer and carefully washed with the above buffer. The antennae were then dehydrated in 30–100% acetone in steps of 10% and embedded in Spurr's resin.

Serial sections were made with a Reichert Ultracut-S microtome, mounted on copper grids, double stained with uranyl acetate and lead citrate, and observed with a Philips CM100 TEM at 60 or 80 kV. Cross and longitudinal serial sections were taken at various levels from five flagella; 33 grooved pegs were serially cross-sectioned with seven complete series. To study the terminal pores, 21 sensilla were serially sectioned in the longitudinal axis.

For scanning electron microscopy (SEM), a mark was made with a fine insect needle on the dorsal face of the flagellum before removing it from the antenna. The flagellum was then fixed on a tungsten electrode and mounted on a stub with adhesive Leit C glue (Provac) and air-dried. It was coated with a 23 nm gold layer in a Baltec SCD 005 sputter apparatus and observed with a Philips XL20 SEM at acceleration voltages of 10–20 kV.

2.3. Electrophysiology

The methods of recording electrophysiological responses from triatomine olfactory sensilla and of stimulus delivery were as described by Guerenstein and Guerin (2001). All electrophysiological recordings were done with nymphs 3–8 weeks post-ecdysis.

Three chemicals were tested on 28 preparations of GP sensilla (using 11 antennae from 11 *T. infestans* nymphs) located ventrally on the antenna, i.e. NH_3 (ammonia), isobutylamine and isobutyric acid. In addition, the following 10 compounds from seven chemical classes were tested on a further 107 GP sensilla of 29 antennae from 25 nymphs located either dorsally ($n = 33$) or ventrally ($n = 74$) on the flagella: NH_3, isopentylamine, formic acid, propionic acid, isobutyric acid, isobutyl isobutyrate, isobutanol, *p*-cresol, *o*-aminoacetophenone, and nonanal. Other synthetic compounds tested were: propylamine, butylamine, 2-butylamine, pentylamine, 3-methyl 2-butylamine, hexylamine, heptylamine, triethanolamine, cadaverine (1,5-diaminopentane), putrescine (1,4-diaminobutane), acetamide, methylamine, dimethylamine, trimethylamine, ethylamine, diethylamine, butyric acid, 2-methyl butyric acid and 2,4-dimethylquinazoline. Volatiles, dissolved in dichloromethane (DCM) or in water, were tested at 10 μg in the stimulus cartridge except isobutyric acid (1 μg). When a response was obtained lower doses were also tested. All chemicals tested on GP sensilla except NH_3, methylamine, dimethylamine, trimethylamine and ethylamine (from ≥25% aqueous solutions) were ≥97% pure; DCM and distilled water were used as solvent blanks.

To establish if responses of the NH_3-sensitive receptor cells in the GP1 and GP2 sensilla types increased significantly with increasing doses, regression analyses were performed using a sigmoid model ($y = a/\{1 + e^{-[(x-x_0)/b]}\}$ where a is the asymptotical maximum Y value, X_0 the value of X for which $Y = a/2$ and $b = a/4s$, where s is the slope at $X = X_0$). In order to compare dose-response trends, each of the three parameters (a, b and X_0)

shaping the sigmoid curves was individually compared using the *t*-test. The spontaneous frequencies of action potentials recorded from different receptor cell types in the GP1, 2 and 3 sensilla types were compared by means of the *t*-test, and a statistical comparison of the different functional classes of GP sensilla on the dorsal and ventral faces of the distal flagellum was made using a R × C G-test of independence (Sokal and Rohlf, 1995). In addition, in order to establish which amine evoked the strongest response from an amine-sensitive receptor, the response to a particular amine was compared pairwise with the response to each of the other amines using the paired two-tailed Wilcoxon signed-rank test, and a ranking of the responses was then established.

3. Results

3.1. Distribution of the GP sensilla

The GP sensilla in fifth instar nymphs of *T. infestans* occur on all faces of the distal two-thirds of the flagellum, the distal antennal segment. Their number varies between 80 and 110 per antenna (mean 94 ± 4.8 (SEM), $n = 7$ antennae from seven animals).

3.2. Structure of the GP sensilla

The overall GP structure is presented semi-schematically (Fig. 1). The short non-articulated cuticular component comprises but a minor part of the sensory apparatus in terms of overall volume; the major part is taken up by the four or five sensory cell soma and the surrounding accessory cells. The cuticular wall is rather strong, especially in the outer distal double-walled part, with a voluminous outer sensillum-lymph cavity compared with the small inner sensillum-lymph cavity.

3.2.1. The cuticular apparatus

The rather uniformly distributed GP sensilla are short and oriented nearly parallel to the antennal cuticle (Figs. 2 and 3). They are of variable length ranging from 8 to 18 μm; 24% of the pegs measure between 13 and 14 μm and 62% between 12 and 15 μm, respectively ($n = 1220$, combined data from 24 flagella from 24 animals). At the non-articulated base they have a diameter of about 2–2.8 μm.

Most of the distal wall of each peg shows 13–18 longitudinal grooves which terminate just short of the tip (Figs. 3 and 4). Near the proximal end of the grooves the peg diameter varies between 1.1 and 1.9 μm. Small terminal pores with a diameter varying between 40 and 340 nm appear to be present in 55% of the sensilla when viewed by SEM ($n = 165$ from seven antennae; Fig. 4(b)). Observation of cross-sections by TEM suggested about 26% with terminal pores ($n = 33$ from three antennae). However, in only one case ($n = 21$) did sectioning in the longitudinal axis allow unequivocal identification of a terminal pore (Fig. 5).

Cross-sections of the grooved distal part of GP sensilla reveal the double-walled structure (Fig. 6(a)). The outer and inner cuticle walls form the borders of the outer and inner sensillum-lymph cavities, respectively. They are connected by small radial cuticular bridges. As a result, the outer sensillum-lymph cavity forms a continuous space which is only traversed by the cuticular bridges that contain the spoke channels (Fig. 6(a)). The inner sensillum-lymph cavity surrounded by the inner cuticular wall contains four or five closely packed outer dendritic segments with microtubules; the segments bathe in the inner sensillum-lymph (Figs. 6(a), 8 and 9).

Radial narrow spoke channels of 15–20 nm in diameter connect the inner sensillum-lymph cavity with the groove channels at the base of the grooves (Figs. 5, 6(a), 8 and 9). After cryofixation, the channels are seen to contain electron-dense material (Figs. 5 and 6(a)), but appear electron-lucent after chemical fixation (Fig. 6(b)). The grooves also contain material, maybe representing lipids from the wax layer (Fig. 6(a) and (b)). The groove channels appear triangular in cross-section (Fig. 6(b)). In the longitudinal axis, the spoke channels are irregularly spaced at between 170 and 330 nm apart. With a mean distance of 240 nm between spoke channels, a mean groove length of 11.2 μm and 13–18 grooves per GP, the total number of spoke channels can be estimated to be between 600 and 850 per sensillum.

Near the proximal end of the grooves, the inner cuticular wall ends and a thinner dendrite sheath continues to enclose the outer dendritic segments down to the level of the thecogen cell (Figs. 11 and 12).

3.2.2. The receptor cells

Of 33 GP sensilla examined, 18 were innervated by four and 15 by five bipolar receptor cells of about the same size. The somata give rise to small inner dendritic segments which are connected to the outer dendritic segments via a short ciliary segment (Fig. 10(a)). The latter contains nine peripheral microtubule doublets which originate from the distal basal body; the number of central microtubules could not be determined with accuracy. Both proximal and distal basal bodies are connected to ciliary rootlets (Fig. 10(a)). The outer dendritic segments are thinner than the inner segments, and as they proceed up the GP sensilla they are tightly encased by the dendrite sheath to the level where the grooves begin, and then by the inner cuticular wall (Figs. 6, 8, 9, 11 and 12). The dendrites become thinner and do not appear to branch. One or two dendrites can be observed near the tip of the GP sensilla.

Most often, the receptor cells are separated by thin extensions of the thecogen cell (Fig. 10(a)). Smaller areas of direct contact, resembling gap junctions, exist between the sensory cells (Fig. 10(a) and (b)). The cytoplasm contains an active euchromatic nucleus with a prominent nucleolus,

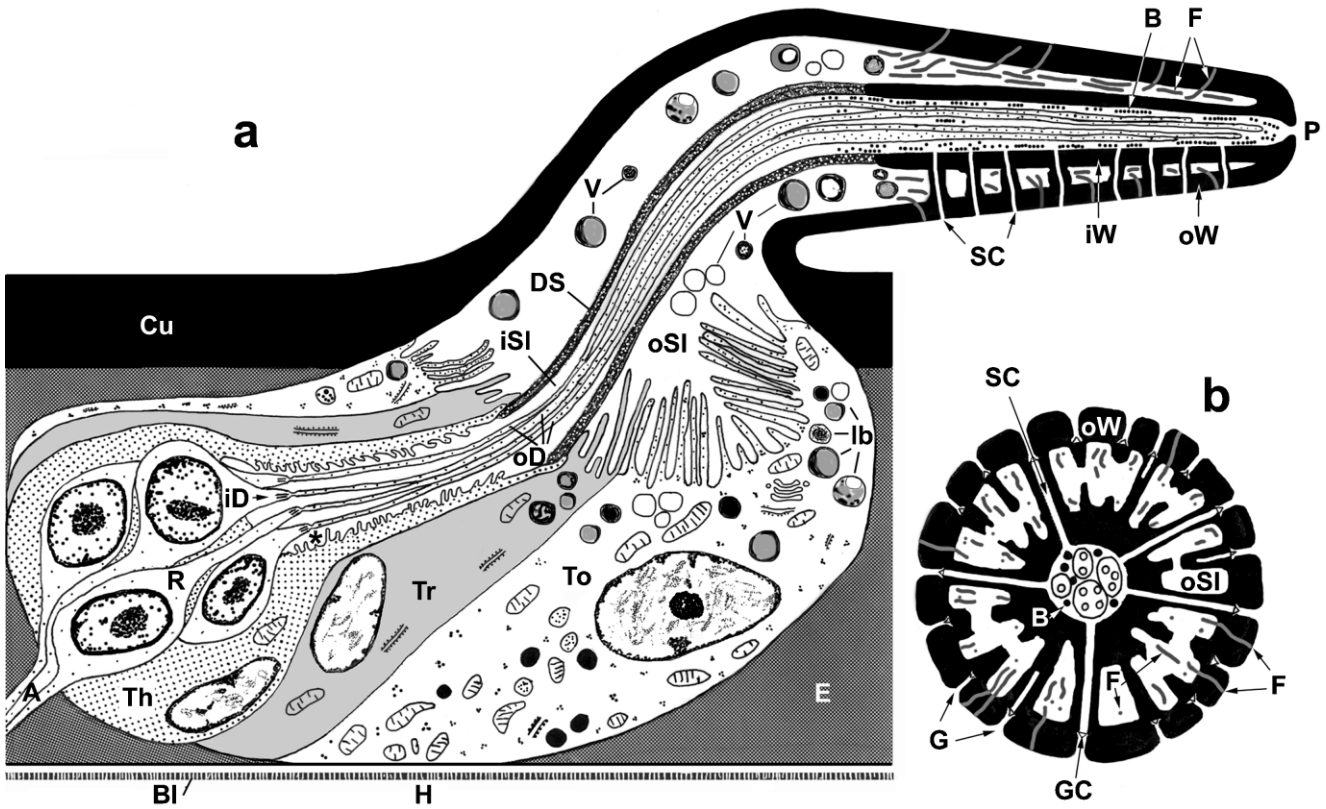


Fig. 1. Semi-schematic drawing of the GP sensillum on antennae of *T. infestans* fifth instar nymphs in longitudinal (a) and in cross-section (b). Axons (A), beads (B), basal lamina (Bl), ciliary constriction (*), cuticle (Cu), dendrite sheath (DS), epidermal cell (E), filaments (F), grooves (G), groove channels (GC), haemolymph (H), inclusion bodies (Ib), inner dendritic segments (iD), inner sensillum-lymph cavity (iSl), inner cuticular wall (iW), outer dendritic segments (oD), outer sensillum-lymph cavity (oSl), outer cuticular wall (oW), terminal pore (P), 4–5 monodendritic bipolar receptor cells (R), radial spoke channels (SC), thecogen cell (Th), tormogen cell (To), trichogen cell (Tr), vesicles (V).

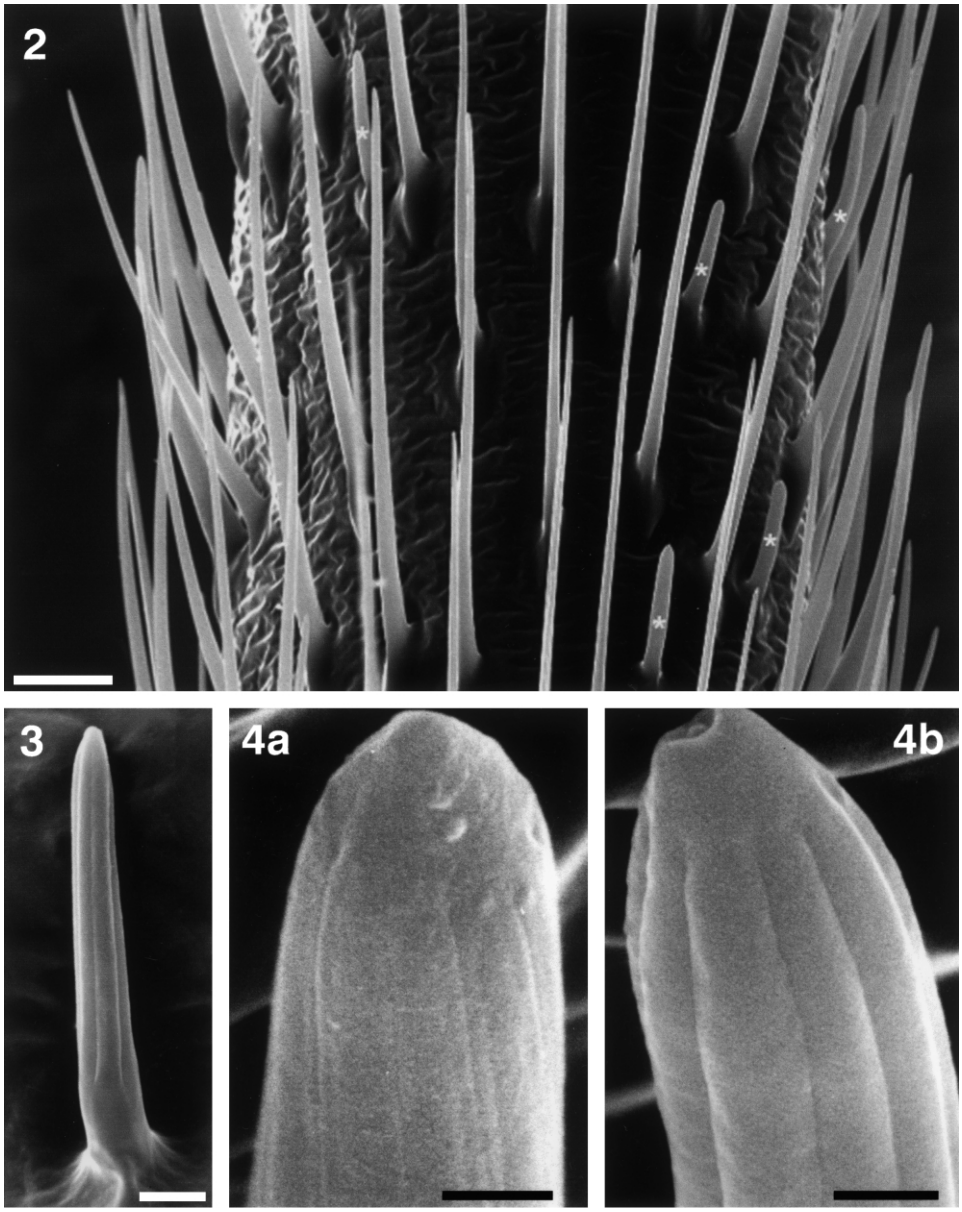
many mitochondria, an endoplasmic reticulum, a Golgi apparatus and lysosomes. Many small electron-lucent vesicles are present in the distal part of the inner dendritic segment (Fig. 10(a)).

3.2.3. The auxiliary cells and sensillum-lymph cavities

Three auxiliary cells are present in the GP sensilla (Figs. 10(a), 11 and 12). The sensory cell bodies and the inner dendritic segments are enveloped by the innermost thecogen cell which is connected distally to the dendrite sheath. The thecogen cell itself is surrounded successively by the trichogen cell and the outermost and larger tormogen cell. The latter contacts the cuticle. Septate junctions are present at the apical regions between all the cells. Ordinary epidermal cells normally separate the auxiliary cells of neighbouring sensilla except in regions with closely packed sensilla, where direct contact between tormogen cells is possible.

Apically, the cell membrane surface of the tormogen and trichogen cells is considerably enlarged by formation of numerous microvilli and microlamellae. The microlamellae can be arranged in a parallel or concentric pattern, especially in the tormogen cell (Figs. 11 and 12). The microvilli contain concentrically arranged thin filaments with a diameter of 7–9 nm-maybe actin-as a cytoskeletal element; comparable filaments appear to form a midline in the microlamellae. The cytoplasmic side of the apical cell membrane of the tormogen cell bears small particles which resemble ‘portasomes’ (Harvey, 1980). Many mitochondria are present in the cytoplasm, together with some endoplasmic reticulum, Golgi apparatus and multivesicular bodies. The trichogen and tormogen cells contain inclusion bodies of variable size and electron density which may correspond to lipoid material (Fig. 12).

The outer-sensillum lymph cavity is bordered by the apical cell membranes of the trichogen and tormogen cell, the outer and the inner cuticular wall, and the dendrite sheath (Figs. 5, 9, 11 and 12). The proximal cavity in the non-grooved region of the peg contains fine granular material and vesicles of different size filled with material of varying electron density presumed to be lipoid in nature. They resemble the inclusion bodies in the trichogen and tormogen cell and are thus likely to be secreted by these cells (Fig. 12). In the grooved distal part of each GP the outer sensillum-lymph space contains filaments with a diameter of 12–20 nm (Figs. 7–9). With cryofixation they may appear hollow (Fig. 7), but with chemical fixation they



Figs. 2–4. Fig. 2: SEM of GP sensilla (∗) on the flagellum of fifth instar *T. infestans* nymphs. Their length varies between 8 and 18 μm; scale bar 10 μm. Fig. 3: Peg with longitudinal grooves; scale bar 2 μm. Fig. 4: (a) Peg without terminal pore, scale bar 500 nm; (b) peg with a terminal pore, scale bar 500 nm.

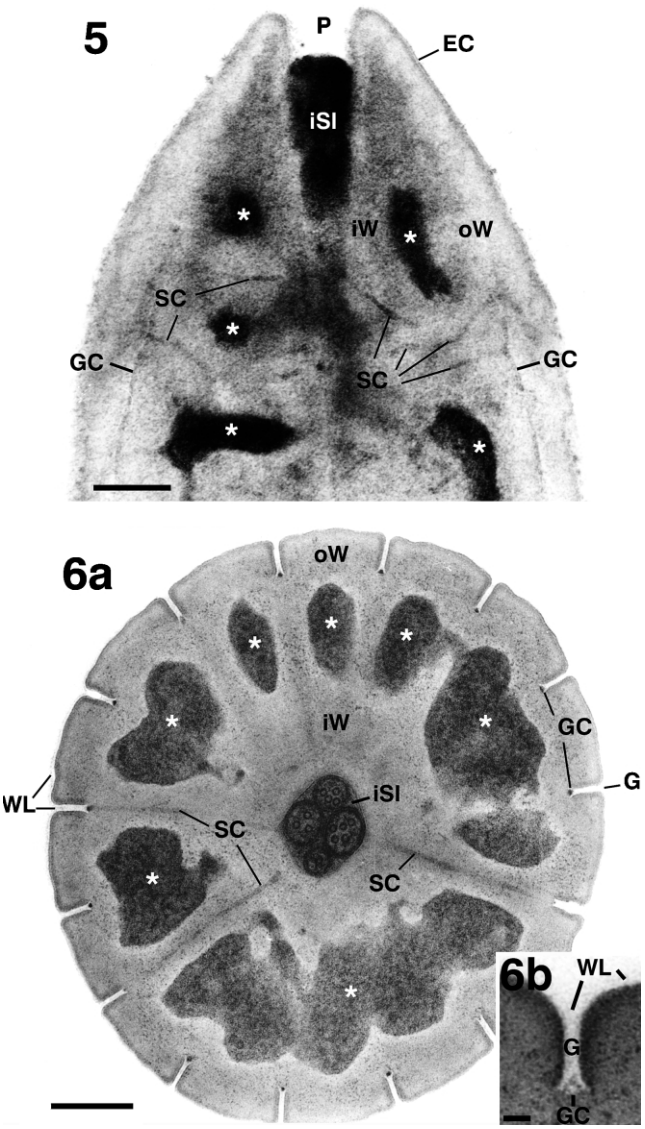
are rather uniformly dense and are contiguous with comparable filaments in the cuticle leading to the epicuticule (Figs. 8 and 9). These structures resemble wax filaments. Vesicles are absent.

The inner-sensillum lymph cavity houses the apical part of the inner dendritic segments, the ciliary constriction and the outer segments of the dendrites. It is bordered by the apical cell membrane of the thecogen cell, the dendrite sheath, and more distally by the inner cuticular wall (Figs. 5, 6, 8, 9, 11 and 12). Its content is fine granular; the distal part of the inner-sensillum lymph cavity contains numerous beads (about 20 nm in diameter), often regularly arranged in a string (Figs. 8 and 9).

3.3. Electrophysiology

3.3.1. Responses of receptor cells in GP 1 and GP 2 sensilla

The existence of two functional GP sensilla sub-types as described by Taneja and Guerin (1997) was confirmed here (Table 1, Figs. 13 and 14). Responses of the NH_3-sensitive receptor cells housed both in the GP 1 and GP 2 sensilla increased significantly with the dose of NH_3 ($p < 0.001$, Fig. 15). The dose-response trends of the two NH_3 receptor cells were not significantly different (ns for the three parameters of the sigmoid curves; Fig. 15) despite the fact that the NH_3-sensitive receptor cells housed in GP 2 sensilla showed a higher spontaneous spike frequency than those in



Figs. 5 and 6. Fig. 5: A near longitudinal section through the tip of a GP sensillum on a cryofixed flagellum of a fifth instar *T. infestans* nymph. A pore (P) leads into the inner sensillum-lymph cavity (iSl). Spoke channels (SC) and groove channels (GC) are filled with electron-dense material; EC, thin epicuticule, iW, inner wall, oW, outer wall, ∗, outer sensillum-lymph cavity; scale bar 200 nm. Fig. 6: Cross-section through a GP sensillum on the flagellum of a fifth instar *T. infestans* nymph half way along the grooved wall: (a) the double-walled wall-pore structure is clearly visible. Fifteen grooves (G) are present in the outer wall (oW) with electron-dense groove channels (GC) at the bottom of the grooves. The inner sensillum-lymph cavity (iSl) is surrounded by the inner wall (iW) and contains four outer dendrites with microtubules. Spoke channels (SC) lead from the groove channels (GC) to the inner sensillum-lymph cavity. Radial cuticular bridges connect the outer and inner walls around the spoke channels, but the outer sensillum-lymph cavity (∗) is a largely confluent space; scale bar 250 nm. (b) The triangular-shaped groove channels (GC) are at the bottom of the grooves (G). The channel content appears electron-lucent after chemical fixation. Probable remains of the wax layer (WL) are found on the cuticle surface and within the grooves; scale bar 100 nm.

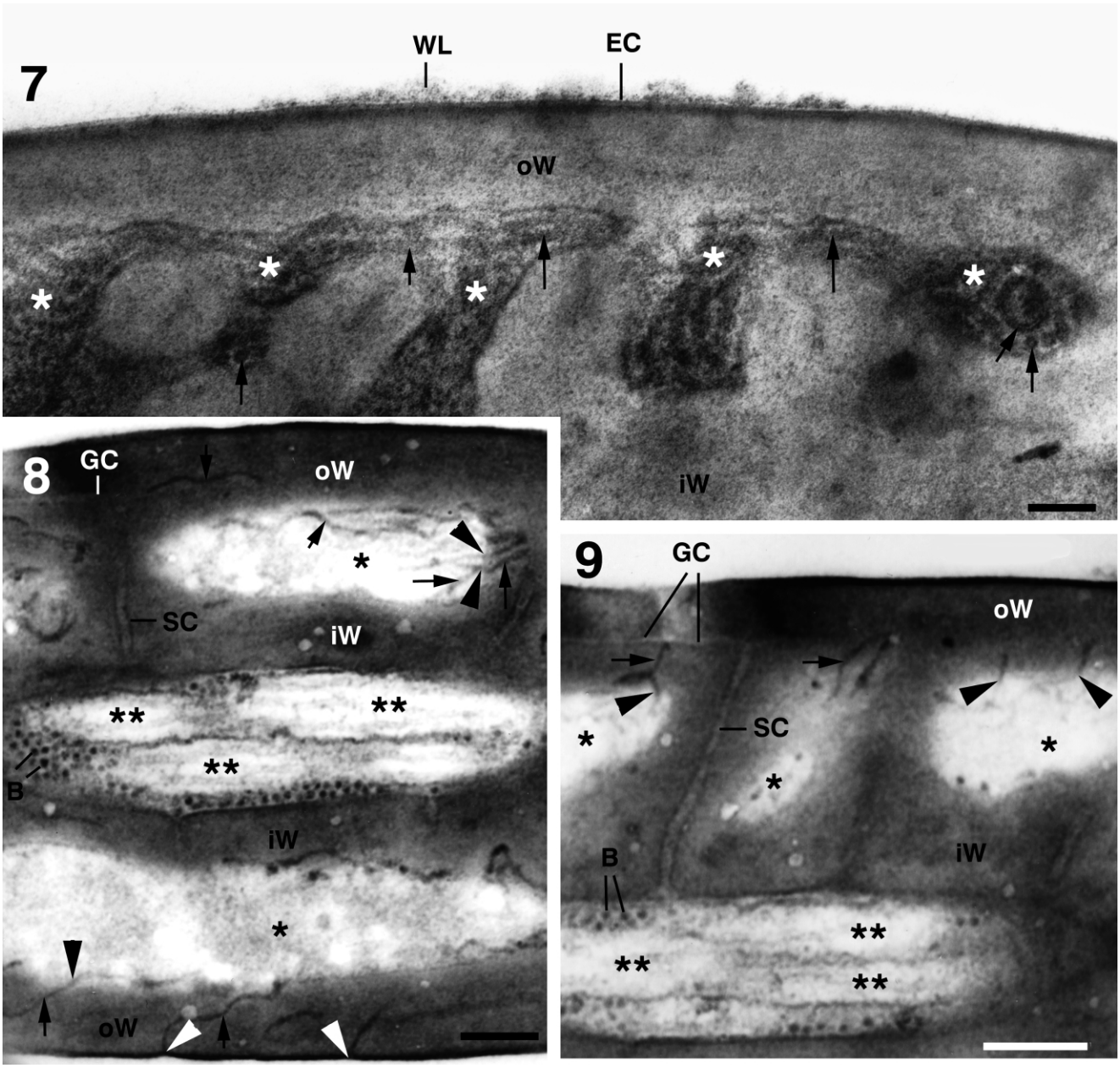
the GP 1 sensilla ($p < 0.05$; Table 1). Furthermore, the response continued long past stimulation with NH_3 for the responding receptor cells in the GP 1 and GP 2 sensilla, as was the case for the acid-excited cell in the GP 2 sensilla (Figs. 13 and 14).

Receptor cells in GP 1 and GP 2 sensilla also responded to short-chain aliphatic amines (ORC3 in Table 1), but the responses of these amine-excited receptor cells were extremely phasic, terminating even before the end of stimulation (Figs. 13 and 14). The spontaneous activity of these cells did not differ between the two sensilla types. When different short-chain aliphatic amines were tested (each at 10 μg in the stimulus cartridge) on the GP 2 sensilla of three *T. infestans* nymphs, isobutylamine was consistently the best stimulus for the aliphatic amine-cell (data not presented). In double-successive stimulations (method described in Steullet and Guerin (1994)) on GP 2 sensilla using 2-methyl butyric acid followed by isobutyric acid only one receptor neurone responded. By contrast, when a stimulation with isobutyric acid was followed by a stimulation with isobutylamine, a cell of a different spike amplitude was excited, suggesting different receptor cells for acids and amines in GP2 sensilla.

3.3.2. Responses of receptor cells in GP 3 sensilla

In addition to the GP 1 and GP 2 type sensilla, a third type of GP sensillum (GP 3) was found with a cell of low spike amplitude excited by short-chain aliphatic amines and another with a high spike amplitude inhibited by the same products (Table 1, Fig. 16). No cell responded to NH_3 or to carboxylic acids in these sensilla in a manner similar to that of the receptor cells in the GP 1 and GP 2 sensilla. When different short-chain aliphatic amines were tested on the GP 3 sensilla, isobutylamine and 2-butylamine were the best stimuli for the amine-sensitive cell ($p < 0.05$, $n > 6$, Wilcoxon signed-rank test; Table 2). This receptor did not respond to the di-amines cadaverine and putrescine, to the aminoalcohol triethanolamine or to acetamide at similar doses. In contrast to the amine-sensitive cells in the GP 1 and GP 2 sensilla, excitation of the amine-excited cell in GP 3 sensilla continued past the period of stimulation (Fig. 16). Furthermore, the firing rate of the amine sensitive cell in the GP 1 and GP 2 sensilla to the best stimulant reached 30 Hz, whereas the firing rate of the amine sensitive cell in the GP 3 sensilla could reach 112 Hz at similar source doses of stimulus (Table 2). The spontaneous activity of the amine-excited cells in the GP 3 sensilla was significantly lower than that of the GP 2 sensilla receptor cells but not different from those of the GP 1 sensilla ($p < 0.05$ and ns, respectively).

2,4-Dimethylquinazoline, a nitrogenous compound present in triatomine faeces (Cruz-López and Morgan, 1995), did not activate receptor cells in any of the GP sensilla types. Methylamine, dimethylamine, trimethylamine, ethylamine and diethylamine did evoke moderate

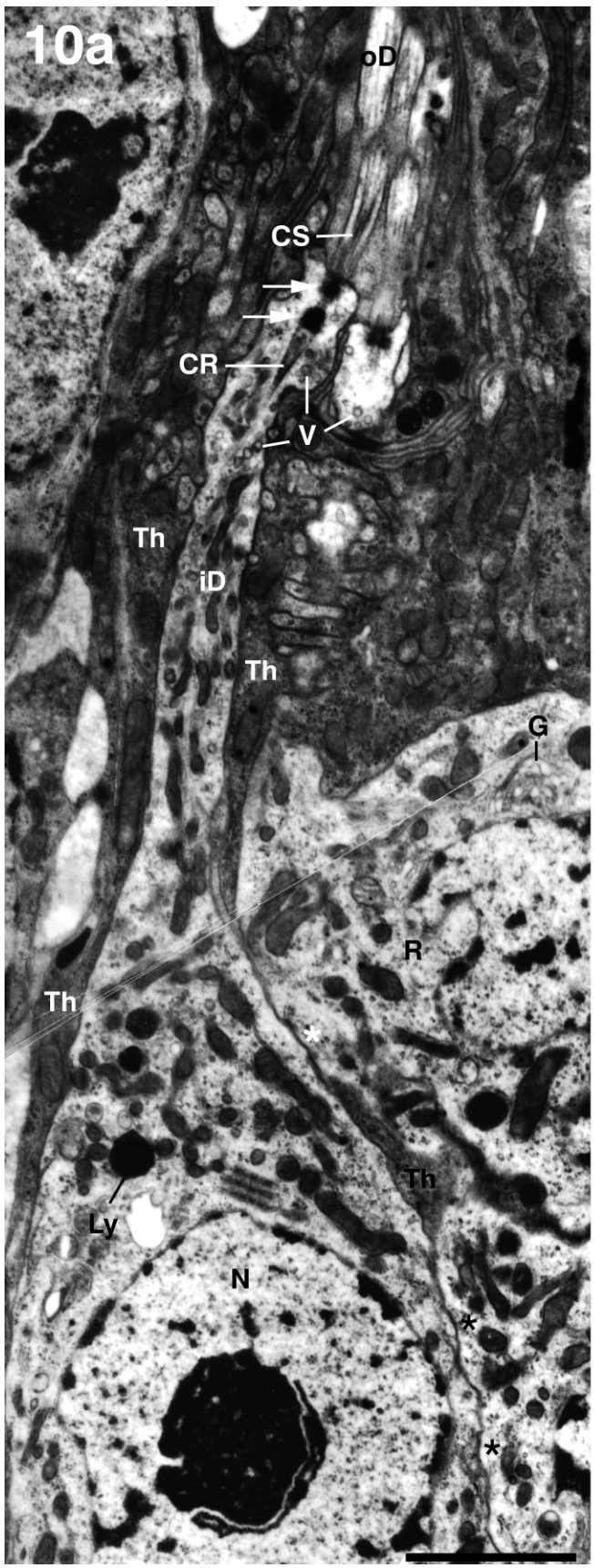


Figs. 7–9. Fig. 7: Longitudinal section through the grooved part of a cryofixed GP sensillum on the flagellum of a fifth instar *T. infestans* nymph. Remains of the wax layer (WL) and the outer epicuticule (EC) are visible at the periphery of the outer wall (oW). Hollow filaments (arrows) are present in the outer sensillum-lymph (*); scale bar 100 nm. Figs. 8 and 9: Longitudinal section through the grooved part of a chemically fixed GP sensillum on the flagellum of a fifth instar *T. infestans* nymph. Fig. 8: Electron-dense filaments (presumably wax filaments, arrows) originate in the epicuticle (white arrowheads) and traverse the outer wall (oW). Figs. 8 and 9: The filaments continue (dark arrowheads) into the outer sensillum-lymph cavity (*). Beads (B) are present in the inner sensillum-lymph surrounding the outer dendritic segments (* *); groove channels (GC), spoke channels (SC); scale bars 200 nm.

to weak responses on both the NH_3 and the amine-sensitive receptor cells in the different GP sensilla types.

3.3.3. Proportions of the three functional GP sensilla types

To study the proportion of different types of GP sensilla on the antennae of fifth instar *T. infestans* nymphs, 28 GP sensilla (located ventrally on the distal flagellum) were stimulated with NH_3, isobutyric acid and isobutylamine. From this, it was estimated that the GP types 1, 2 and 3 represent ca. 36, 10 and 43% of the GP sensilla, respectively (Table 1), whereas the remaining 11% of the GP sensilla contain receptor cells that did not respond to any of the three stimulants tested. No receptor cell other than the electrophysiologically characterized NH_3, carboxylic acid and amine sensitive cells responded when 107 GP sensilla (located either dorsally or ventrally on the flagellum) were stimulated with 10 synthetic compounds belonging to seven chemical classes. No difference was found between the GP sensilla types present on the dorsal and ventral sides of the distal flagellum (ns, R × C G-test of independence).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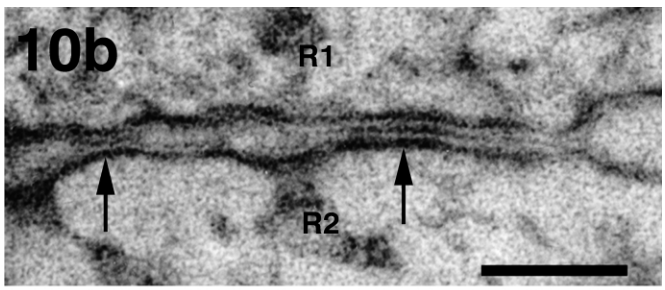
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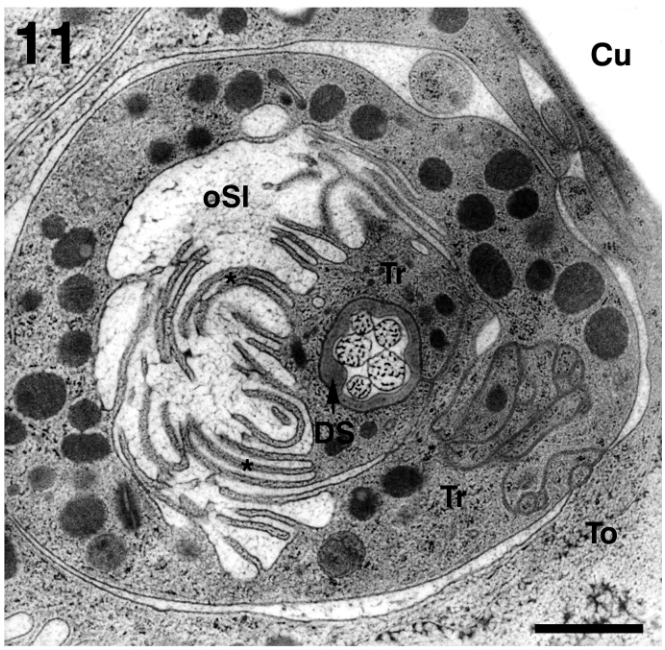
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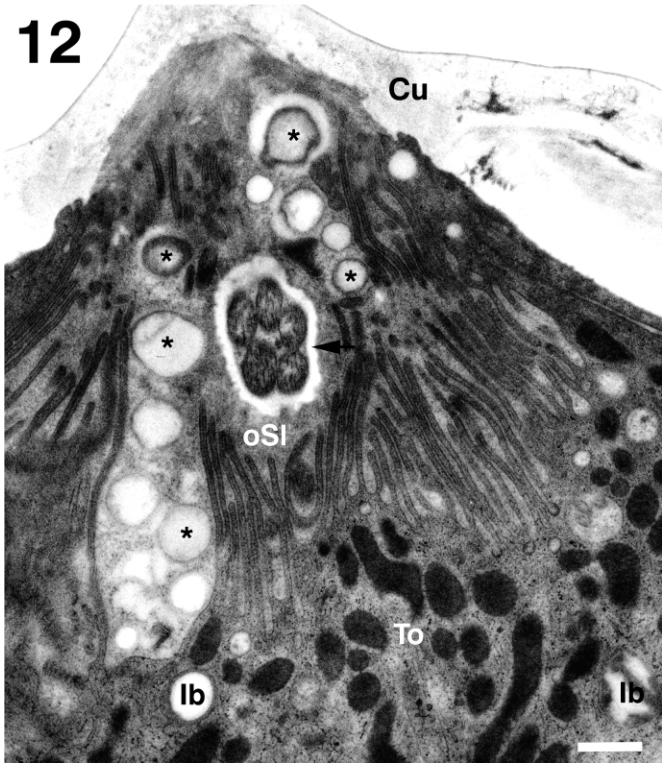
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Table 1
Grooved peg sensilla sub-types on the antenna of fifth instar *T. infestans* nymphs characterized according to the electrophysiological response profiles of their olfactory receptor cells (ORCs 1-3)

	GP 1 (36%)	GP 2 (10%)	GP 3 (43%)
ORC 1	High spike amplitude 0.9 ± 0.4 mV ($n = 10$) s.a.: 2.4 ± 1.1 Hz Excited by *ammonia* Threshold: 1–10 μg	High spike amplitude[a] 0.6 ± 0.2 mV ($n = 6$) s.a.: 4.6 ± 1.7 Hz Excited by *isobutyric acid* Threshold: 0.01–0.1 μg	High spike amplitude 0.8 ± 0.3 mV ($n = 12$) s.a.: 6.4 ± 3.6 Hz Inhibited by C_4 *aliphatic amines* Threshold: 1–10 μg
ORC 2		Intermediate spike amplitude[a] 0.5 ± 0.2 mV ($n = 6$) s.a.: 4.0 ± 1.8 Hz Excited by *ammonia* Threshold: 1–10 μg	
ORC 3	Low spike amplitude 0.6 ± 0.3 mV ($n = 10$) s.a.: 4.2 ± 2.1 Hz Excited by *isobutylamine* Threshold: 10 μg	Low spike amplitude 0.4 ± 0.1 mV ($n = 6$) s.a.: 6.1 ± 3.0 Hz Excited by *isobutylamine* Threshold: 1–10 μg	Low spike amplitude 0.6 ± 0.2 mV ($n = 12$) s.a.: 2.9 ± 2.0 Hz Excited by *isobutylamine* and 2-*butylamine* Threshold: 1–10 μg

Proportion of a sensillum type as percentage of the total GP sensilla population sampled are indicated in parentheses. The table provides information only about cells that could be stimulated among the 4–5 neurons that fire spontaneously in GP sensilla. Chemicals mentioned were the best stimulants found for the particular cell and the threshold refers to the dose in μg in the stimulus cartridge (μg NH_4OH for ammonia); s.a. spontaneous activity (mean ± SD), spike amplitudes are means ± SD.

[a] In three of 11 preparations the spike amplitude of the ammonia-excited cell was the highest and in one case it showed the same spike amplitude as the carboxylic acid-excited cell.

4. Discussion

4.1. Structure

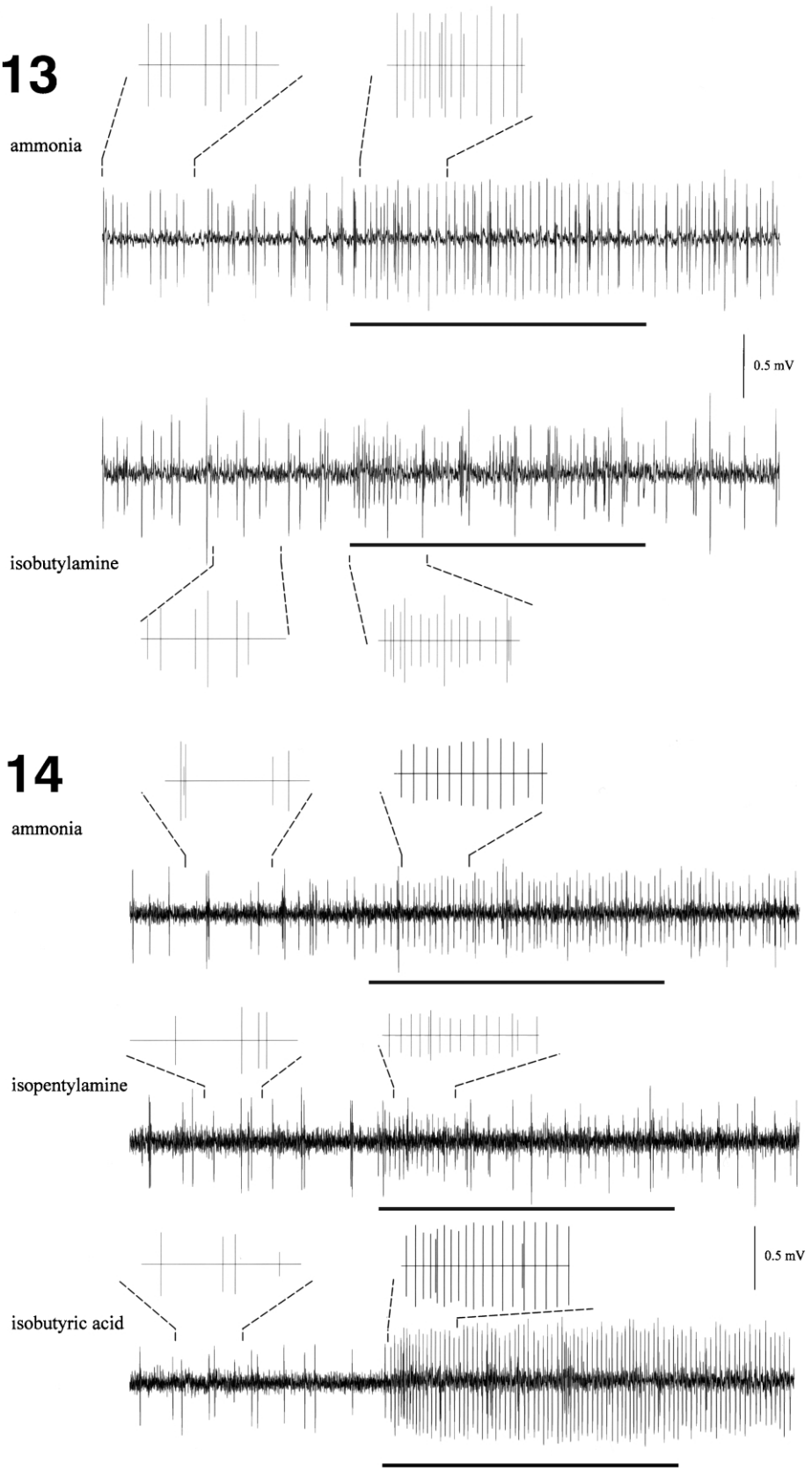
Our microscopic investigations have shown that the GP sensilla of *T. infestans* last instar nymphs fit well into the general picture of insect dw-wp olfactory sensilla (Altner and Prillinger, 1980; Steinbrecht, 1997, 1999). The 8–18 μm long GP sensilla show 13–18 grooves and contain four or five receptor cells, each giving rise to one unbranched dendrite.

Localization. In other insects, the short (≤20 μm) dw-wp sensilla are most often localized in antennal pits where the delicate pegs are assumed to be protected against mechanical damage and/or desiccation (e.g. coeloconic sensilla in *Bombyx*: Hunger and Steinbrecht, 1998). In contrast, the GP sensilla of *T. infestans* protrude from the antennal surface where they are mechanically protected by bigger surrounding sensilla. This is comparable to their placement in *Aedes* (McIver, 1974), *Drosophila* (Shanbhag et al., 1999), *Cimex* (Steinbrecht and Müller, 1976) or *Carausius* (Monteforti et al., 2002).

Cuticular apparatus and outer sensillum-lymph cavity. In contrast to *Bombyx* (Hunger and Steinbrecht, 1998), but comparable to *Cimex* (Steinbrecht, 1997), the outer sensillum-lymph cavities of the cuticular fingers in *Triatoma* fuse extensively to produce a single cavity. Only small radial cuticular bridges connect the outer and inner cuticle walls at the level of the spoke channels. We do not know if this extensive fusion of the outer sensillum-lymph cavities is of any functional significance.

Cryofixation permitted detection of electron-dense tubules in the outer sensillum-lymph cavity of the GP sensilla. Similar structures have been described for dw sensilla from *Bombyx* (Hunger and Steinbrecht, 1998) and from *Drosophila* (Shanbhag et al., 1999). With chemical fixation, they appear as electron-dense ribbon-like structures resembling 'wax-canal filaments' (Locke, 1961). In *T. infestans*, they are clearly connected to filaments in the outer cuticle wall which terminate in the

Figs. 10–12. Fig. 10: (a) Longitudinal section through a chemically fixed GP sensillum of a fifth instar *T. infestans* nymph at the level of the transition region of the inner (iD) to the outer dendritic segment (oD) connected by a small ciliary segment (CS). The somata of the receptor cells (R) contain an euchromatic nucleus (N) with a prominent nucleolus and many mitochondria, rough endoplasmic reticulum, Golgi apparatus (G) and lysosomes (Ly). Many electron-lucent vesicles (V) accumulate in the inner dendritic segment (iD). The two basal bodies (arrows) are anchored to the ciliary rootlets (CR); thecogen cell (Th) surrounding the receptor cell; * indicate regions of direct contact between receptor cells; scale bar 2 μm. (b) Image obtained after cryofixation showing close contacts between cell membranes (arrows) of two receptor cells (R), resembling gap junctions; scale bar 100 nm. Fig. 11: Cross-section below the antennal cuticle and base of a cryofixed GP sensillum from a fifth instar *T. infestans* nymph. Towards its proximal end the dendrite sheath (DS) in the GP sensilla is surrounded by the mitochondria-rich trichogen cell (Tr). Trichogen cell microlamellae (*) in contact with the outer sensillum-lymph (oSl) are often arranged in a parallel or concentric manner. The dendrite sheath borders the inner sensillum-lymph cavity containing five outer dendritic segments in this section; Cu electron-lucent cuticle, To tormogen cell; scale bar 1 μm. Fig. 12: Section of a tormogen cell (To) in a cryofixed GP sensillum on the antenna of a fifth instar *T. infestans* nymph with numerous microvilli and microlamellae-often arranged in parallel-on the basal border of the outer sensillum-lymph cavity (oSl). The cell contains numerous mitochondria and inclusion bodies of variable electron-density (Ib). Many vesicles (*) resembling the inclusion bodies are found in the outer sensillum-lymph space. The outer dendritic segments are enclosed by the dendrite sheath (arrow); Cu cuticle; scale bar 500 nm.



Figs. 13 and 14. Fig. 13: Responses of olfactory receptor cells in a GP 1 sensillum on the antenna of a fifth instar *T. infestans* nymph to NH_3 and isobutylamine, both at 10 μg in the stimulus cartridge. The bar beneath each recording indicates stimulation for 1 s; extracted spike trains of 25 ms are presented to show the spike amplitudes of the receptor cells before and after stimulation. A cell characterized by high amplitude spikes responded to NH_3, whereas a cell with spikes

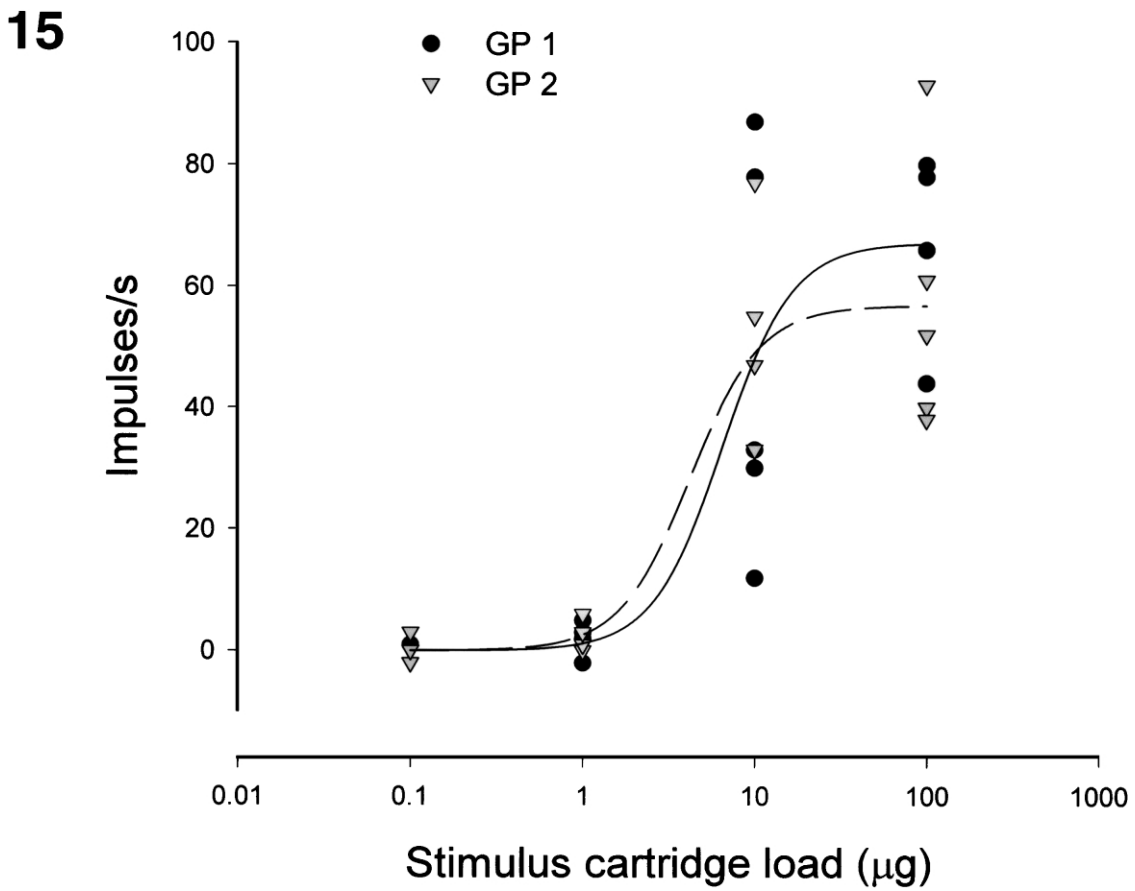


Fig. 15. Dose–response relationships of NH_3-excited receptors in the GP 1 and GP 2 sensilla on the antennae of fifth instar *T. infestans* nymphs: ordinate-spike frequency of the responding receptor cells calculated from the first 1 s of the response after subtraction of the blank control, abscissa-dose of compound in the stimulus cartridge. Each electrophysiological preparation of the GP 1 and GP 2 sensilla was subjected to NH_3 vapour for 1 s released from a stimulus cartridge containing increasing doses of NH_4OH on filter paper. Five nymphs (one receptor per nymph) were used for each GP sensillum type. The trend lines (GP 1, solid line; GP 2, dashed line), drawn by using a sigmoidal equation with three parameters ($y = a/\{1 + e - [(x - x_0)/b]\}$, Sigma Plot for Windows V. 4.01, SPSS Inc., USA), are not significantly different (refer text).

epicuticle. These wick-like filaments could transport waterproofing lipids from the outer sensillum-lymph cavity to the wax layer on the cuticle surface. The cuticular lipids could originate from the vesicles or droplets of variable electron-density which are present in the proximal part of the outer sensillum-lymph cavity. Comparable inclusions have also been reported from several insects such as *Bombyx* (Hunger and Steinbrecht, 1998), *Periplaneta* (Altner et al., 1977), *Aedes* (McIver, 1974), *Drosophila* (Shanbhag et al., 1999), *Psylliodes* (Bartlet et al., 1999), or *Carausius* (Monteforti et al., 2002). They appear to be released by the trichogen and tormogen cells into the outer sensillum-lymph cavity.

Sensory and auxiliary cells. The GP sensilla contain four or five sensory cells each giving rise to an unbranched inner and outer dendritic segment separated by a ciliary segment. As in other corresponding insect sensilla, they are surrounded successively by the auxiliary thecogen, trichogen and tormogen cell. We observed gap junction-like intimate contacts between sensory cell soma. Such close contacts between receptor cells have also been reported in thin sections of the olfactory dw sensilla coeloconica of *Bombyx* (Hunger and Steinbrecht, 1998), in the single-walled peg-like sensillum in the coleopteran *Ceutorhynchus* (Isidoro and Solinas, 1992), in gustatory sensilla (Isidoro et al., 1994; Moulins and Noirot, 1972) and between thermo/hygro-receptor cells of *Bombyx* (Steinbrecht, 1989). Physiological cross-talk such as peripheral inhibitory or excitatory interactions, mediated possibly through gap junctions, has been recorded in olfactory dw sensilla coeloconica in *Bombyx* (Pophof, 1997) and in sensilla placodea of the beetle *Popillia* (Nikonov and Leal, 2002), in gustatory

of low amplitude responded to isobutylamine. Fig. 14: Responses of olfactory receptors in a GP 2 sensillum on the antenna of a fifth instar *T. infestans* nymph to NH_3 and isopentylamine (each at 10 μg in the stimulus cartridge) and to isobutyric acid (1 μg in the stimulus cartridge). The bar beneath each recording indicates stimulation for 1 s; extracted spike trains of 20 ms are presented to show the spike amplitudes of the receptor cells. A cell of intermediate spike amplitude responded to NH_3, one with a low spike amplitude to isopentylamine and another with a high spike amplitude to isobutyric acid.

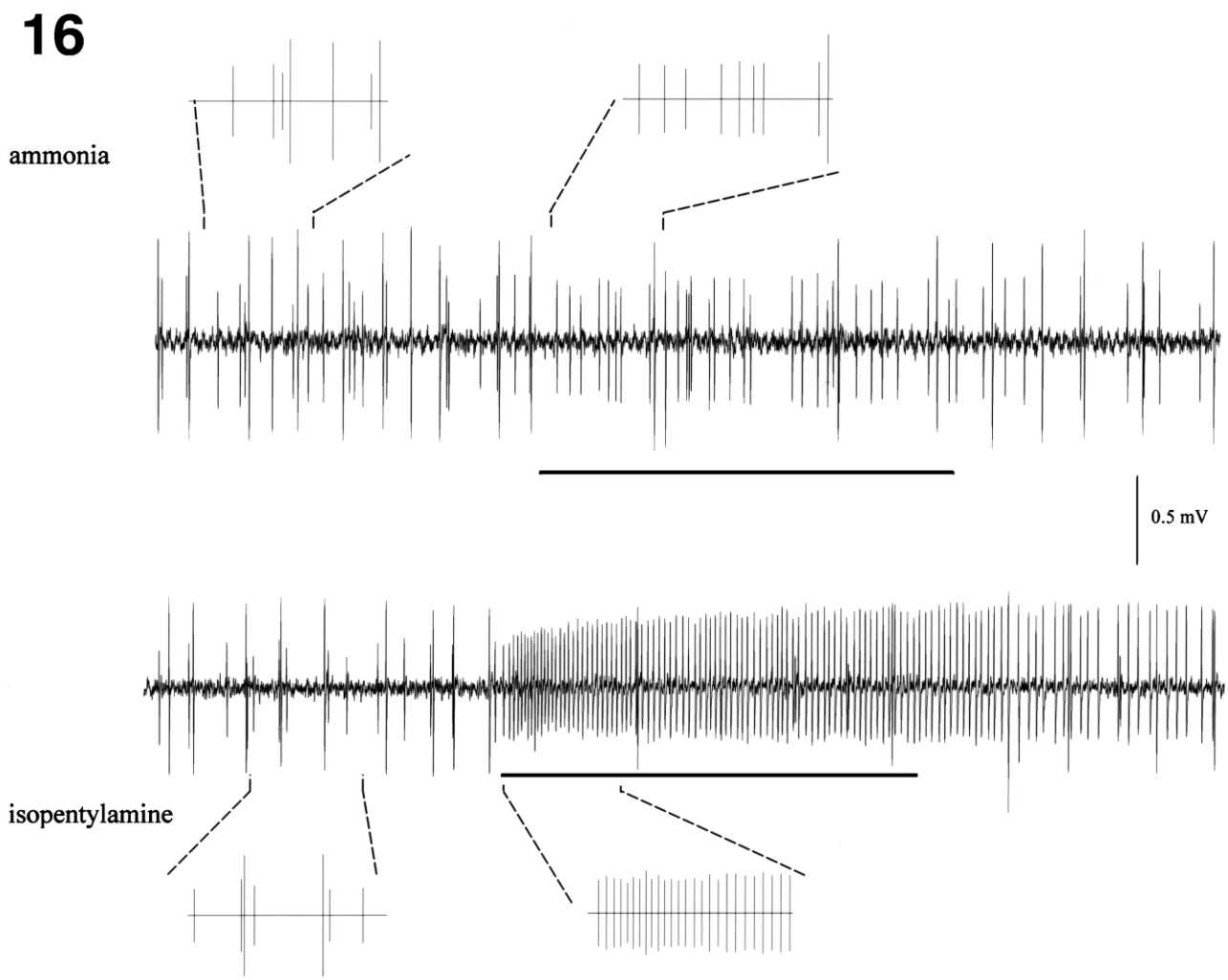


Fig. 16. Responses of olfactory receptor cells in a GP 3 sensillum on the antenna of a fifth instar *T. infestans* nymph to NH_3 and isopentylamine both at 10 μg in the stimulus cartridge. The bar beneath each recording indicates stimulation for 1 s; extracted spike trains of 25 ms are presented to show the spike amplitudes of the receptor cells. A cell with low spike amplitude was excited by isopentylamine whereas a cell with high amplitude spike was inhibited. Note the absence of a response to NH_3 by any receptor in this sensillum type.

sensilla (Mitchell and McCashin, 1994; Simmonds et al., 1990; White et al., 1990) and in sensilla with thermo/hygroreceptor cells (Gödde and Haug, 1990; Waldow, 1970). In all cases more precise ultrastructural work is clearly needed to definitively confirm the presence of gap junctions by using additional techniques such as cryofracture or immunocytochemistry with antibodies directed against gap-junction proteins.

Table 2
Responses (impulses/s) of the amine-sensitive receptor housed in GP 3 sensilla on the antenna of fifth instar *T. infestans* nymphs to different amines at 10 μg in the stimulus cartridge

	A	B	C	D	E	F	G	H	Medians
Ammonia	6	4	−2	20	15	7	−1	−1	5
Propylamine	6	47	Nt	Nt	11	31	6	Nt	11
Butylamine	20	75	53	23	6	17	16	68	21.5
Isobutylamine	50	63	63	23	30	40	47	95	48.5
2-Butylamine	21	85	77	27	28	29	24	112	28.5
Pentylamine	10	15	Nt	−3	6	13	2	Nt	8
Isopentylamine	18	57	41	−6	10	22	6	24	20
3-Methyl 2-butylamine	22	Nt	45	Nt	26	21	9	40	24
Hexylamine	Nt	Nt	−2	Nt	−3	2	Nt	6	0

Responses were calculated from the first 1 s of the response after subtracting control values for eight different preparations (A–H). Isobutylamine and 2-butylamine were the best stimulants ($p < 0.05$, Wilcoxon signed-rank test); nt, not tested.

Stimulus transport in dw-wp sensilla. It is currently admitted that the odorant stimulus is captured by the epicuticle and then diffuses from the groove channel through the spoke channel towards the inner sensillum-lymph cavity where it may get solubilized in order to reach the dendrite membrane by diffusion (Steinbrecht, 1997). In *T. infestans*, we have estimated a total of about 600–850 spoke channels per peg (mean peg length: 13–14 μm). This is quite comparable with the situation in *Bombyx* of about 600 spoke channels per peg (10 μm; Hunger and Steinbrecht, 1998), in *Aedes* with 456 per peg (5 μm; Cribb and Jones, 1995) and in *Drosophila* with 200 per peg (5 μm; Shanbhag et al., 1999). The surface to capture odorants on the outside of these tiny pegs is small and there are only a limited number of stimulus-conducting spoke channels present compared with the numerous pore tubules in the longer single-walled olfactory sensilla. Therefore, the dw-sensilla do not seem to be designed for the detection of low odorant concentrations (Steinbrecht, 1997). However, the smaller capture surface of the GP sensilla may be compensated by a higher solubility within the sensillum lymph of the polar stimuli for the GP sensilla receptor cells.

Terminal pore. SEM observations indicate the presence of a terminal pore at the tip of about half of the GP sensilla examined in *Triatoma* nymphs. A pore was unequivocally confirmed in one sensillum by serial thin sections in the longitudinal axis showing the tips of the dendrites to be very close to the opening. Thus, it appears possible that volatiles could enter through this pore into the inner sensillum-lymph in addition to transport through the groove-spoke channel system. On the other hand, the pore could simply represent a molting pore containing a molting plug not permitting access to volatiles. At present, we cannot decide if these structures are of any functional importance. The presence of a terminal pore in comparable dw-sensilla of other insects seems to be rare. A terminal pore was demonstrated in the G dw-wp sensillum in male *Periplaneta* by Toh (1977). In *Aedes* females a terminal pore was reported by McIver (1974), but could subsequently not be confirmed by Cribb and Jones (1995). Similarities between dw-wp sensilla and terminal-pore sensilla have been suggested by Hunger and Steinbrecht (1998).

4.2. Physiology

The electrophysiological data presented suggest three functional sub-types of GP sensilla on the antennae of *T. infestans* (Guerenstein and Guerin, 2001; Taneja and Guerin, 1997). Both GP 1 and GP 2 sensilla house an NH_3 and an aliphatic-amine excited cell and, in addition, the GP 2 type has a carboxylic acid-excited receptor. Even though the response characteristics of the NH_3 receptor cells are similar they are distinct in terms of their spontaneous firing rates between sensilla. The GP 3 type sensilla are characterized by the absence of both NH_3 and carboxylic acid-sensitive receptor cells, and the presence of an aliphatic-amine excited cell with response characteristics that are different from those in GP types 1 and 2. Isobutylamine, a constituent of mouse urine (Nishimura et al., 1989) and human vaginal secretions (Jones et al., 1994), was among the compounds that evoked the strongest responses from the amine-sensitive receptor cells in all three GP sensilla types of *T. infestans* nymphs. This compound and butylamine, a volatile present in human effluvia (Ellin et al., 1974) that also excites the amine receptor cells of *T. infestans*, may constitute part of the host odour that attracts triatomines (Taneja and Guerin, 1995). Short-chain aliphatic amine-excited receptor cells have been reported in GP sensilla of mosquitoes where pentylamine is the best stimulus (Pappenberger et al., 1996). In our studies, the number of effective stimuli for receptor cells in GP type sensilla of *T. infestans* exceeded in no case the number of cells the GP sensilla contain.

The existence of different functional GP sensilla subtypes was already reported from mosquitoes where a correlation between sensillum length and functional subtype occurs, i.e. short GP sensilla contain a neurone that is excited by lactic acid whereas long ones do not (Bowen, 1995). Lactic acid-inhibited cells can occur in either short or long GP sensilla depending on the mosquito species. The length of the *T. infestans* GP sensilla varies between 8 and 18 μm, but we could not observe size classes. The only noticeable structural differences between GP sensilla were (a) absence or presence of a terminal pore and (b) the variable number of receptor cells (four or five). At present we are unable to correlate the physiologically characterizable sensilla subtypes to sensilla of different structures. This would need considerably more work with GP sensilla tested individually by electrophysiology, subsequently being marked and then serially cut.

4.2.1. Single-walled versus double walled sensilla

It was proposed that receptor cells in single-walled wall-pore sensilla (sw-wp) might be adapted to respond to more apolar compounds such as long-chain aliphatic mono- and sesquiterpenes, aldehydes, alcohols or esters whereas those in double-walled wall-pore sensilla (dw-wp) would specialize for the perception of more polar and hydrophilic volatiles such as short-chain carboxylic acids (Altner et al., 1977). This difference was associated with the distinct stimulus transport systems reigning in each sensillum type (Steinbrecht, 1997). This hypothesis is supported by the responses of the receptor cells in the sw-wp basiconic sensilla on the antenna of *T. infestans* which are sensitive to long-chain aldehydes and terpenes (Guerenstein, 1999; Guerenstein and Guerin, 2001) whereas the dw-wp GP sensilla receptor cells are sensitive to more polar products such as NH_3, short-chain carboxylic acids and short-chain amines (this paper). This is comparable to the results reported from the dw sensilla in *Aedes* responding to lactic acid (Davis, 1977; Davis and Sokolove, 1976), in *Locusta* to green leaf volatiles such as hexenal, hexanol and aliphatic

organic acids with a chain length of 3–8 carbons (Boeckh, 1967; Kafka, 1970), and in *Periplaneta* to acids with a chain length up to six carbons and to amines (Altner et al., 1977; Altner and Prillinger, 1980). In addition, most receptor cells in the dw-wp sensilla coeloconica from *Bombyx* responded best to acids and aldehydes (Pophof, 1997). At present, we do not understand the physiological function of the structural differences between the sw-wp and dw-wp sensilla using, respectively, the pore tubule system and spoke channel system of stimulus entry (Hunger and Steinbrecht, 1998; Steinbrecht, 1997).

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