Carbon-Dioxide Sensing Structures in Terrestrial Arthropods

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ABSTRACT Sensory structures that detect atmospheric carbon dioxide have been identified and described to the subcellular level in adults of Lepidoptera, Diptera, Hymenoptera, Isoptera, Chilopoda, and Ixodidae, as well as in lepidopteran larvae. The structures are usually composed of clusters of wall-pore type sensilla that may form distinct sensory organs, often recessed in pits or capsules. In insects, they are located on either the palps or the antennae, in chilopods on the head capsule, and in ixodids on the forelegs. In the two cases where the central projections have been examined (Lepidoptera and mosquitoes), the clustering is preserved to the level of second order neurons, which are located in the deutocerebrum. Individual sensilla usually contain a single receptor neuron that is sensitive to CO2; it may be accompanied by other neurons that respond to other olfactory qualities. The distal dendritic processes of CO2-sensitive neurons invariably show an increased surface area, dividing into many cylindrical branches or into lamellar structures. Lamellar membranes are often closely linked to arrays of microtubules. Fine pore canal tubules are usually associated with the cuticular pores. Microsc. Res. Tech. 47:416–427, 1999. © 1999 Wiley-Liss, Inc.

INTRODUCTION

A considerable body of evidence shows that atmospheric carbon dioxide is detected by many terrestrial arthropods, in a wide range of habitats and behavioural contexts. CO2 is clearly an olfactory stimulus, but its properties are sufficiently distinct to warrant a separate discussion. First, CO2 is a major, ubiquitous atmospheric trace gas. Its background concentration is 10^16 molecules/ml in the open, and up to 10^18 molecules/ml in confined environments. This far exceeds the threshold concentrations for detection of other significant olfactory stimuli by arthropods: pheromone-specific sensilla in male moths can respond to single molecules released by conspecific females. Furthermore, temporal or spatial gradients of CO2 are created by the activity of nearly all living matter, raising the question of where and when CO2 signals can be an unambiguous source of ecologically relevant information. Finally, osmotically active molecules are usually highly lipophilic organic molecules with 5–16 carbon atoms and with distinct structural features. In comparison, CO2 is a very volatile and relatively inert small molecule. The literature on CO2-related behaviour and electrophysiology has been reviewed by Stange (1996), and the purpose of this article is to provide a corresponding overview of structural aspects of CO2 reception.

At the outset, it must be emphasized that work in this area is just beginning and that many receptor neurons that may be CO2 sensitive on anatomical or behavioural grounds are still undescribed or incompletely characterized in terms of their electrophysiology. For lack of an obvious alternative, the material is presented as a series of case studies across the arthropod phylum, beginning with those systems about which most is known. The terminology used is that of the original papers.

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of Von Rath (sic) and suggest an olfactory function but mention that ablation of the organ in females of *Tinea pelionella* did not impair their capability to perceive the odour of the substrate where they oviposit.

A detailed electron-microscopical analysis of the structure of the labial palp organ of *Pieris rapae*, and of the sensilla contained in it, was performed by Lee et al. (1985), who provided the description on which the following is based. The palps are about 3 mm long and are held pointing rostrally and slightly upward. The third, terminal segment is about 1 mm long and has an opening at the tip with a diameter of about 30 µm. The pit is about 100 µm deep and contains, at its bottom, a field of about 80 sensilla. They are about 20 µm long and slightly club-shaped, with a basal diameter of about 3.5 µm, tapering to about 1 µm and then slightly expanding to about 1.2 µm. The surface of the pegs is furrowed, with numerous irregular longitudinal ridges. Pores in the furrows between the ridges are clearly present, and within them are pore tubules. Ninety-four percent of the pegs house the dendritic outer segment of one single sensory cell, while two cells are found in the remainder. The inner segment is structured as usual in insect sensilla. The basal ciliary section of the outer segment is followed distally by a cylindrical section, proximally about 500 nm thick and tapering to about 400 nm. Within its most distal 6 µm, the dendrite splits into 4–6 flattened and folded branches, 80–90 nm thick, which are tightly interdigititated; the diameter of this lamellated region increases to about 600 nm. The cell membranes of adjacent lamellae are only 8–12 nm apart. The branches contain about 140 microtubules, regularly spaced at about 15–25 nm intervals, lying 20–30 nm from the dendritic membrane to which they can sometimes be seen to be attached with fine filaments. The surface area of the lamellated part of the dendritic membrane is 40 µm² while the cylindrical section of the outer segment, which is three times as long, has an area of 25 µm². A dendritic sheath or theca is present but it fully encloses only the proximal, cylindrical portion of the dendritic outer segment. Distally, at about the level where the dendrite begins to branch and becomes lamellated, the sheath splits into narrow tapering flaps, most of which end after only a short distance.

The widespread occurrence of lamellation implies a strong selective advantage. Amplification of membrane area to provide for a greater number of receptor sites is unlikely to be the sole factor since, as pointed out by Lee et al. (1985), cylindrical rather than flat dendrite branches, if tightly packed, would provide a greater surface area. Other considerations may include ease of cytoplasmic flow and exchange of metabolites. Lee et al. (1985) also emphasize the need for more detailed evaluation of the microtubule-membrane complex.

In sphingid moths, the labial palps point upward at the sides of the proboscis. The second segment is often quite large and is mainly occupied by a tracheal sac; the
third, much smaller segment is located on its top and contains a flask-shaped cavity with a narrow neck and a wide interior. It houses a densely packed array of sensilla. In the large sphingid moth, Manduca sexta, the estimated number of sensilla in each organ is 1,750 (Kent et al., 1986). The sensilla are about 30 µm in length and 7 µm in diameter. The surfaces carry longitudinal grooves and the walls are pierced by numerous pores. Pore tubules extend into the outer lymph cavity surrounding the dendrite of a sensory cell, which is also lamellated in its distal portion.

The 3-segmented labial palps of the arctiid moth Rhodagastria spp. (Bogner et al., 1986) lie on each side of the proboscis and are entirely covered by scales. An opening near the tip of each palp is surrounded by pin-pointed microtrichia and is of variable diameter (35–70 µm). It extends to a cavity up to 150 µm deep and 80 µm wide, which is densely packed with approximately 200 sensilla. As in other labial palp sensilla, a cross-section reveals wall pores and a lamellated dendrite, although the overall structure differs slightly from the norm in the preponderance of forked tips, relatively smooth walls and a slight taper rather than a clubbed shape. In the same paper, it is clearly demonstrated electrophysiologically, for the first time, that the labial palp sensilla are primarily tuned to the detection of CO₂.

On the tip of each labial palp of the European sunflower moth, Homoeosoma nebulicella, there is a pit organ whose opening has a diameter of approximately 22 µm and is surrounded by pin-pointed microtrichia. Each pit organ houses approximately 40–50 multporous sensilla coeloconica (Faucheux, 1991).

In the pyralid moth Cactoblastis cactorum, the labial palps show a pronounced sexual dimorphism (Stange et al., 1995). In females (typical body length 21 mm), the palps point forwards and measure approximately 12% of body length. The terminal segment, which contains the labial palp organ with the CO₂-receptors, measures 4% of body length. In males (17 mm long), the palps are held pointing upwards and are only about half as long in proportion to body length. In Lepidoptera in general, the labial palps tend to be of the same size in both sexes.

Each terminal segment contains a pore at the tip that opens into the labial palp organ, which is lined with a dense, canopy-like layer of approximately 200 sensilla (Fig. 1b). Although the length of the grooved sensilla, about 10 µm, is similar to that described in other species, the shape is distinctive. While the basal part of the sensillum, 5–8 µm long and 1.5 µm in diameter, is thin and cylindrical, the distal part bends sharply towards the tip of the palp and expands into a leaf-like structure 3–6 µm long and 3–4 µm wide. Since the leaf-like portions overlap to some extent, the effect is to form a canopy or inner wall within the labial palp organ. Each sensillum is grooved with pores at the base of the grooves and an underlying system of pore tubules, most evident in freeze-substituted material. The pattern of grooves and ridges is often quite regular, flatter strips of cuticle alternating with thinner, sharper ridges (Fig. 1c). Within the sensillum is a single dendrite containing densely arranged microtubules. In the proximal shaft, there is some degree of infolding and lamellation, resulting in an estimated membrane surface area of 20–35 µm², comparable to that found in P. rapae by Lee et al. (1985). However, the degree of lamellation is vastly increased within the leaf-like terminal portion, to 200 µm² or more, compared to 40 µm² for the lamellated portion of P. rapae. In antennal olfactory hairs, the total membrane area can be considerably larger: in males of the silkmoths, Antheraea polyphemus and Antheraea pernyi, Keil (1984) measured up to 600 µm², but in this case the membrane is not concentrated within a small volume.

While a large part of the lamellated membrane is interleaved with regular rows of microtubules, as reported in many other species, in many sensilla a substantial portion appeared as very closely appressed sheets, separated only by a few nm of cytoplasm. This was particularly evident in freeze-substituted material. Lee et al. do not describe anything similar in P. rapae, but in the digitiform organ of Helicoverpa armigera caterpillars Keil (1996) illustrates lamellar areas with and without microtubules, although in this case the membranes are much less closely associated. In C. cactorum, there was no obvious evidence of any internal structure being distributed differentially between the two sides of the expanded portion of the sensilla, that is, between the surface facing into the central cavity and that facing into the restricted space between the sensilla canopy and the wall of the labial palp organ.

Central Projections

As the labial palps are mouth parts, we could expect that the sensory neurons carried by them innervate the corresponding part of the central nervous system, which is the suboesophageal ganglion. On the other hand, it could be that CO₂-receptor neurons form a projection in the vicinity of receptor neurons sensitive to other olfactory qualities. Those neurons are commonly located on the antennae and project to the antennal lobes. Indeed, Kent et al. (1986) found in M. sexta that axons stained with cobalt from the cut end of the third labial palp segment enter the suboesophageal ganglion via the first labial nerve, and some fibres have an arborisation on the ipsilateral side of that ganglion, but others ascend to the brain, arborizing within a single glomerulus in both ipsilateral and contralateral antennal lobes. In A. polyphemus and Bombyx mori, Kent et al. (1986) observed similar projections that were less pronounced, corresponding to the smaller number of receptor neurons in those species.

Lepidoptera: Larval Maxillary Palps

There are few interspecies differences in the types and distribution of the sensilla found on the larval maxillary palps. Several types of sensilla are observable externally: eight sensilla basiconica on the apex of the distal segment, a varying number of campaniform sensilla in larvae of the spruce budworm, Choristoneura fumiferana, and Devitt and Smith (1982) described the external anatomy and the innervation patterns of mouthpart sensilla in larvae of the European spruce bark beetle, Ips typographus, with a varying number of campaniform sensilla in larvae of the spruce budworm, Choristoneura fumiferana, and Devitt and Smith (1982) described the external anatomy and the ultrastructure of the larval maxillary palp of the noctuid moth Euxoa messoria by Grimes and Neunzig (1986). Faucheux (1995) exam-
in the sensilla on the mouth parts of larval sunflower moths, *Homoeosoma nebullella*.

Electrophysiological evidence (Roessingh, 1989, in the moth *Yponomeuta cagnagellus*; Stange, unpublished data, in the moths *C. cactorum* and *H. armigera*) shows that there is at least one, and probably no more than one, CO$_2$-sensitive sensillum on the larval maxillary palp. Keil (1996) has investigated the ultrastructure of the sensilla on the maxillary palps of *H. armigera* larvae, with the specific intention of examining possible candidates for the CO$_2$-receptors. The eight chemosensory pegs on the apex have channels at their tips, strongly indicating that they are contact chemoreceptors. One campaniform sensillum and three internal scolopidia are undoubtedly mechanoreceptors, leaving two pore plates and one digitiform sensillum as possible CO$_2$ receptors. Keil draws attention to similarities between the distal pore plate and the organ of Tomosváry in chilopods (porous cuticle, large lymph cavity, numerous thin dendritic branches), but sees the digitiform sensillum (Fig. 2b) as the most likely prospect. This structure has very fine slits in the cuticle (10 nm in *H. armigera* but as large as 50 nm in *H. nebullella*; Faucheux, 1995). The small size of these slits, and pores in other structures, means that reports of non-porous sensilla based purely on SEM observations, particularly of conventionally gold-coated material, should be treated with caution.

**Adult Trichoptera: Labial and Maxillary Palps**

Most of the sensilla on the palps of caddisflies can be assigned to well-known categories (Ljungberg and Hallberg, 1992); among the previously undescribed types of sensilla are hairs that usually occur on the dorsal surface of the outer segments of the maxillary, in some genera also on the labial palps. They contain one single receptor cell with a lamellated outer dendritic segment. The surfaces of the hairs are longitudinally striated and several hairs may be grouped together in depressions on the palp or even in distinct pits. While those sensilla have a number of features in common with the sensilla in the labial palp organ of the Lepidoptera, which are clearly tuned to the detection of CO$_2$, there is no evidence of either wall pores or pore tubules, and the dendritic sheath encloses the dendrite along its entire length. The authors conclude that the sensilla are clearly not olfactory.

**Diptera nematocera: Lutz's Organ**

In amputation experiments on males of *Aedes aegypti*, Bässler (1958) identified a group of 20–30 large, recessed peg-shaped sensilla on the maxillary palps as CO$_2$-receptor neurons. In males, this group is located on the second segment, while females carry it on the terminal segment. For the latter, CO$_2$ sensitivity was confirmed in electrophysiological recordings from single sensilla by Kellogg (1970) and Grant et al. (1995). The recordings showed that there are several neurons in each sensillum; only one responded to CO$_2$ while the others responded independently to other chemical vapours.

The fine structure of the palpal pegs in females of three species of culicine mosquitoes was examined by McIver (1972). The pegs are located mainly on the distal half of the ventral part of segment 4 of the characteristically short, five-segmented palp. The external cuticular peg, 13–17 μm long, with numerous pores in the surface, is accompanied by three bipolar sensory neurons and by sheath cells. The exocuticle of the peg is up to 250 nm thick and contains numerous perfora-
tions, 23 pores/µm² in Culex pipiens and 12 in A. aegypti. The external openings have diameters of 15–18 nm, expanding into a spherical chamber region of 100 nm diameter. Pore tubules radiate from that chamber towards the dendrites. The sensory neurons are of two distinct types, both at the level of the cell bodies and of the dendritic processes. The large dendritic process of a single neuron is located in the central area of the lumen and branches distally to form lamellae. Several small, peripheral processes originate from two relatively more electron dense neurons (Fig. 3c,d).

It is not evident from those observations which of the neurons is CO₂-sensitive, but a comparative study has provided an indication that the lamellated dendrite is the CO₂ receptor. If palpal pegs with lamellated dendrites are involved in the detection of host-emitted CO₂ by haematophages, they ought to be absent in species that do not feed on blood. McIver and Siemicki (1984) examined this question in females of the mosquito Toxorhynchitis brevipalpis, which never feed on blood. Here, each peg sensillum is digitiform and contains 3, sometimes 2, neurons; the dendrites of all the neurons extend the length of the peg lumen and divide into branches but are not lamellated. This contrasts with the situation in blood feeders where the sensilla are of capitate shape and where one of the dendrites is lamellated.

The capitate shape of maxillary palp sensilla is particularly pronounced in biting midges (Forcipomyia fairfaxensis: Desser and Hong, 1992; Fig. 3a,b). Of the five segments of the maxillary palp, the third one is enlarged and contains a superficial pit with 21–31 stalked, bulbous sensilla. The bulbs are almost spherical in shape, of diameter 2.3 µm; the stalks are 3.2 µm long, with a diameter of 0.65 µm. There are pores in the cuticle which open to a lamellate dendrite within the bulb.

**Central Projections**

Like the labial palps in Lepidoptera, the maxillary palps are not part of the segment where integration of olfactory inputs occurs. Using both HRP labelling and induced degeneration techniques, Distler and Boeckh (1997) found that afferents from the fourth segment of the maxillary palp of A. aegypti project into the subesophageal ganglion and ascend further into the antennal lobe, where they terminate ipsilaterally within a distinct glomerulus. Afferents from the antennae do not innervate this glomerulus but they do project into all other ipsilateralglomeruli.

Thus, on the one hand, the palpal receptor neurons project into the deutocerebrum where other olfactory information is processed, but, on the other hand, antennal and palpal projections are mutually exclusive on the level of the individual glomerulus. As appendages other than the maxillary palps do not carry CO₂-receptor neurons, all CO₂ afferents project into this glomerulus, but the glomerulus also receives signals from other maxillary receptor cells that respond to other odors.

**Diptera brachycera: Antennal Sensilla**

Feeding behaviour in the haematophagous stable fly Stomoxys calcitrans is controlled by a range of chemical factors including CO₂; six types of sensilla with perforated cuticle are present externally on the fusiculus (third segment) of its antenna (Lewis, 1971). Of those, the clavate sensilla are 15 µm long, each with a stem of 2 µm in diameter, swelling to a tapered bulb of 4 µm diameter. A single dendrite is generally present as a...
simple tubular structure at the base of the sensillum but becomes folded into the form of a scroll more distally in the stem. In the bulb, the dendritic folds become less regular in arrangement but the membranes of adjacent folds remain in contact for much of their length. Small branches run from the lamellated structure to the pores. Regularly arranged neurotubules are located between the spiral membranes. Some clavate sensilla possess two receptor cells, the dendrites of both exhibiting a folded structure. Thus, the clavate sensilla could well be CO2 receptors.

The ultrastructure of antennal sensilla of the Queensland fruit fly, *Bactrocera tryoni* (Tephritidae), was examined by Hull and Cribb (1997). The funiculus carries several types of sensilla that are multiporous, suggesting an olfactory function (Fig. 4a). One subtype, with a single thin wall, is innervated by 3 sensory cells and is characterized by a large dendrite that shows lamellar folding within the apical section. The dendrites of the other two sensory cells are extensively branched (Fig. 4b,c). Given the similarity to CO2-sensitive sensilla in mosquitoes, the authors suggest a CO2-detecting function for the lamellated dendrite and suspect that the other dendrites respond to general environmental odors. In action potential recordings from the sensillum, Hull (personal communication) found that one of the cells can detect CO2; it also responds to other odorants. Behavioural responses to CO2 were reported by Rice (1989) who observed an arousal response to high concentrations, and by Stange (1999) who observed that flies aggregate at a point source of air that is enriched, above background, with 100 ppm CO2 or more.

**Hymenoptera: Sensilla Ampullacea**

The sensilla ampullacea, bottle organs (Forel, 1908) or Forel's flasks, are distinctive structures located on the antennae of many hymenoptera. In worker honeybees, the antennal flagella carry approximately 12,000 sensilla of various types, containing approximately 100,000 sensory neurons (Lacher, 1964). Most of them are sensitive to olfactory stimuli such as floral odours or several types of pheromones involved in social commu-
communication. Two morphologically distinct sensillum types (sensilla coeloconica and sensilla ampullacea), are clustered together in small groups close to the distal ends of each of the eight distal flagellar segments. They are 10–30 µm long conical pegs of 10 µm diameter at the base, and are completely sunken in individual pits. They number 236 per antenna, with the sensilla ampullacea forming the majority. In recordings from their vicinity, Lacher (1964) observed a cell type that responds to CO2. He suggested that, of the two types, the sensilla ampullacea were more likely to be the CO2 receptors.

So¨tz (1989) conducted a morphological and ultrastructural examination of the sensilla coeloconica and sensilla ampullacea, with the specific objective of obtaining cues on their functions, in particular with regard to CO2 sensitivity. The sensilla coeloconica carry, at least in their distal parts, longitudinal cuticular folds and are double-walled; spoke channels provide a connection between the outside and the sensillum lymph space. These features are reminiscent of sensilla found elsewhere that clearly serve a general olfactory function. Each sensillum usually contains four dendrites. The sensilla ampullacea are single-walled and longitudinally grooved, reminiscent of CO2-receptive sensilla in the Lepidoptera. As in the latter, each sensillum contains a single dendrite with a strongly enlarged surface area; a major difference lies in the fact that this surface enlargement comes about by extensive branching rather than lamellation of the dendrite. Pore tubules were not clearly identified. A branched dendrite, containing approximately 80 branches with a single microtubule each, was also observed by Keil (personal communication, Fig 5b).

The suggestion that the sensillum ampullaceum is the CO2-sensitive organ is further corroborated by the observation that nerve impulses from CO2-sensitive sites are consistently observed in isolation (Lacher, 1964; Stange and Diesendorf, 1973), indicating that recordings were from a sensillum that contains a single neuron.

Each of the antennae of the leaf-cutting ant *Atta cephalotes* carries about 1,400 sensilla (Kleineidam and Tautz, 1996), which predominantly respond to olfactory, contact chemical, or mechanical stimuli. The apertures of an elliptical array of sensilla coeloconica and sensilla ampullacea are located in a distinct patch on the ventrolateral side of the distal flagellar segment. Both types of sensilla are pegs located in cavities with only small apertures to the outside. In contrast to the sensilla coeloconica, which are 10 µm deep and reside completely within the thick antennal cuticle, the sensilla ampullacea are sited deep within the lumen of the antenna and are connected to the outside by a narrow duct, 20–50 µm long and 1.5 µm in diameter (Fig. 5a). As in honeybees, the dendrites are extensively branched (120 branches, Kleineidam, personal communication).
the CO₂-receptive cells are associated with temperature-sensitive neurons in the same sensilla.

**Coleoptera: Adult Head Appendages**

The red flour beetle, _Tribolium castaneum_ , is strongly attracted by low (< 15% or 150,000 ppm) concentrations of CO₂ and strongly repelled by high (> 60% or 600,000 ppm) concentrations (Willis and Roth, 1954). Successive amputation of antennal club segments progressively reduced and finally eliminated orientation towards a CO₂ source, while removing one or both maxillary palps did not have a recognizable effect on CO₂ orientation. The antennae carry two types of putative olfactory receptors, firstly trigichoid sensilla, concentrated on the terminal segment, and secondly basiconic sensilla, more evenly distributed across all segments. Removing the terminal segment alone does not eliminate the CO₂ response, suggesting that it is mediated by the latter.

Honemichl and Guse (1981) speculate that digitiform sensilla, which have a widespread distribution including the distal maxillary palp, may function as CO₂ receptors. A pore system was not found in those sensory hairs. The dendrites, which extend to the tip of the hair, contain many microtubules, and a dendrite-free lumen runs beside the dendrite-containing one. The hairs are unusual in that they lie within a cuticular groove for most of their length.

**Coleoptera: Larval Head Appendages**

In the process of localizing the respiring roots of host plants, the polyphagous larvae of the weevil _Otioryynchus sulcatus_ detect CO₂-concentration gradients in the soil. Klingler (1966) conducted amputation experiments on the antennae and on the maxillary and labial palps in order to identify the location of the sensory organs that mediate this capacity. He concluded that the CO₂-receptor neurons are predominantly located on the maxillary palps, in particular the terminal segment, but that there is also a measurable but weak influence of the antennae on orientation performance.

The same distribution was evident in larvae of the wood-boring beetle _Orthosoma brunneum_ , which move toward high concentrations of CO₂ while in a gradient (White et al., 1974). Sensilla are concentrated on the distal segments of all three appendages and are clustered within conspicuous apical depressions.

**Isoperta: Antennal Sensilla With Combined CO₂/Odorant Sensitivity**

CO₂-concentration signals carry information that is relevant to termites: they are adapted to a life in the nest or in enclosed galleries and are prone to perish quickly when exposed to the open atmosphere. One type of single-walled sensillum on the antennal flagellum of _Schedorhnotermes lamanianus_ (Kaib et al., 1993; Ziesmann, 1996) contains two receptor neurons that respond with similar specificities to odours such as primary and secondary alcohols with 5–6 carbon atoms. They differ in that one is not responsive to CO₂, while the other is inhibited completely by CO₂ concentrations likely to occur in the nest. Morphologically, the sensilla are similar to other olfactory receptor neurons: they are 10 µm long hairs with a pore-tubule system penetrating the wall; in common with other CO₂-sensitive structures, the two dendrites are branched. The sensilla occur on all flagellar segments of the antenna and are not located in pits.

**Chilopoda: Organ of Tömösváry**

Some soil arthropods such as the Chilopoda, Diplopoda, Pauropoda, Symphyla, and Apterygotta possess a pair of sensory organs on the head, referred to as postantennal organs, temporal organs, organs of Tömösváry, or pseudoculi. Although behavioural experiments had suggested that the temporal organ of _Lithobius forficatus_ is a hygroreceptor (Tichy, 1973), Yamana and al. (1986) found in electrophysiological experiments that the temporal organ of the Japanese house centipede, _Thereuonema hilgendorfi_ , contains receptor neurons that strongly respond to CO₂ at ecologically relevant concentrations. The structure, examined by Yamana and Tob (1990), is remarkable in that it bears little resemblance to other sensory structures in arthropods that respond to CO₂ or general odorants. Each organ, situated between the base of the antenna and the pseudo-compound eye, appears externally as a cuticular dome, 40 µm in diameter, with a central opening of 5 µm, leading into a cavity of 40 µm diameter and 20 µm depth. A mushroom-shaped projection of approximately half this size extends from the base of the cavity, towards the opening. The cuticle that covers the projection is neither layered nor does it contain the more common pore-tubule structures; instead it consists of a fabric of fibrils of about 10 nm in diameter, forming a water-permeable layer of less than 0.5 µm thickness. Of more than 100 epithelial cells contained in the projection, only about ten are receptor neurons. The apical sections of the receptor cell dendrites consist of pairs of unbranched cilia that extend distally about 50 µm towards the cuticle where they become swollen, from 0.25 µm at the base, to 2 µm. They may course horizontally along the inner surface of the cuticle. The supporting cells that surround the dendrites extend single distal processes, leaving little extracellular space. An intercellular space that might be functionally equivalent to the sensillum lymph space of other olfactory sensilla surrounds the bases of the sensory cilia.

The trichobothria of myriapods and other arthropods are generally mechanoreceptive fine hairs sensitive to slow air currents. However, the trichobothria of _Polyxenus ssp._ (Pselaphognatha, Tichy, 1975), which are located in the same position as the organ of Tömösváry in related myriapods, have developed some significantly different features. The dendrites, surrounded by thin processes and lamellae of enveloping cells, extend into the hair shaft, which has numerous pores with pore tubules in the same region and lies distally within a cuticular cavity. Two ciliary processes form from each sensory cell. The dendrites themselves are not further branched or lamellated, but maintain a pattern of ciliary doublets rather than single microtubules. The dendrites are connected by electron dense filaments, apparently as a complex with dense material extending from the membrane internally to adjacent microtubule doublets. The basal bulb, seen in related groups, is reduced. Tichy and later reviewers (Haupt, 1979; Hopkins and Read, 1992) point out that the resulting structure has some features found in mechanoreceptors but also others found in olfactory, hygro- and thermosensitive sensilla. A thorough electrophysiological investigation would be of great interest.
Ixodidae: Haller’s Organ

Olfactory sensilla of several different types are located on the tarsi of the first pair of legs, which breath-stimulated ticks lift in the air to sample their surroundings, as insects do with their antennae. Haller’s organ on the dorsal side of the tarsus of both ixodid and argasid ticks consists of an anterior pit and a posterior capsule with a generally narrow opening, and contains many of the olfactory sensilla; among the 19 tarsal olfactory sensilla of the tropical bont tick *Amblyomma variegatum*, 3 belong to the anterior pit and 7 to the capsule. Two of the sensilla in the capsule contain receptors sensitive to changes in CO₂ concentration, one with an inhibitory response to small changes, maximally to 0.001–0.002% or 10–20 ppm increases in ambient (360 ppm), the other with an excitatory response to changes above 0.1% (Steullet and Guerin, 1992). Waladde and Rice (1982) found in *Boophilus microplus* that all 4 capsular sensilla were of the thin-walled type, with relatively large (0.2 µm diam) plugged pores (Fig. 6). Sonenshine (1991) shows considerable variation in the form of the posterior capsule.

Fig. 6.  (a) anterior pit and (b) anterior pore sensilla of the tick *Boophilus microplus*, with cross-section through the pored sensillum of the anterior pit (c), and detail of plugged pore (d). After Waladde SM, Rice MJ. 1982. The sensory basis of tick feeding behaviour. In: Obenchain FD, Galun R, editors. Physiology of ticks. New York: Pergamon Press. p 71–118, with permission.
roof, from completely open to an almost closed transverse slit.

Haller’s organ is one of several examples of CO₂-responsive sensilla located inside a pit. Steullet and Guerin (1992) point out that it could be an advantage if a sensory organ is well protected from physical damage or desiccation and that the rigid architecture of the capsule does not restrict the passage of volatiles, at least highly diffusible CO₂ molecules, to the capsular sensilla.

Other orders of arachnids (reviewed by Foelix, 1985) have not been thoroughly investigated for possible CO₂ sensitivity. Pore sensilla rather similar to those in ticks are found in whip scorpions (Amblypygi), but are rare or absent in most spiders, opilionids and scorpions.

**DISCUSSION**

**Common Structural Features**

CO₂-sensitive structures in land arthropods are clearly a subset of olfactory sensilla. They are hairs or pegs that contain dendrites and have a thin cuticle that almost invariably has many pores. The pores may be in grooves, they may be plugged, most have pore tubules. Sensilla are frequently located within a depression or a pit with a restricted opening. What is certain is that there is an enormous diversity on all levels, ranging from the macroscopic to the ultrastructure and allowing few if any generalizations, giving the strong impression of a multiple, polyphyletic origin.

**Functional Contexts**

CO₂ receptors are most consistently present in haematophages, including the Ixodidae and the Diptera, but also the triatomid bugs where CO₂ sensitivity has been demonstrated (Núñez, 1982) but the associated structures remain to be identified. It remains open as to how common CO₂ sensitivity is in non-haematophages. There are some examples of CO₂ sensitivity in species that are confined within enclosed environments such as centipedes, beetle larvae, and termites, as well as ants and bees that spend parts of their life cycles within those environments. It is conceivable that the CO₂ sensitivity of the herbivorous lepidopteran larvae constitutes a similar adaptation, because CO₂ gradients in the immediate vicinity of metabolically active plant organs can be significant; the same applies for tephritid fruit flies (Stange, 1999). Adult females of the pyralid moth *Cactoblastis cactorum*, after alighting on a potential host, probe the surface of the food plant with labial palps that are pointing forward and elongate; the same behaviour is observable in other pyralids (Stange, unpublished data). However, in many adult Lepidoptera the labial palps point upwards in such a way that major contortions would be required to bring them into contact with the substrate, suggesting a function that does not involve probing. It is worth mentioning that CO₂-sensitive structures remain to be identified in entire groups of herbivorous arthropods such as adult Coleoptera and Orthoptera.

**Distinct Sensory Organs**

The organ of Tömösváry on the head capsule of centipedes, Hailer’s organ in ticks, Lutz’s organ on the maxillary palps of mosquitoes, and vom Rath’s organ (labial palp organ) of the Lepidoptera are examples where clustering of CO₂-sensitive structures has been noticed by early observers. The sensilla ampullacea of bees and ants (Forel’s flasks) are concentrated in distinct patches on the antennae. In the two cases where the central projections have been examined (Lepidoptera and mosquitoes), the clustering is preserved to the level of second order neurons, which are located in the deutocerebrum, suggesting that afferent signals from CO₂ receptors are separately processed initially but are then integrated with other olfactory afferents from the antennae. While CO₂ receptors in termites and flies are also located on the antennae, there is no evidence that they are concentrated in patches. There is no known case where CO₂ receptors are simultaneously present on different appendages.

**Adaptive Value of Clustering**

Sensilla that detect other olfactory qualities are often distributed across the full length of the antennae, presenting a large cross-sectional area for the capture of molecules from air that is flowing past them. Enlargement of cross-sectional area is particularly pronounced in the antennae of many male moths, which form branched structures and carry very long pheromone-sensitive sensilla trichodea.

A highly lipophilic pheromone molecule that has been carried towards an antennal “filter” by air moving relative to the animal is likely to be adsorbed to the lipophilic cuticle of a sensillum. This step is essentially irreversible, with the consequence that the sensory organ can only function if adsorbed odour molecules are processed and deactivated, at least at the same rate as adsorption, by mechanisms other than desorption (Kaisling, 1998). Thus, a pheromone-sensitive sensillum is a flux detector where the rate of adsorptive uptake is proportional to the product of the relative velocity of the organ and the external medium and to the stimulus concentration.

Different considerations apply for the detection of CO₂. As CO₂ is only moderately lipophilic and extremely volatile, it is unlikely that its adsorption to sensory structures is irreversible. Moreover, the large background concentration, together with high diffusivity, has the consequence that a flux detector for CO₂ would have to continuously adsorb and process a considerable number of stimulus molecules. This leads to the conjecture that the interaction between stimulus and CO₂ receptor is reversible and that the receptor is a concentration detector (Kaisling, 1998), which responds independently of wind speed. Such an independence was observed electrophysiologically in honeybees (Stange and Diesendorf, 1973), mosquitoes (Grant et al., 1995), and moths (Stange, unpublished data). A detector that is independent of wind speed will convey more reliable information about the environment than one that cannot distinguish between changes of speed and concentration.

A capsule or a pit that surrounds a detector constitutes an obstacle to the diffusion and mass movement of stimulus molecules. In a concentration detector, there is no net mass transport and the presence of an additional diffusion resistance is inconsequential to resolution in the concentration domain because, after a concentration change, a new equilibrium will be reached, albeit with an additional delay, so that the resolution in
the time domain will be reduced. Indeed, there is evidence that the extremely slender duct found in Forel’s flasks (Fig. 5a) acts as a low-pass filter (Kleineidam and Tautz, 1998). For the case of the labial palp organ of the moth Helicoverpa armigera (Stange, 1992), any low-pass filtering effect of the labial palp cavity seems to be negligible because the responses of the CO₂-receptor neurons to stimulus concentration changes are at least as fast as those found in free-standing olfactory sensilla responding to other stimuli.

This leads to the suggestion that a capsule surrounding a concentration detector simply provides protection from desiccation, without impairing sensory function. The cuticle and pore structures of a sensillum cannot be perfectly impermeable to water vapour, and the rate of water loss, which is affected by net mass transport, will be reduced by the presence of an additional diffusion resistance.

Finally, it is possible that clustering simply occurs by default, due to the absence of selective pressure to present a large cross-section to a moving air current. However, receptive structures that are concentrated in the smallest possible volume will behave like a point sensor, maximizing spatial resolution. The presence of a capsule reduces the incidence of damage and provides protection against disturbances from outside, as suggested by vom Rath (1888).

Perireceptor Environment

Even more than the whole sensory organs, the dendritic structures involved in pheromone reception and those involved in CO₂ reception appear to be the products of opposing selective pressures. The former are long and slender, with no or only limited branching, while the latter are short and either lamellated or contain densely packed branches, without currently known exceptions. Thus, the primary design principle of pheromone sensilla, where a large membrane surface exposed to the outside world maximizes capture efficiency, cannot apply to CO₂ reception. Instead, a large but highly concentrated membrane surface suggests that there is an advantage in packing the, yet unidentified, molecular receptors for CO₂ as densely as possible. Other examples of organelles with densely stacked cell membranes are the outer dendritic segments of insect thermoreceptors, rhabdomeres, and rod outer segments in visual systems and the thylakoids in the chloroplasts of plants.

McIver (1972) observed that if the functional role of lamellation consists of an increase of the receptive surface area, then reception of the stimulating molecule should be possible at places on the dendritic membrane other than at the pore tubules. Since then, evidence has accumulated, for other olfactory receptor neurons, that the pore tubules are less important for stimulus conduction than previously thought and that, instead, soluble binding proteins are of crucial importance. The involvement of a carrier could also be important in CO₂-receptive structures: Maleszka and Stange (1997) cloned cDNAs expressed in the labial palps of Č. cactorum and found pronounced homology to OS-D (McKenna et al., 1994; Pikielny et al., 1994), a putative olfactory binding protein that is found exclusively in the funiculus of the antenna of Drosophila.

The process of stimulus transport poses an interesting problem, regardless of whether it occurs via pore tubules or via binding proteins: the densely appressed sheets in the dendritic lamellae in the labial palp organ leave a limited amount of extracellular space between them and it remains open whether diffusion of a carrier protein can be sufficiently rapid to account for the time course of the receptor responses. Also, in contrast to other olfactory stimuli CO₂ is readily soluble in water but also sufficiently lipid soluble to cross cell membranes. Therefore, it is possible that CO₂ molecules can reach any place on the dendritic membrane without involvement of a carrier mechanism.

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References


CARBON-DIOXIDE SENSING STRUCTURES


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