J Comp Physiol A (1986) 158:741–749

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

CO_2 sensitive receptors on labial palps of *Rhodogastria* moths (Lepidoptera: Arctiidae): physiology, fine structure and central projection

Franz Bogner, Michael Boppré, Klaus-Dieter Ernst, and Jürgen Boeckh
Universität Regensburg, Institut für Zoologie, Universitätsstrasse 31, D-8400 Regensburg, Federal Republic of Germany

Accepted March 24, 1986

Summary. The tips of the labial palps of *Rhodogastria* possess a pit housing uniform sensilla (Fig. 1), histologically characterized by wall-pores and receptor cells with lamellated outer dendrites (Fig. 2). The receptor cell axons project to glomeruli in the deutocerebrum (cf. Fig. 3) which are not innervated by antennal receptors. From their histology as well as from their central projection these sense organs are identical with palpal pit organs of other Lepidoptera (Lee et al. 1985; Kent et al. 1986; Lee and Altner 1986).

Physiologically, the palp-pit receptors respond uniformly; they are most excitable by stimulation with carbon dioxide (Fig. 6) while they exhibit relatively moderate responses to various odorants (Fig. 4). The responses to CO_2 (Fig. 7) show a steep dose-response characteristic. In ambient atmosphere (i.e., ca. 0.03% CO_2) the cells are in an excited condition already; the seeming 'spontaneous activity' exhibited in air is decreased if the preparation is kept under N_2 or O_2 or CO_2-free air (Figs. 7, 10). There is hardly any adaptation of the responses to continuous or repeated stimulation (Fig. 8). Perhaps CO_2 sensitivity is correlated with sensilla characterized by both wall-pores and lamellated dendrites. Pilot tests indicate that CO_2 perception might be widespread in the Lepidoptera (cf. Fig. 12), but the biological significance remains obscure.

Introduction

Behavioural tests have revealed that carbon dioxide has attractive and/or repellent effects for a variety of insect species (for refs. see Lacher 1967; Klingler 1958; cf. Discussion). The physiology of CO_2 sensitive exteroreceptors, however, has been only investigated in antennal sensilla of the honey bee (*Apis mellifica*; Lacher 1964; Stange and Diesendorf 1973; Stange 1974) and of a blowfly (*Lucilia cuprina*; Stange 1975) and on maxillary palps of mosquitoes (*Aedes aegypti*; Kellogg 1970). To date, no further physiological studies on CO_2 receptors in insects have been undertaken.

In our survey of olfactory receptors in *Rhodogastria* (Arctiidae) moths (Bogner and Boppré 1985, unpubl.) we discovered that cells in the pits of their labial palps are responsive to CO_2. The histology of the pit sensilla and their central projection have been studied in *Pieris rapae* (Pieridae) by Lee et al. (1985) and Lee and Altner (1986) and in *Manduca sexta* (Sphingidae) by Kent et al. (1986). These authors found a peculiar combination of sensillum wall structure and foldings of the dendritic outer segments of the receptor cells as well as bilateral projections of the receptor axons in special deutocerebral glomeruli (cf. Discussion). Hence, it seemed important for us to investigate *Rhodogastria*'s palp-pit organs in greater detail in order to obtain more information on CO_2 receptors. Indeed, insight into the physiology of such a morphologically unusual chemoreceptor and its central projection might contribute fundamentally to concepts of structure-function relationship in insect sensory systems (cf., e.g., Altner and Loftus 1985).

Material and methods

We investigated the labial palps of both sexes of *Rhodogastria* (Lepidoptera: Arctiidae). The moths were from our laboratory cultures originating from field-caught material from Kenya, East Africa, and maintained on a semi-artificial diet (Bergomaz and Boppré 1986). Species studied include *R. luteibarba* Hamp-

Table 1. Odorants tested at 10^{-2} concentration on palp-pit receptors. +: >25% increase, −: >25% decrease, 0: no effect on cell activity, compared to air control

Test compound	Resp.	Test compound	Resp.	Test compound	Resp.
Cyclopentanone	+	Acetic acid	−	Formic acid	0
Methyl-pentanone	+	Propanoic acid	−	Eugenol	0
Benzylalcohol	+	Butyric acid	−	α-Ionone	0
Octanol	+	3-methyl-butyric acid	−	Heptanol	0
Limonene	+	Hexanal	−	Geraniol	0
Citral	+	Butanal	−	HCl (pH = 1)	0
Fenchone	+	Pentanal	−	Hexane	0
Terpineol	+				
Acetic ester	+				
Propylacetate	+				
Benzylacetate	+				
Pentylcaproate	+				

son, *R. phaedra* (Weymer), and a yet undescribed species (*Rhodogastria sp.C*) named elsewhere (Boppré manuscript). *Rhodogastria* data figured in this paper were obtained from *Rhodogastria sp.C* exclusively.

Since degeneration experiments revealed clearer results with *Achaea lienardi* (Boisduval), this noctuid moth was employed for more detailed histological studies as well as for pilot and comparative physiological tests. The cultures of *Achaea* came from Kenya and were kept under the very same conditions as *Rhodogastria.*

Scanning electron microscopy (SEM). SEM was employed for studying the gross morphology of the palps in order to aid the electrophysiological investigation. Palps were deprived of covering scales by means of sticky tape, mounted on aluminium stubs with carbon cement, air-dried, then gold-coated in a sputtering chamber (Hummer II, Technics Inc.) and investigated with a Novascan 30 (Zeiss), operated at 15 kV. To allow a view into the pit organ, palpal tips were dissected prior to drying.

Histology. For investigation of the fine structure of the sensilla, terminal segments of palps of *Rhodogastria* pupae (< 10 h prior to eclosion) were cut, fixed in 5% glutaraldehyde in 0.1 mol/l Na-cacodylate buffer (pH = 7.3; buffer 185 mosmol) followed by postfixation with 2% OsO_4 in the same buffer solutions. For degeneration studies, the procedure of Boeckh et al. (1969) and Ernst et al. (1977) were employed. About 4 days after eclosion of the *Rhodogastria* and *Achaea* moths, the tips of their right labial palps were removed. 3 to 5 days after this operation the brains were excised, fixed in buffered glutaraldehyde after Sabatini et al. (1962) and postfixed in 2% OsO_4 in veronal-acetate buffer according to Palade.

Palps and brains were dehydrated and embedded in Epon (Luft) according to standard methods. Serial 3 μm sections were stained with azur II-methylene blue after Richardson et al. (1960), modified according to Andres (1966), and inspected under the light microscope. Thin sections of silver to gold interference colour were constrasted with uranylacetate and lead citrate and inspected with a Siemens Elmiscope IA.

Electrophysiology. For recording, 1- to 4-day-old moths were fastened in a perspex holder with their labial palps immobilized by means of tungsten hooks; the apical segment was slightly raised with plasticine to make the pit accessible for the electrode. Covering scales were taken off with tweezers.

Activity of single cells was recorded extracellularly with tungsten microelectrodes with tip diameters of ca. 1 μm. These were inserted into the pit cavity until cell responses could be recorded. The reference electrode was placed in the adjacent segment of the palp. Cell signals were fed into a conventional amplifying and recording system (cf. Sass 1976). Stimuli were delivered from a syringe olfactometer (Kafka 1970; Sass 1976) or from a tube system controlled by an electric valve with the outlets pointing towards the opening of the palpal pit.

Odour stimuli applied are listed in Table 1. All substances were of at least 99% purity. Except for HCl, they were diluted (in general to 10^{-2}) in liquid paraffin and 1 ml was filled into an 8 ml glass vial inside a disposable 25 ml syringe.

CO_2, N_2 and O_2 stimuli were provided from commercially available gas cylinders and delivered with a flow rate of 0.01 l/s via an electric valve through an empty syringe towards the palp tip. Different concentrations of CO_2 were obtained by mixing pressurized air with CO_2 from the cylinder through a mixer valve (Nigretti/Zambra, modified by C. Albers); the CO_2 content of the mixture was checked spectroscopically with a gas analyzer (Binos-IR; Leybold-Heraeus). As controls, freshly produced CO_2 ($Na_2CO_3 + HCl$), dry ice as well as air led through a saturated aqueous solution of $Ca(OH)_2$ were employed.

To test the effect of humidity, syringes were loaded with drip-wet filter paper. Warm and cold air for stimulation was obtained by heating or cooling syringes containing brass cylinders (20 × 20 mm); at the organ, the air stream had a temperature of 13 ° or 33 °C.

Pilot tests had revealed that the head space air of confined conspecifics elicited cell responses. Thus, syringes were used to house specimens of each sex of *Rhodogastria* as well as non-food plant material (e.g., leaves of *Brassica* sp. (Cruciferae), *Solanum tuberosum* (Solanaceae), *Gynura scandens* (Asteraceae)) to analyse their stimulatory effects. The foodplants of *Rhodogastria* are unknown. In order to determine whether responses were due to odours (e.g., pheromones or other allelochemicals) or primary products of the organisms' metabolism (e.g., CO_2, H_2O), we not only used living and freshly freeze-killed conspecifics but also the same of *Autographa gamma* (Lep.: Noctuidae), *Periplaneta americana* (Blattoidea) and *Locusta migratoria* (Orthoptera). These tests were qualitative since the status of each living organism's metabolism was not known.

In general, stimulus duration was 1 s and intervals between stimuli were at least 3 min. The preparations were kept in room air (i.e., ca. 0.03% CO_2) between stimuli. The temperature in the laboratory was 23 °C.

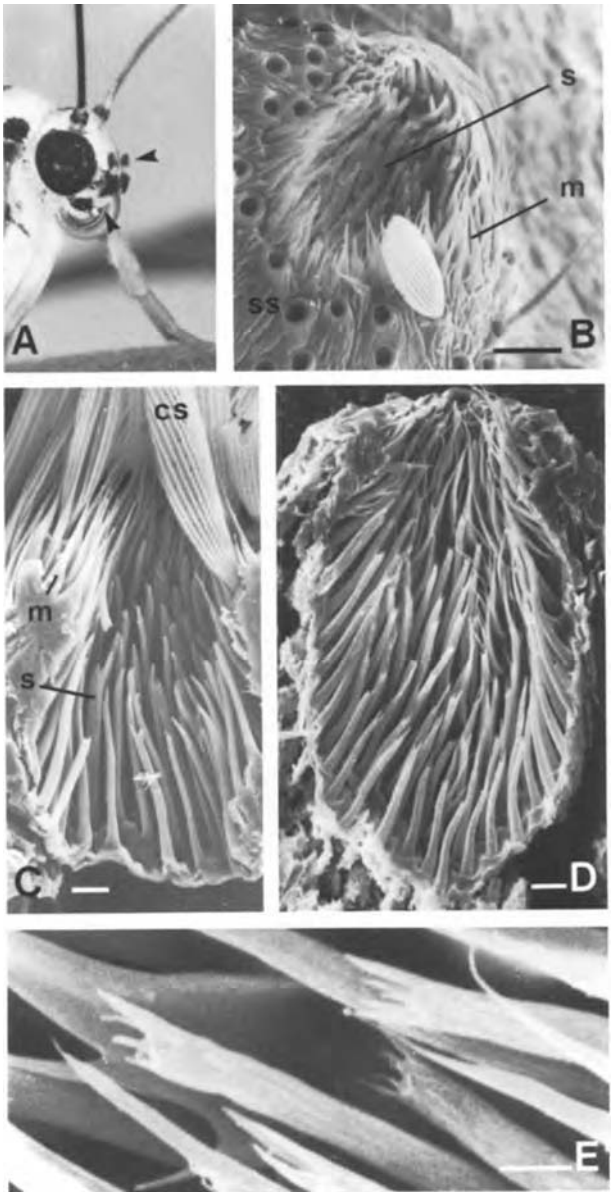

Fig. 1. Head of *Rhodogastria sp.C* showing labial palps (arrows) (**A**) and SEM micrographs of pit organs (**B–D**). **B** palp tip deprived of scales showing sockets of covering scales and the opening of the pit organ with microtrichia and sensilla; **C, D** longitudinal sections through pit cavity demonstrating the opening to be hidden by scales (**C**) and allowing a view on microtrichia (**C**) and sensilla (**C, D**). **E** forked tips of sensilla. *m* microtrichia; *s* sensilla; *cs* covering scale; *ss* scale sockets. Scale bars: **B** 100 µm; **C** 10 µm; **D** 10 µm; **E** 3 µm

Results

Morphology

The 3-segmented labial palps of *Rhodogastria* lie on each side of the proboscis (Fig. 1A) and are covered entirely by scales. Removal of scales reveals an opening near the tip of each palp, surrounded by pin-pointed microtrichia (Fig. 1B, C). It is of variable diameter (35 to 70 µm) and extends to a cavity, up to 150 µm deep and 80 µm wide. This pit organ is densely packed with smooth-walled sensilla of uniform appearance (Fig. 1B–D), which arise from prominent sockets. Each pit organ houses approximately 200 such sensilla (Fig. 1B–D). They are about 35 µm in length and 3.5 µm in diameter at their bases, each tapering and ending in folked tips (Fig. 1E).

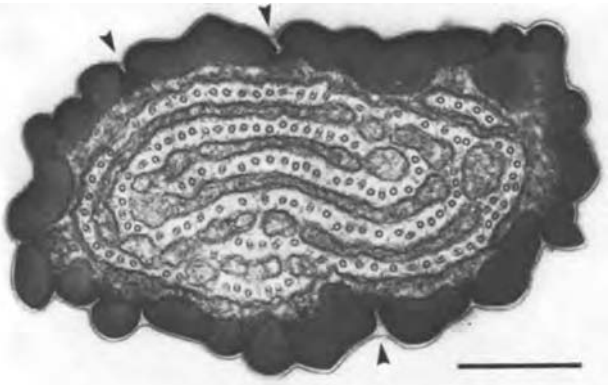

Fig. 2. Cross section of palp pit sensillum showing wall pores (arrows) and lamellated dendrite. Scale bar: 0.5 µm

Histology and neuroanatomy

Electron microscopical inspection of sections of the pit organ clearly reveals pores in the sensillum walls as well as lamellated outer sections of the receptor cell dendrites (Fig. 2), features described to be characteristic for the pit organ receptors of *Pieris* (Lee et al. 1985) and of *Manduca* (Kent et al. 1986). The same is true for other structural features so that a fully detailed description of the fine structure of the *Rhodogastria* sensilla needs not to be elaborated upon here.

The central projections of the receptors of *Rhodogastria* and *Achaea* are as described for the aforementioned and several other lepidopteran species (Kent et al. 1986; Lee and Altner 1986). Removal of the labial palp of only one side results in degeneration of processes in one distinct glomerulus located ventro-medially in each, the ipsi- as well as the contra-lateral deutocerebrum (Fig. 3A–D). Degenerated material was also visible in the suboesophageal part of the brain, indicating another projection area of the palps. If antennae were removed, degenerated material was found in all glomeruli of the antennal lobe except in the above mentioned distinct one.

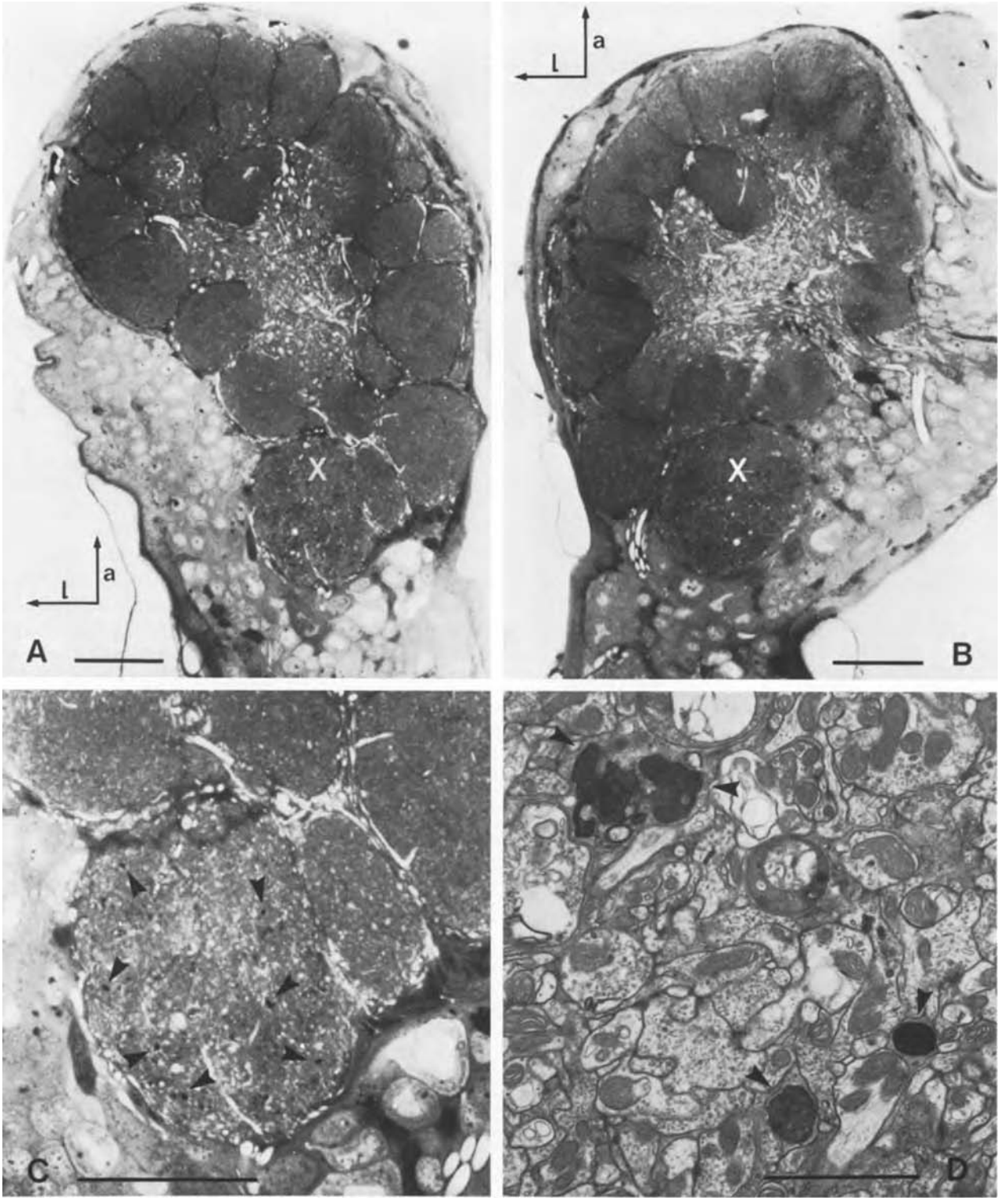

Fig. 3A–D. Palpal projection in deutocerebral glomeruli in *Achaea lienardi*. Ipsilateral (**A**) and contralateral (**B**) antennal lobe in frontal plane showing glomerulus (X) with degenerated material; **C** Enlarged glomerulus (X) from **A**; **D** EM of glomerulus (X) from **B**. Arrows point to degenerated material. *a* anterior; *l* lateral. Scale bars: **A–C** 50 μm; **D** 2 μm

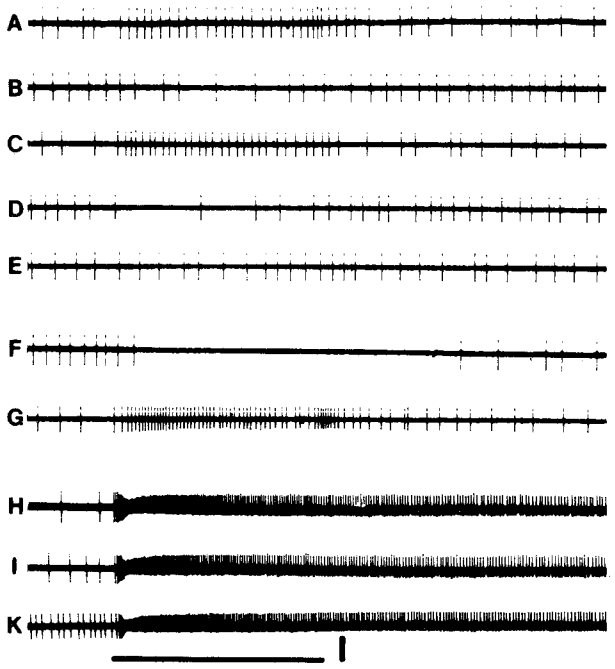

Fig. 4. Responses of a palp-pit receptor cell to ambient air at ambient temperature (**A**), to warmed (**B**) and cooled (**C**) air, to moistened + warmed (**D**) and moistened + cooled (**E**) air, to acetic acid (**F**) and to cyclopentanone (**G**), to human breath (**H**), to CO_2 from a cylinder (**I**) and to CO_2 made from sodium carbonate (**K**). Bars: stimulus duration (=1 s), 2 μV. (In **K**, the relatively high activity prior to stimulus onset is due to CO_2 leaking from the reaction vial.)

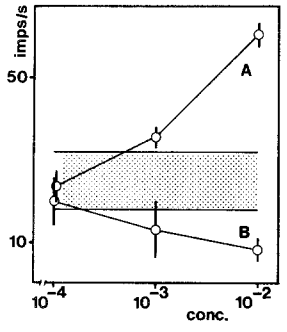

Fig. 5. Dose-response curves of responses of palp-pit receptor cells ($n=6$) to stimulation with cyclopentanone (*A*) and acetic acid (*B*). Bars: standard deviation. Dotted area: range of activity to control stimuli (air)

Electrophysiology

Data were evaluated from 21 recordings of 16 specimens. In the great majority of recordings, impulses of ca. 2 mV amplitude from only one sensory cell were detected. In two cases, a second element with a spike amplitude below 200 μV responded in the same manner as did the other. No other differences between recordings were apparent.

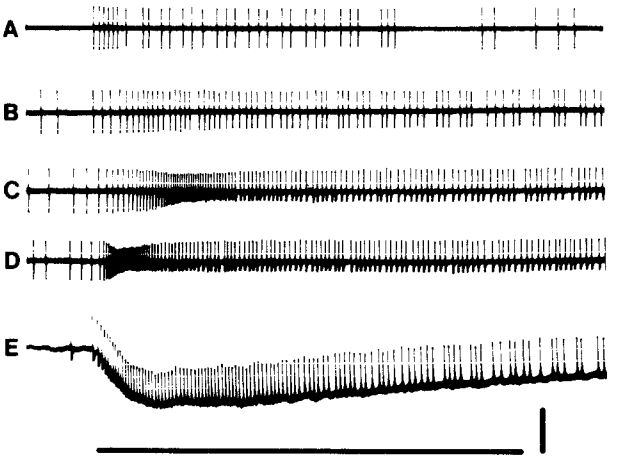

Fig. 6 Responses of a palp-pit receptor cell to ambient air (**A**) and to different concentrations of CO_2 (**B–E**); **B** 0.1%; **C** 1.0%; **D** 10.0%; **E** 5% (unfiltered recording). Bars: stimulus duration (=1 s), 2 μV

In room air, cell activity was about 13 imps/s and was only slightly affected by puffs of air from a syringe (i) at room temperature (Fig. 4A, cf. Fig. 7), (ii) warmed or cooled (Fig. 4B, C) or (iii) with altered humidity (Fig. 4D, E). Odorants either increased, decreased or failed to influence cell activity (cf. Table 1, Fig. 4F, G). Dose-response characteristics for cyclopentanone and acetic acid are provided in Fig. 5.

While responses to such odorous substances did not exceed 75 imps/s (e.g., undiluted cyclopentanone), up to 120 imps/s were elicited by breathing and blowing upon the preparation as well as by CO_2 stimuli (Fig. 4H–K). Details of the dose-response characteristics of 11 cells to stimulation with CO_2 are shown in Figs. 6 and 7. The time-course of the response showed a marked peak followed by a very steady plateau, e.g., for 10% CO_2 the values were 300 imps/s (i.e., 15 spikes in the first 50 ms of stimulation) and 80 imps/s, respectively. The plateau remained strikingly constant during sustained stimulation (Fig. 8); no decrease was apparent even after continuous stimulation for 5 min nor did repetitive stimulation reveal fatigue (Fig. 8). This held for both high and low concentrations.

Identical effects to CO_2 from the commercial cylinder were obtained with freshly produced CO_2 and from dry ice (cf. Fig. 4I, K).

Sequential stimulation with CO_2 or inhibitory or stimulatory odorants, respectively, demonstrated that the very same cells are affected by these stimuli (cf. Fig. 9). While inhibitory odours (e.g., acetic acid) diminished responses to CO_2 drastically (Fig. 9B), stimulatory ones (e.g., cyclopen-

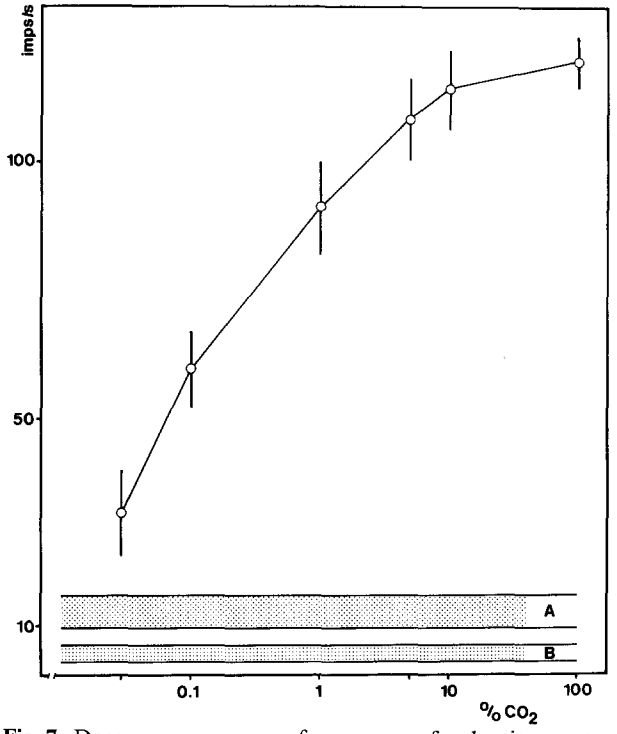

Fig. 7. Dose-response curve of responses of palp-pit receptor cells ($n=11$) to stimulation with CO_2, and ranges of spontaneous activity of preparation kept in room air (*A*) or blown with nitrogen (*B*). The value for 0.03% CO_2 was obtained by stimulation with ambient air

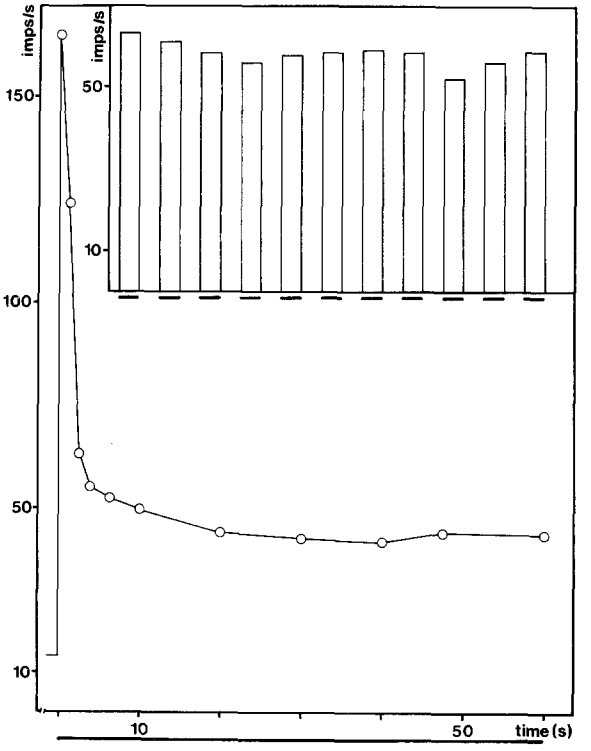

Fig. 8. Spike frequencies during continuous (main diagram) and repeated (inset; for 1 s (bars) with 1 s intervals) stimulation with 1% CO_2. All data points reflect spike numbers during 1 s. Thick bars: stimulus duration

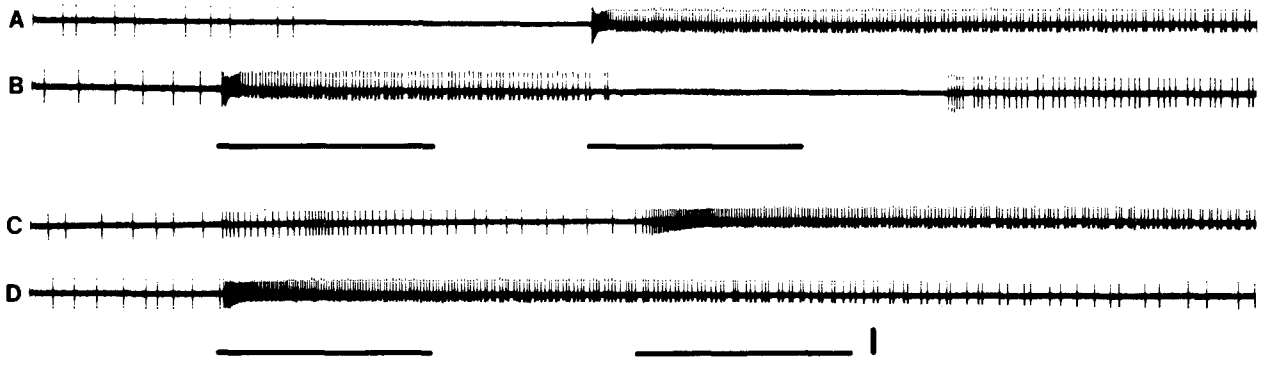

Fig. 9. Responses of a palp-pit receptor cell to acetic acid (10^{-2}) followed by 10% CO_2 (**A**) and vice versa (**B**), and to cyclopentanone (10^{-2}) followed by 10% CO_2 (**C**) and vice versa (**D**). Bars: duration of stimuli (=1 s), 2 mV

tanone) did not influence cell activity increased by CO_2 (Fig. 9D).

Puffs of N_2 or O_2 as well as CO_2-free air caused a decrease of the cell activity (cf. Fig. 10A, C, respectively); when either of these gases was delivered continuously (Fig. 10B), a steadily small activity (4.0 ± 1.8 imps/s), significantly smaller than in room air (12.6 ± 1.9 imps/s), was the result (cf. Fig. 7). Warming or cooling CO_2-free air had no effects.

Responses to head space air of plant leaves or insects confined in syringes were highly dependent upon time of confinement and state of the material. Living insects and fresh leaves confined for some time elicited strong responses (Fig. 11A–E, I) independent of species. Freshly confined insects evoked lower activity (Fig. 11F). Insects or leaves deep-frozen in the syringe and brought to room temperature prior to use elicited responses hardly different from those to air puffs (Fig. 11G, H, K).

Results of physiological pilot experiments with *Achaea* are consistent with the findings obtained with *Rhodogastria* (Fig. 12).

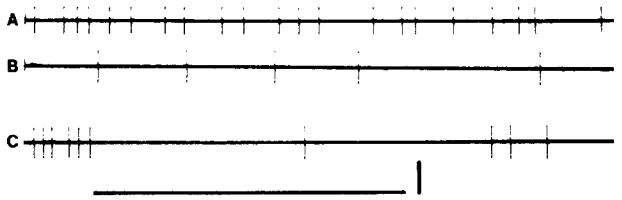

Fig. 10. Activity of a palp-pit receptor cell kept under ambient air (**A**), nitrogen (**B**) and stimulated with puff of nitrogen (**C**; bar: stimulation = 1 s)

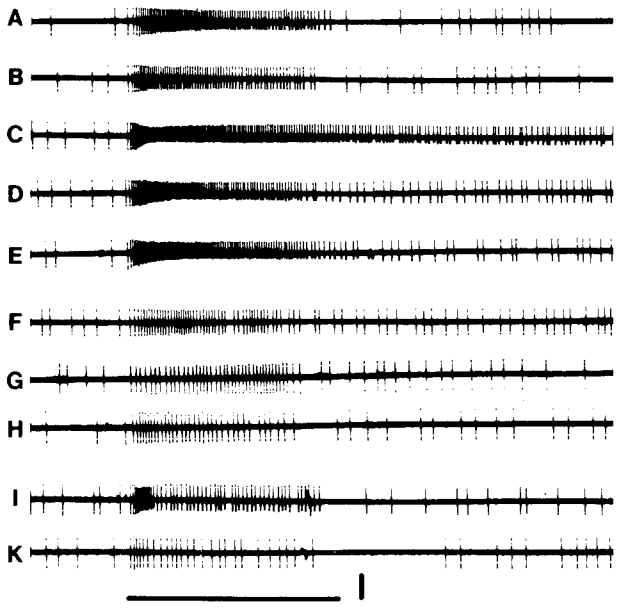

Fig. 11. Responses of a palp-pit receptor cell to stimulation with head space air of living specimens of *Locusta* (**A**), *Periplaneta* (**B**), *Autographa* (**C**), a male (**D**) and a female (**E, F**) of *Rhodogastria sp. C*, of a dead female *Rhodogastria sp. C* (**G**), of fresh leaves of *Brassica* sp. (**I**) and of previously frozen leaves of *Brassica* sp. (**K**). For comparison: stimulation with ambient air (**H**). Note that specimens were confined for 30 min (**A–E, I**) or 60 min (**G, K**) or 1 min only (**F**).Bars: duration of stimulus (=1 s), 2 mV

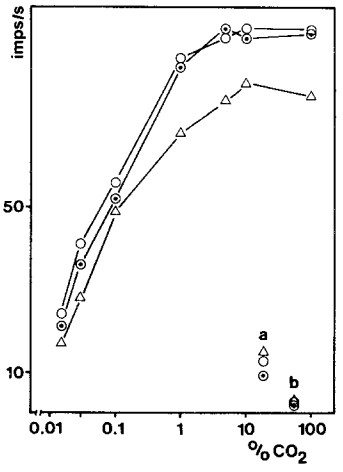

Fig. 12. Dose-response curve of responses of three palp-pit receptor cells of *Achaea* to stimulation with CO_2, and their activity when the preparations were kept in room air (*a*) and under an O_2 stream (*b*). Value for 0.03% CO_2 was obtained by stimulation with ambient air that for 0.015% CO_2 with ambient air diluted with O_2 (1:1)

Discussion

Receptor cells with lamellated outer dendrites have been found primarily in poreless sensilla and were considered to serve as sensory structures for detecting changes in either humidity or temperature (e.g., Loftus and Corbière-Tichané 1981; for reviews see Altner and Prillinger 1980; Steinbrecht 1984; Altner and Loftus 1985). In some cases, however, lamellated outer dendrites have also been observed in wall-pore sensilla, suggesting an olfactory function (for refs. see Lee et al. 1985). Evidence for a chemoreceptive role of such peculiar receptor structures has been advanced by the results of an electrophysiological exploration with *Pieris* (Lee et al. 1985, see below).

Our morphological and histological investigations with *Rhodogastria* clearly reveal that the moths' labial palp pit-organs with their sensilla and underlying receptor cells are in all aspects of fine structure and arrangement so similar to those described for *Pieris* that they can be regarded as at least structurally homologous. With respect to functional aspects, however, our physiological findings add a new facet to the discussion.

The sensory cells in the labial pit organ of *Rhodogastria* and *Achaea* are chemoreceptors sensitive to CO_2. The decrease of their activity in ambient air seen under N_2 and O_2 is not due to inhibitory effects of these gases because CO_2-free air causes the very same low activity (cf. Figs. 7, 10) but rather demonstrates that at atmospheric values of CO_2 the cells are in an excited state. This and the dose-response characteristics (see also Fig. 12) clearly indicate that their activity is indeed influenced by this compound under natural conditions. None of the other chemicals tested were found to be such powerful stimuli, and it appears that the palp-pit receptors are more sensitive to changes of CO_2 concentration than to those of odorants. It requires further data to clarify the physiological effects of odorants,but all this nevertheless justifies the view that the palp pit receptors of our moths are CO_2 receptors having only a secondary sensitivity for odorants. Different from many other chemoreceptors (cf., e.g., Kaissling 1971), the stable plateau under continuous stimulation would permit precise monitoring of the environmental CO_2 levels.

The responses of the *Rhodogastria* and *Achaea* receptors are broadly similar to those of the antennal CO_2 receptors studied in the honeybee (Lacher 1964; Stange and Diesendorf 1973; Stange 1974, 1975) and the maxillary palpal CO_2 receptors of the mosquito *Aedes* (Kellogg 1970). They differ from those, however, in that several odorants have been found to be effective stimuli in our moths.

From their histology, the palp-pit receptors of *Rhodogastria* appear identical to those of *Pieris*. Their physiological identity must nevertheless remain an open question because Lee et al. (1985) did not test for CO_2 as a *Pieris* stimulus; however, several aspects can be established. As we did with *Rhodogastria*, Lee et al. (1985) with *Pieris* demonstrated only moderate responses to stimulation with such compounds as esters. Their results from natural stimuli (head space air from foodplants and conspecifics) are also similar to ours, but in the light of our own findings on CO_2 sensitivity we would question their interpretation that this was due to 'natural complex flavours'. Instead, the CO_2 emitted by plants and insects may have been the primary causative stimulus. Our comparison of excitatory effects of head space air of different species, different sexes, as well as of dead and living specimens, demonstrates that this requires further investigation with *Pieris*.

Although a final understanding of structure-function relation must await further data, our findings support the idea that CO_2 sensitivity might be generally correlated with lamellated dendrites in wall-pore sensilla. Indirect evidence for this hypothesis comes from reports on Diptera. On the one hand, Kellogg (1970) on electrophysiological evidence discerned three types of receptor cells in the sensilla of *Aedes* maxillary palps, one of which responded to CO_2. On the other hand, McIver (1972) characterized these pegs to contain three dendrites, one of which had a lamellated outer segment. Such a correlation has recently also been forwarded by McIver and Siemicki (1984) who compared the occurrence of receptors with lamellated dendrites and hematophagy in Diptera. Hence, with respect to the hypothesis a further survey appears highly rewarding of additional species possessing wall-pore sensilla with lamellated dendrites or CO_2 receptors for their physiology and histology (cf., e.g., Lewis 1971; Hechler and Rühm 1983; Klingler 1958).

For the central projections of the axons of the labial pit-organ in *Rhodogastria* and *Achaea*, our degeneration studies confirm the findings of Kent et al. (1986) and Lee and Altner (1986) for *Manduca*, other moths and *Pieris*. Although in our case no label with cobalt or Lucifer Yellow was applied to reveal the exact course of the axons in the brain, the degenerations in the suboesophageal part of the brain and in a ventro-medially located deutocerebral glomerulus on each side are clearly recognized. This is another example for transganglionic primary projection of chemosensory organs from a head appendage as was found for the first time in *Locusta migratoria* and *Periplaneta americana* by Ernst et al. (1977).

According to prior results (Boeckh et al. 1969; Ernst et al. 1977), it can be assumed that the deutocerebral glomeruli with degenerated profiles represent the termination regions of the ablated sense organ. It is not apparent that antennal inputs reach these glomeruli, and on these grounds we are inclined to ascribe this glomerulus an input for the labial pit organ and thus for processing of information on CO_2. That this is the exclusive role of this glomerulus remains an open question since there are other possible inputs to the deutocerebrum which have not been examined including those from the proboscis. It might be interesting to see, however, if there is – second to the sexually dimorphic macroglomerular complex – another glomerulus in the lepidopteran brain characterized as the distinct projection area for a specific set of chemoreceptors separated from the major inputs to the deutocerebrum from other head appendages.

We have not investigated whether the degenerated material in the suboesophageal ganglion represents solely the tract between the palps and the deutocerebrum or whether it also contains terminals.

The biological significance of CO_2 sensitivity is well established for a wide range of haematophagous arthropods, including not only insects but ticks and mites too, and root-feeding insects where CO_2 plays an important role in host seeking behaviour (for refs. see, e.g., DeFoliart and Morris 1967; Hocking 1971; Friend and Smith 1977; Klingler 1958; Gillies 1980) and for hive ventilation in *Apis* (Seeley 1974). It also seems reasonable that CO_2 reception would be important for orientation of saprophagous insects. With respect to Lepidoptera, however, the biological function(s) served by CO_2 perception remains entirely open to speculation. The non-adapting responses of the receptors to 'physiological' concentrations of CO_2 makes them good candidates for monitoring changes in the CO_2 content of the immediate environment (be those caused by other animals such as conspecifics and predators or by foodplants) or the less localized changes in the general environment. As with other environmental features (humidity, temperature, light), CO_2 is a ubiquitous stimulus, but compared to the other modalities we lack knowledge of spatial and/or temporal gradients in the CO_2 concentration of the 'microclimate', and are

thus left without an obvious functional explanation for CO_2 sensitivity in Lepidoptera. Perhaps, CO_2 detection is an attribute which remains to be discovered in a far greater range of arthropods than has been hitherto suspected.

Acknowledgements. This study was supported by the Deutsche Forschungsgemeinschaft (SFB4-B3/B6). We are also grateful to M. Bairov for rearing the moths and to C. Albers for providing the mixer valve and the gas analyzer. We are indebted to A.W.R. McCrae for his efforts in revising the manuscript linguistically and to him as well as to H. Sass and K.E. Kaissling for stimulating criticism.

References

Altner H, Loftus R (1985) Ultrastructure and function of insect thermo- and hygroreceptors. Annu Rev Entomol 30:273–295

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

Andres KH (1966) Über die Feinstruktur der Rezeptoren an Sinneshaaren. Z Zellforsch 75:339–365

Bergomaz R, Boppré M (1986) A simple instant diet for rearing several Arctiidae and a variety of other moths. J Lep Soc (in press)

Boeckh J, Sandri C, Akert K (1969) Sensorische Eingänge und synaptische Verbindungen im Zentralnervensystem von Insekten. Experimentelle Degeneration in der antennalen Sinnesbahn im Oberschlundganglion von Fliegen und Schaben. Z Zellforsch 103:429–446

Bogner F, Boppré M (1985) A special olfactory receptor for alkaloid perception in adult Lepidoptera. Verh Dtsch Zool Ges 78:281

DeFoliart GR, Morris CD (1967) A dry ice-baited trap for the collection and field storage of hematophagous Diptera. J Med Entomol 4:360–362

Ernst K-D, Boeckh J, Boeckh V (1977) A neuroanatomical study on the organization of the central antennal pathways in insects. II. Deutocerebral connections in *Locusta migratoria* and *Periplaneta americana*. Cell Tissue Res 176:285–308

Friend WG, Smith JJB (1977) Factors affecting feeding by bloodsucking insects. Annu Rev Entomol 22:309–331

Gillies MT (1980) The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae): a review. Bull Ent Res 70:525–532

Hechler J, Rühm W (1983) Licht- und elektronenmikroskopische Untersuchungen über das ‚Lutz'sche Organ' der Simuliiden (Diptera, Simuliidae). Mitt Hamburger Zool Mus Inst 80:231–249

Hocking B (1971) Blood-sucking behavior of terrestrial arthropods. Annu Rev Entomol 16:1–26

Kafka WA (1970) Molekulare Wechselwirkungen bei der Erregung einzelner Riechzellen. Z Vergl Physiol 70:105–143

Kaissling KE (1971) Insect olfaction. In: Beidler LM (ed) Chemical senses 1. Olfaction. (Handbook of sensory physiology, vol IV/1) Springer, Berlin Heidelberg New York, pp 523–533

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

Kent KS, Harrow ID, Quartararo P, Hildebrand JG (1986) An accessory olfactory pathway in Lepidoptera: the labial pit organ and its central projections in *Manduca sexta* and certain other sphinx moths and silk moths. Cell Tissue Res (in press)

Klingler J (1958) Die Bedeutung der Kohlendioxid-Ausscheidung der Wurzeln für die Orientierung der Larven von *Otiorrhynchus sulcatus* F. und anderer bodenbewohnender phytophager Insektenarten. Mitt Schweiz Entomol Ges 31:205–289

Lacher V (1964) Elektrophysiologische Untersuchungen an einzelnen Rezeptoren für Geruch, Kohlendioxyd, Luftfeuchtigkeit und Temperatur auf den Antennen der Arbeitsbiene und der Drohne (*Apis mellifica* L.). Z Vergl Physiol 48:587–623

Lacher V (1967) Verhaltensreaktionen der Bienenarbeiterin bei Dressur auf Kohlendioxid. Z Vergl Physiol 54:75–84

Lee J-K, Altner H (1986) Primary sensory projections of the labial palp-pit organ in *Pieris rapae* L. (Lepidoptera: Pieridae). Int J Insect Morphol Embryol (in press)

Lee J-K, Selzer R, Altner H (1985) Lamellated outer dendritic segments of a chemoreceptor within wall-pore sensilla in the labial palp-pit organ of the butterfly, *Pieris rapae* L. (Insecta, Lepidoptera). Cell Tissue Res 240:333-342

Lewis CT (1971) Superficial sense organs of the antennae of the fly, *Stomoxys calcitrans*. J Insect Physiol 17:449–461

Loftus R, Corbière-Tichané G (1981) Antennal warm and cold receptors of the cave beetle, *Speophyes lucidulus* Delar., in sensilla with a lamellated dendrite. I. Response to sudden temperature change. J Comp Physiol 143:443–452

McIver SB (1972) Fine structure of pegs on the palps of female culicine mosquitoes. Can J Zool 50:571–576

McIver SB, Siemicki R (1984) Fine structure of pegs on the maxillary palps of adult *Toxorhynchites brevipalpis* Theobald (Diptera: Culicidae). Int J Insect Morphol Embryol 13:11–20

Richardson KC, Jarett I, Finke EH (1960) Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Technol 35:313–323

Sabatini DS, Bensch K, Barnett RJ (1962) New fixative for cytological and cytochemical studies. In: Breese SS (ed) 5th Intern Congr Electron Microscopy 2. Academic Press, London, pp L3–L4

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

Seeley TD (1974) Atmospheric carbon dioxide regulation in honeybee (*Apis mellifera*) colonies. J Insect Physiol 20:2301–2305

Stange G (1974) The influence of carbonic anhydrase inhibitor on the function of the honeybee antennal CO_2-receptors. J Comp Physiol 91:149–159

Stange G (1975) Linear relation between stimulus concentration and primary transduction process in insect CO_2-receptors. In: Denton DA, Coghlan JP (eds) Olfaction and taste V. Academic Press, New York, pp 205–209

Stange G. Diesendorf M (1973) The response of the honeybee antennal CO_2-receptors to N_2O and Xe. J Comp Physiol 86:139–158

Steinbrecht RA (1984) Chemo-, hygro- and thermoreceptors. In: Bereiter-Hahn J, Matoltsy AG, Richards KS (eds) Biology of the integument, vol 1, Invertebrates. Springer, Berlin Heidelberg New York Tokyo, pp 523–533

Note added in proof. Recently, *Pieris brassicae* L. became available and electrophysiological studies showed that their palp-pit receptors exhibit the very same characteristics as those of *Rhodogastria*. Details will be reported elsewhere.