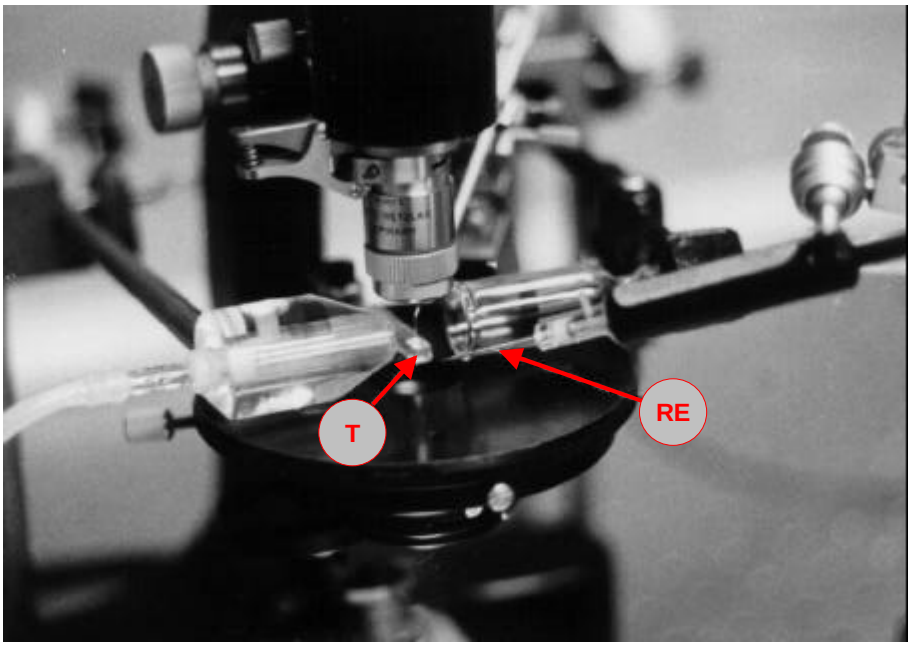
V. RESPONSE CHARACTERISTICS OF THE CO_2 RECEPTOR CELL



Experimental set-up for electrophysiological recording of single CO_2 receptor cells. The ant is mounted on a table (T) and the neural activity is measured with a recording electrode (RE).

Introduction

Neurons in sensory systems have specialized feature detection properties that promote the perception of biologically relevant cues. The design of an animals receptors limits what information the receptor cells can collect and relay for further processing (Alcock 1993). Thus, sensory physiology provides a basis for understanding how animals perceive and react to their environment.

In olfaction, sensory neurons translate some of the chemical properties of an odor stimulus into electrical impulses. The sensory neurons convey information about quality and concentration of odors, they can track pulsed signals and measure stimulus durations.

In insects, odor perception takes place on the antennae. Electrophysiological evidence was first provided by Schneider who recorded voltage changes between the base and the tip of antenna in dependency of an odor stimulus; a technique termed electroantennogram (EAG; Schneider 1957). This technique was originally developed in the silk moth *Bombyx mori* (Lepidoptera) and in the following years successfully used in investigations on pheromone perception in other Lepidopteran species. An EAG simultaneously records the summed receptor potential of sensory neurons located in several sensilla. Thus, the recorded signal depends not only on stimulus intensity but also on number and size of different receptor cells. A requirement for quantitative EAG's is a large number of sensory neurons that share the same stimulus specificity, as is the case in the pheromone receptor cells of Lepidopteran species. Therefore, quantitative response measurements at different stimulus intensities are possible only in some olfactorial systems.

Activity recordings from single olfactorial sensory neurons were first performed by Boeckh only five years after EAG had been established (Boeckh 1962). By inserting sharp tungsten or filled glass electrodes (filled with electrolyte) into the sensillum he was able to measure action potentials generated from neurons in a single sensillum. The technical demands for single cell recordings are much higher than for EAG mainly because of the extremely small size of the investigated neurons. The somata of the sensory neurons measure only few microns and are densely packed in the antenna. Thus, the currents which generate the action potentials are very low and often hardly assessable. The recordings are extracellular, thus currents of action potentials which spread out electrotonically through the receptor lymph or via accessory cells (sheath cells) of the sensillum are measured. So far no intracellular recordings of sensory neurons in situ (in insect antennae) are possible.

The advantages of single cell recording in sensory physiology are obvious: by recording action potentials generated by single cells one can describe the information of a stimulus available to the animal. This is the information the animal can then act upon. Therefore, electrophysiology on sensory neurons contributes considerably to a better understanding of behavior and its constraints.

Sensory neurons for CO_2 perception

Extracellular single cell recordings of CO_2 receptor cells in arthropods have been performed in several species (recently reviewed by (Ziesmann 1996). The prevailing majority of sensory neurons have a working range of about 0-5% CO_2. Only in mosquitoes has a higher sensitivity been found and in honey bees some receptor cells have been shown to have a much lower sensitivity (Lacher 1964; Grant *et al*. 1995). Ambient air has a CO_2 concentration of about 0.05%. Under these conditions, neural activity lies between a few and 40 impulses (action potentials) per second.

In Hymenoptera, the activity of CO_2 receptor cells was first recorded by Lacher for the honey bee (*Apis mellifera*) (Lacher 1964). Later, different characteristics of the CO_2 response were investigated with antagonistic stimuli like N_2O and with a carbonic anhydrase inhibitor (Stange and Diesendorf 1973; Stange 1974; Stange 1975). For ants a qualitative electrophysiological study on CO_2 perception was carried out with the species *Lasius fulliginosus* (Dumpert 1972).

Biological systems normally do not receive precise information about absolute intensities. Rather, a common feature in the stimulus response in olfactory receptor cells as well as in other sensory systems is adaptation. Adaptation is defined as a reversible decrease in sensitivity of a receptor cell as a result of continuous or repeated stimulation (sensory fatigue). The ability to discriminate changing intensities over a wide range is enhanced by adaptation, but the ability to code absolute intensities is inevitably reduced. For social insects the knowledge about absolute CO_2 concentrations is essential because it plays a crucial role in the context of microclimatic control of the nest. However, neither behavioral nor electrophysiological studies have so far supplied information whether social insects are able to continuously assess the absolute CO_2 concentration.

The present study describes the response of CO_2 receptor cells of leaf-cutting ants to different CO_2 stimuli. The aim was to describe the working range of the CO_2 receptors and to analyze the temporal resolution of CO_2 perception in leaf-cutting ants. Since in social insects and particularly in the leaf-cutting ants exceptional demands for CO_2 perception are expected (for microclimatic control; see chapter one and two) the influence of long-term stimulation was examined.

Methods

Workers of *Atta sexdens rubropilosa* were obtained from the same laboratory colony as was used for the studies reported in chapters 3 and 4. Workers were collected from the feeding site, thus it is supposed that only foragers were investigated.

The electrophysiological recordings were made under optic control with a Leitz microscope equipped with a long distance objective (NPL-Fluotar L25/0.35). The total magnification was 250, and the illumination of the flagellum was from below with a standard microscope light.

Animals were fixed on a plastic holder with adhesive tape. The scapus was mounted with tungsten hooks and the flagellum was glued onto the holder with water soluble Tipp-Ex.

Electrolytically sharpened tungsten electrodes were used as indifferent and different electrodes. The indifferent electrode was inserted deep into the last flagellar segment, while the recording electrode was superficially inserted into the cuticle beside a visible hole of a sensillum coeloconicum or ampullaceum. By stimulation with temperature (microscope lamp), human breath and CO_2 the stimulus specificity was determined.

Later the different tungsten electrode was replaced by a glass electrode filled with 0.15 M KCl. The sharp tip glass electrodes (like a intracellular electrode) were prepared with a laser electrode puller (Sutter, Model P2000). The advantage of this different technique was a increased signal to noise ratio, while the disadvantage was that only few sensilla could be checked for CO_2 receptors, since the very tip of the electrode broke easily.

The headstage of an intracellular amplifier (A-M Systems, Neuroprobe Amplifier Model 1600) was mounted on a micromanipulator (Märzhäuser, HS6) and was used with tungsten and glass electrodes. The signal was filtered with 3 kHz low-pass, 60 Hz high-pass and amplified with a gain of 1000 (Kemo, VBF8). Data were digitalized with a sampling rate of 12 kHz (CED, 1401plus) and stored for analysis on a PC. For data analysis commercial software was used (CED, Spike2 V2.01). Optic and acoustic control of the signal was obtained with an oscilloscope (Gould, DSO1604) and a self-made audio monitor.

Threshold curve

Stimulation with increased CO_2 concentration was produced by injection of CO_2 into a continuous air flow over the flagellum. The laminar flow (about 7 cms^{-1}) with a flow rate of 1 $lmin^{-1}$ was adjusted with a flow meter (Progressive technology, UFR/B/1-1Y-A) and carried moistened air with a CO_2 concentration of 0.05% (±25 ppm) from pressure tanks. Injection of CO_2 was controlled with a magnetic valve (Lee Co, LFYX0500250AB) and the flow rate of injected CO_2 could be varied by changing the pressure (10-60 mbar). CO_2 concentrations up to 12% could be produced.

Stimulus duration was 3 s with an interstimulus interval of 10 s. The mean neural activity was measured during the last second before stimulation and during the second and third second of stimulation (tonic response). The intensity of the phasic response was assessed with the maximum mean frequency over 0.7 s (bin size). Mean frequency is defined by the number of action potentials per unit time (bin). For each neuron at least four repeated measurements were performed with each concentration and the mean response was calculated.

Temporal resolution

In order to determine the temporal resolution of the sensory neurons to changing CO_2 concentration a pulsed stimulation with 100 ms was used. The continuous air flow had a CO_2 concentration of 0.05% and stimulation was via a canula which was directed in close vicinity (<100 µm) onto the sensillum. The emerging gas jet had a velocity of 7.4 ms^{-1}. Stimulus intensity was 0.15% CO_2 (±75 ppm) Four pulses (phrase), separated by three seconds without stimulation (interphrase interval, IPI) and different pauses between the pulses (interstimulus interval, ISI) were used (see Fig.5.5).

Interstimulus intervals of 900, 400, 250, 150, and 25 milliseconds in successive phrases were used. The sequence of phrases were randomly chosen and computer controlled (with a sequencer script of the Spike program). The delay between stimulus onset and second action potential at peak frequency was measured for each ISI. Peak frequency is defined by the minimum time between two successive action potentials. Peak frequency always occurred between the first and second action potential during stimulation. Since the sensory neurons generated action potentials at the 'background' concentration of 0.05% CO_2 the first action potential during stimulation (which marked the beginning of peak frequency) cannot be counted with certainty as stimulus response. If stimulation occurs long after the last action potential the response is immediate, if stimulation occurs simultaneously with an action potential the period until the next action potential is generated is longer (see also next section). Using the second action potential at peak frequency as criterion this variation in response is reduced.

Delay of perception

In order to evaluate whether the duct of the sensilla ampullacea causes a delay of perception the delay between stimulus onset and response of the sensory neuron was measured. Natural variation in duct length between different sensilla was used and the times until the associated neurons responded to stimulation were compared. After recording the neural activity the lengths of the ducts were measured.

Since high stimulus intensities cause a fast neuronal response, in the beginning of the experiments a CO_2 concentration of 10% (±1%, obtained from pressure tanks) above a background concentration of 0.05% was used. Such tremendous changes in CO_2 concentration probably never occur in the natural environment of the ants, but they make it possible to evaluate minimum neuron response times. More likely in the biological range is an increase in CO_2 concentration to 0.15%. Therefore, this concentration was used in the second part of the experiments. The stimulation was coupled to the neural activity in order to reduce the variability in peak frequency and response delay.

Under stable neural activity (at background concentration) depolarization of the neuron leads to subsequent generation of action potentials. Stimulation induces a faster depolarization between action potentials. Thus, at the beginning of stimulation the period until the a action potential is generated depends on the period between last action potential before stimulation and stimulus onset. Stimulus onset is defined by the opening of the magnetic valve. After stimulus onset the CO_2 concentration at the sensillum increases rapidly but with an unknown time course. The time course of depolarization at the beginning of stimulation is also unknown. Thus, the peak frequency also can be different depending on the period between last action potential before stimulation and stimulus onset.

Therefore, the generated action potentials of the recorded neuron were used to trigger the stimulation (by a window discriminator, WPI, Model 121). Using this experimental design, stimulation always occurred with a fixed delay after an action potential.

In all experiments the stimulation was applied via a canula as described in the section above, always for 100 ms, and with an ISI of 5 s.

During electrophysiological recordings the sensillum was simultaneously stained with fluorescent dye DiI (MW 6000, Sigma) deluded in 1 µl of the electrolyte (0.15 M KCl) and filled first into the recording electrode. After the electrophysiological experiments the flagellum was cut in ringer solution and fixed with tungsten hooks onto a sylgard plate. The last flagellar segment was cut oblique, by hand with a razor blade and immediately investigated for the stained sensillum with an invert microscope (Zeiss, Axiovert 405P). The duct length of the stained sensillum was measured at the same microscope.

Adaptation

Using glass electrodes the neural activity could be recorded continuously over long periods (once more than three hours). The adaptation characteristics of the sensory neurons were investigated by continuos stimulation over one hour with a concentration of 1.2% CO_2. This concentration was obtained by mixing air with 10% CO_2 (±1%) from pressure tanks with air containing 0.05% CO_2.

Throughout the experiment the mean neural activity was measured for periods of 30 s at different times. Before stimulation: 10 minutes before (at the background concentration of 0.05% CO_2) and immediately before stimulation. During stimulation: After one minute and every 10 minutes during stimulation. After stimulation: 10 minutes after stimulation was terminated, thus at the background concentration of 0.05% CO_2.

Results

The CO_2 receptor cells of the leaf-cutting ants show a phasic-tonic response to an increased CO_2 concentration. The initial phasic response has a duration of less then one second followed by a tonic plateau. After termination of the stimulus the activity of the receptor cell is reduced for a period depending on the preceding stimulus intensity. Fig.5.1 shows an example of an extracellular recording during stimulation with an increased CO_2 concentration. The recorded voltage changes of the CO_2 receptor cells were in the range of 1 to 6 mV. These fast voltage changes last for about 1 to 2 ms and correspond to action potentials generated by the sensory neuron.

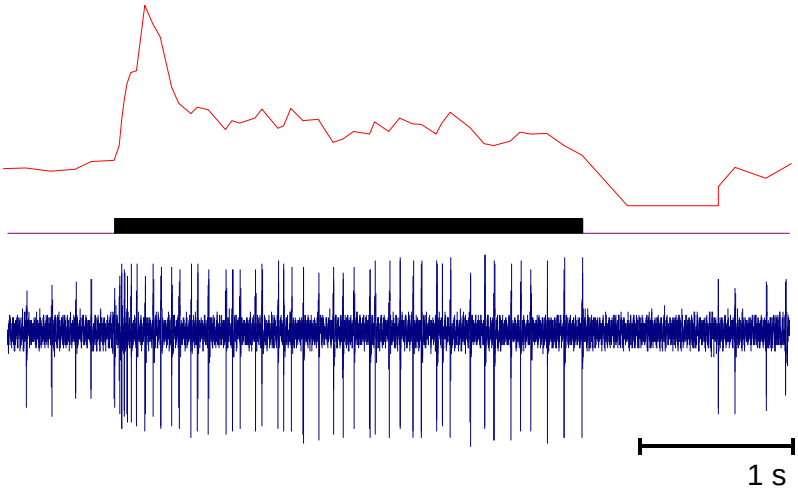


Fig.5.1 Extracellular recording of a CO_2 receptor cell (lower trace) and mean frequency of action potentials (upper trace; bin size 1 s). Bar in the middle indicates a stimulation during 3 s with a CO_2 concentration of 0.15% over a background concentration of 0.05% (before and after stimulation).

Threshold curve

Repeated stimulation with different intensities revealed that the response is an almost linear function to low stimulus intensities, whereas the response is proportional to the logarithm of high stimulus intensities. Fig.5.2 shows as an example the tonic response of a single receptor cell over stimulus intensities up to 12% CO_2. The relation between CO_2 concentration (c_{CO2}) and neuron response (n_a) was calculated using a logarithmic model as $n_a = a+b*\log(c_{CO2})$. For the sensory neuron described the tonic response was $n_a = 29.8+19.3*\log(c_{CO2})$ with R = 0.96 at stimulus intensities up to 7.4% CO_2.

At CO_2 concentrations higher than 7.4% the cell showed bursts of neural activity as shown in Fig.5.3. This response characteristic indicates a stimulus overload of the receptor cell and will be refered to as saturation in the following. Other cells were saturated at even lower concentrations.

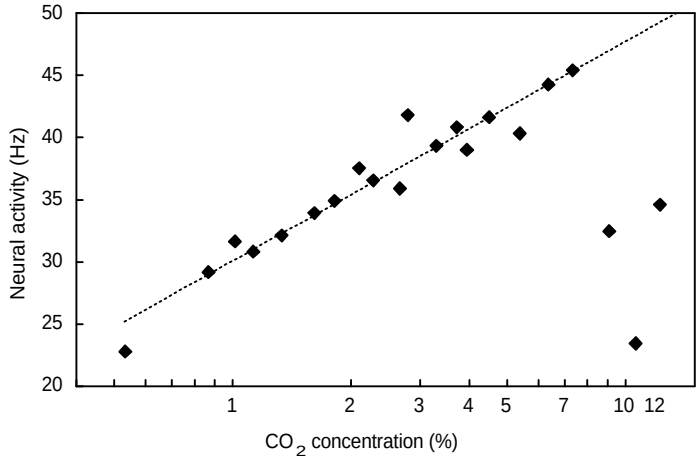


Fig.5.2 Neural activity (frequency of action potentials) vs. stimulus intensity of a single receptor cell. Note that at high stimulus intensities the cell activity does not increase any more. The log-regression fit the data well up to a concentration of 7.4% CO_2 (R = 0.96).

Fig.5.3 Bursts of neural activity indicating stimulus overload at a constant but high stimulus intensity of 10.6% CO_2.

The mean sensitivity of the CO_2 receptor cells was determined with recordings of eight different cells (each from a different ant). Sensory neurons which showed saturation by clear burst activity were excluded at this and higher stimulus intensities. Fig.5.4 shows the mean response of phasic and tonic part up to a stimulus intensity of 1.5%. A doubling of the concentration from 0.5% to 1% CO_2 causes an increase in activity by a factor of 1.3 for the tonic and the phasic response. For both phasic and tonic response the model ($n_a = a+b*\log(c_{CO2})$) fits well the data ($R > 0.98$) at stimulus intensities ranging from 0.15% to 1.52% CO_2. The tonic response (without burst activity) to a concentration of 10% CO_2 was measured for six different neurons and their mean activity was 40.7 Hz (SD ±13.1, n = 6).

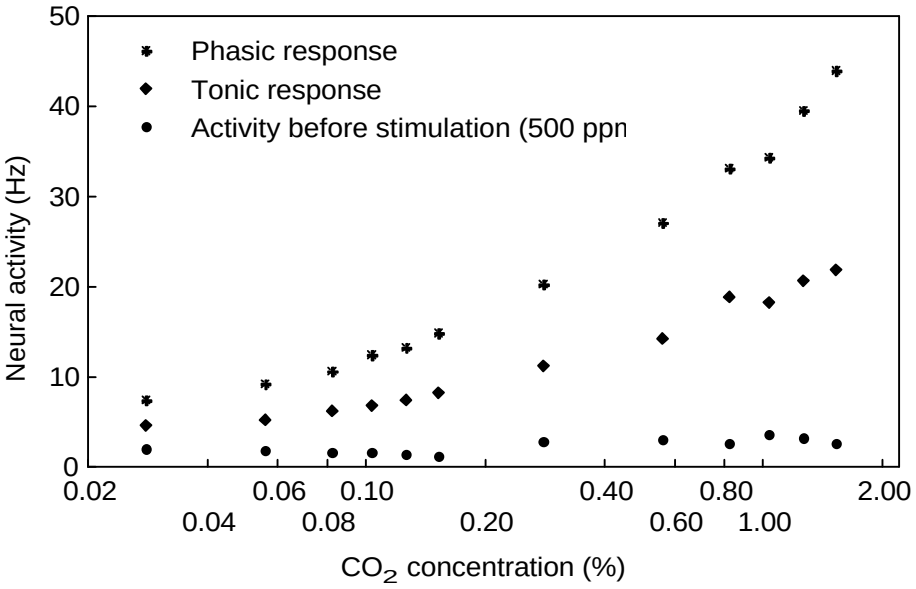


Fig.5.4 Threshold curve of the mean neural activity of eight receptor cells vs. stimulus intensities up to 1.5% CO_2.

Temporal resolution

Phasic response and reduced activity after stimulation result in strong emphasis on the signaling of changes in CO_2 concentration (contrast enhancement). The temporal resolution depends on different parameters of the stimulus as well as on the response characteristic of the neuron. The investigated sensory neurons showed a high variability in temporal resolution to the same fluctuations in CO_2 concentration. This variability seemed to be caused by the different response characteristic after stimulation. The period of reduced activity after stimulation was markedly different between sensory neurons. After a stimulation with 1% CO_2 for 3 s the period without generation of an action potential was 4.6 s (SD ±3.4, n = 6).

No attempt was made to quantify the differences in temporal resolution between neurons. Fig.5.5 shows as an example the activity of a single neuron to a pulsed stimulation with 0.15% CO_2 (against 0.05% CO_2). The bursts of activity separated by phases without generation of action potentials illustrate the effect of contrast enhancement.

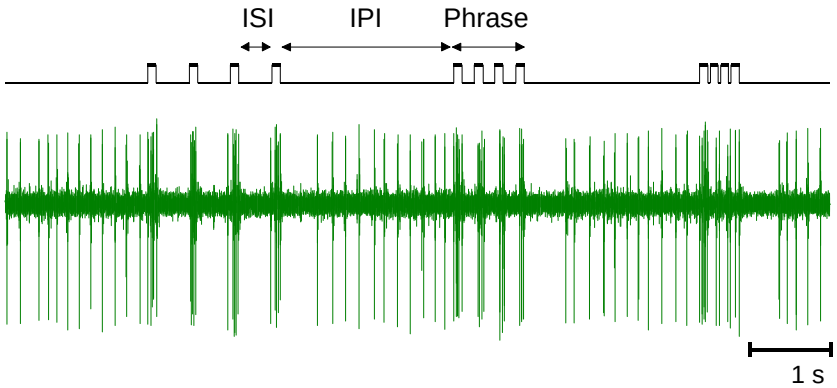


Fig.5.5 Example of contrast enhancement of changing CO_2 concentrations by the phasic response characteristic and the reduced neural activity after stimulation. Pulsed stimulation with 0.15% CO_2 (upper trace) with stimulus duration of 100 ms and ISI's of 400, 150, and 25 ms, respectively.

Even short interstimulus intervals (ISI of 150 ms) are mapped by the neuron. At very short ISI's (25 ms) the single pulses can barely be discriminated by the sensory neuron. However, already at an interstimulus interval of 150 ms the peak frequency is reduced and the response is delayed as it is shown in Fig.5.6. Thus, at this ISI the reduced activity caused by the previous stimulation and the phasic response character of the neuron are overlapping. Note that due to the stimulation at different phases between two action potentials the variability in peak frequency after a ISI of 3 s is much higher than after shorter ISI's.

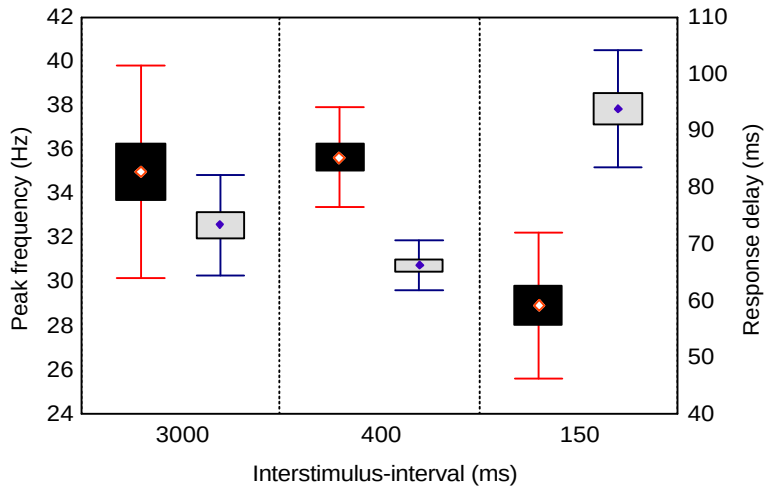


Fig.5.6 Reduction of contrast enhancement. Peak frequency (left; dark) is reduced and response delay (right; light) is increased at a ISI of 150 ms (stimulus duration of 100 ms).

Delay of perception

The response characteristic of the sensory neurons allows to map fast fluctuations of CO_2 concentrations as shown in the previous section. Therefore it is of interest whether perception of the stimulus is also fast and whether the ducts of the sensilla cause a delay in perception.

In order to determine the minimal delay between stimulus onset and response of the neuron a high stimulus intensity of 10% CO_2 (corresponding to a steep onset of stimulation) was used.

For three sensilla the delay of the response and the duct length of the simultaneously stained sensillum could be measured after stimulation. The total delay (delay of apparatus and delay of perception) was in the range of 22 to 25 ms. The apparative delay was measured with an anemometer and was in the range of 13 to 17 ms. Thus, the delay of perception to high stimulus intensities is less than 10 ms. For two sensilla the total delay was measured at stimulation with 0.15% CO_2. The total delay was longer at low than at high stimulus intensities as shown in Fig.5.7.

Note that the response delays for each duct length are mean values (with SD and SE) of repeated measurements (n = 12) of a single neuron. The stimulation with low intensity was coupled with the cell activity before stimulation (see methods) in order to reduce the variability of the response delay. Still the variability in response delay was high, e.g. 58 ms (SD ±13) for the neuron shown at the very right of Fig.5.7.

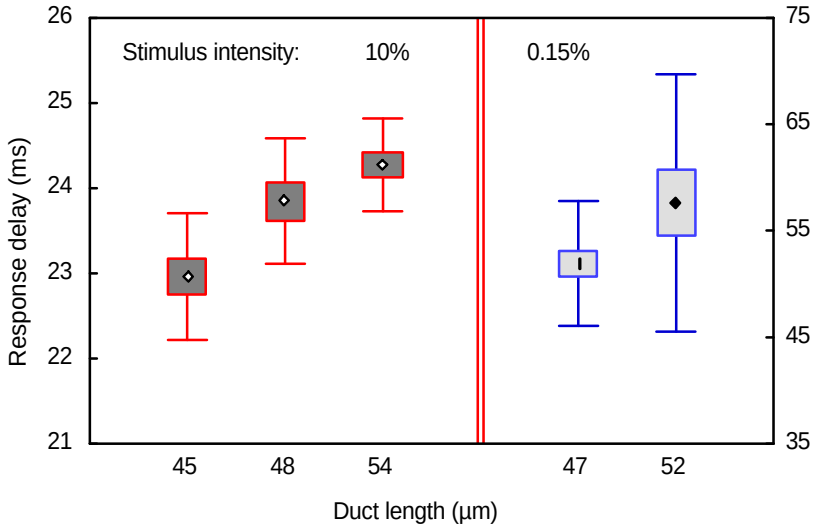


Fig.5.7 Delay of stimulus perception vs. duct length of different sensilla ampullacea to a high stimulus intensity of 10% CO_2 (left) and a low stimulus intensity of 0.15% CO_2 (right). To high stimulus intensities which correspond to a steep onset of stimulation the total response delay is short.

The duct length of the investigated sensilla differed in the range from 45 to 54 µm. The response delay differed between the investigated sensilla. However, due to experimental problems with the staining technique not enough data were obtained to allow a statistical analysis.

Adaptation

For the threshold curve the tonic response was calculated from the mean activity shortly after stimulus onset. In this part the tonic response is investigated during long-term, continuous stimulation. As shown in chapter one, such a situation occurs for the ants when they enter their nest. The question arises whether the ants are still able to assess the concentration of CO_2 after a long exposure to a constant high CO_2 concentration or whether no precise measurement is possible due to adaptation.

A continuos air flow with 1.2% CO_2 was blown over the antenna for one hour. Fig.5.8 shows the mean activity of nine receptor cells for the different phases of stimulation. The mean activity was 5.8 Hz (SD ±3.0) at a CO_2 concentration of 0.05% just before stimulation and 5.2 Hz (SD ±2.4) at the same concentration 10 min after stimulation which is not significantly different (T-test, $p = 0.67$). During stimulation the mean activity was 15.1 Hz (SD ±5.8) and significantly different from the activity at 0.05% CO_2 (T-test, $p < 0.01$). Note that there is a high variability of neural activity. This variability is caused by different levels of neural activity of the neurons and not by high variability of single neurons during stimulation.

The mean relative activity of all nine sensory neurons was 38% (SD ±8) at a concentration of 0.05% compared to the activity during stimulation with 1.2% CO_2. Mean neural activity did not change significantly between the different phases of stimulation (T-test, $p > 0.1$ for all combinations). Thus, no reduced mean activity was found during long-term stimulation. The sensory neurons do not adapt at a CO_2 concentration the ants might encounter inside the nest.

Therefore, the CO_2 receptor cells provide the ants with the necessary information to continuously measure the absolute CO_2 concentration.

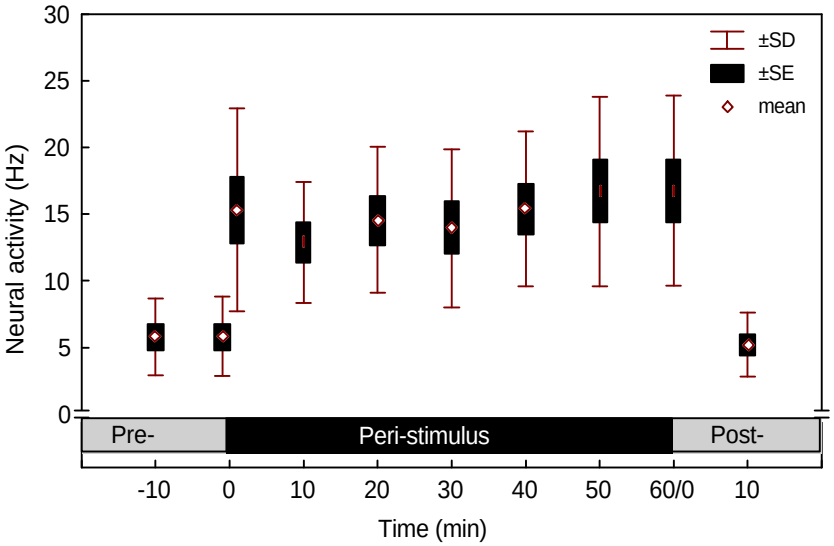


Fig.5.8 Neural activity vs. stimulus intensity during long-term stimulation over 1 hour with 1.2% CO_2. Mean value of nine cells with standard deviation and standard error shows that no adaptation occurs after 1 min up to the end of stimulation after 60 min.

Discussion

The CO_2 receptor cells of the leaf-cutting ant (*Atta sexdens*) show a phasic-tonic response to stimulation. This is the common response characteristic found in most sensory neurons including olfactory neurons of insects (Kaissling 1971). However, the relation between both characteristics can differ considerably as well as the time course of excitation. During the phasic response neural activity increases rapidly followed by a fast decrease to a tonic level. Tonic response is defined by a more or less stable neural activity. With the working range of 0 to 10% CO_2 the neurons respond to the highest concentration the ants might encounter in their natural environment. Contrast enhancement by phasic response accomplished with a reduced activity after stimulation results in the ability to track fast fluctuations in CO_2 concentration. Phasic-tonic response has also been described for the CO_2 receptor cells of the honey bee (Apis mellifera) (Lacher 1964). The most exciting finding of the present study is that the CO_2 neurons of the leaf-cutting ants do not adapt to continuous stimulation. This enables the ants to continuously monitor the actual CO_2 concentration.

Although the neurons of the sensilla ampullacea are located deep below the antennomere cuticle the recorded signals were large compared to recordings from different other chemosensory neurons. The reason for this might be the larger size of the neuron (as shown for the soma in the previous chapter) that might in turn result in larger currents generating action potentials. The good signal to noise ratio and the fact that in each recording only one single neuron was measured allowed a precise description of the response characteristic.

Phasic and tonic response were analyzed during short stimulations and revealed a logarithmic (log) relation between stimulus intensity and neural activity. In addition to this

traditional analysis long term recordings were performed in order to investigate how stable the tonic response actually is.

The kinetic primary processes of chemoreception (perireceptor events) have been described by models derived from odor perception in *Bombyx mori* (Kaissling 1972). The models are based on the Michaelis-Menten equation (Stryer 1988) in which stimulus molecules reversibly interact with acceptors forming a complex which ultimately leads to the generation of spikes. The complex can also decay releasing a product. Although a log relation results, the primary processes cannot explain the log response of sensory neurons completely. The log response of sensory neurons exists over a range of several log units of stimulus intensities. Since intracellular processes (second messengers) mediate receptor events, their kinetic also determine the neural activity. Low stimulus intensities are highly amplified by second messengers, while high stimulus intensities successively cause reduced amplification. A logarithmic relation between stimulus intensity and neural activity has the consequence that the neurons are sensitive over a large range of stimulus intensities.

The ability to discriminate between two CO_2 concentrations by means of the tonic response of the investigated neurons is fairly low. The neural activity at high CO_2 concentration (10%) is only a tenfold of the activity at lowest concentration (0.05%) but in the range also described for the tonic response of temperature receptors (Loftus 1968). Due to the logarithmic relation any detectable increase of CO_2 concentration depends on the background intensity.

Adaptation and different ranges of sensitivity of neurons with the same stimulus specificity often further increase the range of sensitivity for an animal (Davis 1984; Kaissling *et al*. 1987; Mustaparta 1990; Almaas *et al*. 1991). No markable differences in range of sensitivity was found between the investigated CO_2 receptor cells. Although the level of neural activity to the same stimulus intensity was different between receptor cells, the relative increase in activity to increased concentration (sensitivity) was similar between the neurons.

Between neurons, saturation levels differed. With the assumption that the level of saturation is achieved at same neural activity this variability in neuron response would increase the range of sensitivity. In contrast, no clear relation between neural activity and level of saturation was found. Neurons with high neural activity and saturation at high stimulus intensities as well as neurons inverse to that were found. A notable increase in sensitivity range might be obtained if many sensory neurons are available, this however is not the case for the sensilla ampullacea. Each antenna is equipped with a total number of only 20 to 30 sensilla ampullacea (see chapter four). For the CO_2 receptor cells of the honey bee different levels of activity have been described, but in contrast to the findings of this study, the sensitivity of the neurons differed greatly between neurons (Stange and Diesendorf 1973).

Saturation is characterized by burst activity. This kind of response differs from a phasic response, since the instant frequency (neural activity defined by the period between two successive action potentials) during a single burst does not change over time. The observed saturation was reversible, since tonic response activity recovered as soon as stimulus intensity was reduced. No damaging effects even after long lasting saturation were found. From six neurons a tonic response to a stimulus intensity of 10% CO_2 was obtained in 14 successful recordings of neural activity. The conclusion from these data is that the range of stimulus

intensity assessable to the ants is up to a concentration of 10%, even though many of the neurons show burst activity at this intensity.
The phasic response is characterized by a peak frequency shortly after stimulus onset followed by a reduction of instant frequency. In the 'threshold experiments' the stimulus was applied by injection into a continuous air flow, while in the 'temporal resolution' and 'delay' experiments it was applied by directly blowing air onto the sensillum. Thus, stimulus onset differed between these two types of experiments, since by diffusion or mixing of injected air in the continuous flow the stimulus onset might be changed. However, neuron response to stimulation was very fast in both experimental procedures. The delay of perception of the CO_2 receptor cells was less then 10 ms to a high stimulus intensity. The delay measurements could not be performed more precisely with the used apparatus. Unfortunately, only few data are available concerning the response delay of chemosensory neurons. In gustatory neurons, where stimulus onset can easily be controlled, the shortest delay reported is 2.5 ms (Hansen 1999). With the delay experiments in this study the idea was tested that the duct of the sensilla ampullacea causes a delay in perception.

The hypothesis was already formulated in the previous chapter: The temporal resolution of the receptor cell is modulated. The perception of changing CO_2 concentrations is delayed by diffusion through, and adsorption in the duct. Thus, the perception of short term fluctuations in CO_2 concentration is affected.

Comparative morphology and electrophysiology were carried out with the aim of showing the consequences of different duct length on perception. If CO_2 is adsorbed by the inner wall of the duct considerably larger delays are expected than would be caused by diffusion only. Adsorption would reduce the velocity of diffusion of CO_2 to the peg. Unfortunately, the method of measuring the duct length revealed to be unsuited, since in many cases the duct of the sensillum recorded from electrophysiologically could not be identified afterwards or was not complete any more after sectioning. This kind of experiments needs an improvement of the histological methods in order to be feasible in a reasonable time.

Since the measured delays are short and the variability, even with a coupling to the neural activity was high the data do not support the hypothesis that CO_2 is adsorbed to a greater extent at the wall of the duct until the stimulus is perceived. Obviously, there is no pronounced buffer capacity of CO_2 caused by the duct in the range which would have a biological significance. Besides the results of the delay experiments the temporal resolution of the CO_2 receptor cells does not support the hypothesis concerning duct function mentioned above.

The CO_2 receptor cells investigated in this study can track fluctuations in CO_2 concentration up to high pulse rates. Similar results have been reported for sensory neurons of *Periplaneta* (Blattodea) to general odors (Lemon and Getz 1997) and for pheromone neurons of *Antheraea* (Lepidoptera) to pulsed stimulation (Rumbo and Kaissling 1989). For CO_2 perception pulsed stimulation was used in experiments with *Helicoverpa* (Lepidoptera) which revealed a impressively high resolution concerning the discrimination ability of different intensities. The CO_2 receptor cells have been found to track pulses (ISI 0.4 s) down to differences of only 9 ppm above 350 ppm (Stange 1992; Stange and Wong 1993). In the investigated leaf-cutting ants the ability to discriminate small differences in CO_2 concentration

by the phasic response is much lower than in *Helicoverpa*. Although not measured with pulsed stimulation, this result can be derived from the threshold curve.
The biological relevance of the phasic response might be using klinotactic orientation as discussed in chapter three. Moving one antenna back and forth in a CO_2 gradient smaller differences in concentration can be detected than with a static measurement.

The second hypothesis presented in chapter four concerning the functional significance of the duct is: The duct is an accessory structure increasing the specificity of perception. CO_2 is selectively transported to the ampulla and/or non-CO_2 substances which can excite the receptor cell are prevented from reaching the peg.

This hypothesis is hard to examine since the duct cannot be removed experimentally. Selective transport of CO_2 through the duct faster than diffusion is hard to imagine, however, at the time when the electrophysiological recordings were made an exciting paper was published in a physics journal. Laser light was used to guide atoms through glass fibers (Renn *et al*. 1995; Service 1995). Since laser light can guide atoms, light might also guide the small CO_2 molecule through the duct. Therefore, a pilot experiment was performed and during pulsed stimulation with CO_2 a strong light was pulsed onto the sensillum (via a small optical fiber) in order to investigate whether CO_2 perception can be modulated by light. No effects were found, thus, irrespective of whether other light sources can modulate CO_2 perception no biological significance of this mechanism can be expected.

A function of the duct as a filter for stimulus quality is unlikely since beside CO_2 so far no substance has been found to excite the CO_2 receptor cell of honey bees and more than 30 substances have been tested. This stands in contrast to the CO_2 receptor cells of *Rhodogastria* (Lepidoptera) where several odors have been found to be effective stimuli (Bogner *et al*. 1986). This would mean that the duct either increases the specificity to 100%, or more likely the specificity is achieved later on at perireceptor events. However, in the same study a temperature dependency of the CO_2 receptor cells has been described above 32°C(Lacher 1964). For *Helicoverpa* even a compensation of temperature effects has been shown (Stange and Wong 1993). Isolation as function of the duct against environmental temperature fluctuations has been discussed in the previous chapter. The experiments in this study were performed at room temperature, however temperature dependency was tested with the heat of the microscope lamp (increase of about 5°C) and only a slight increase in neural activity was found.

Inhibitory effects of N_2O and Xe have been described for the phasic response of CO_2 receptor cells of honey bees in experiments which have been performed in order to analyze the perception mechanism (Stange and Diesendorf 1973). Only the phasic response is reduced while the tonic response character is unaffected. This inhibition has been suggested to occur via molecular ordering effects in the aqueous or lipid phases of either the cell membrane or the surrounding medium. However, this inhibition might also occur by molecular ordering effects in the duct, but a biological relevance of this kind of inhibition is unclear.

In contrast, the biological significance of the non-adapting character of the investigated CO_2 receptor cells is obvious. It is a prerequisite to measure the absolute concentration of CO_2 inside the nest.

A non-adapting response has been reported for several humidity receptors (Loftus 1976; Yokohari *et al*. 1982; Altner and Loftus 1985) and for temperature receptors (Loftus 1968; Davis and Sokolove 1975; Ehn and Tichy 1996). For both temperature and humidity a translation into a mechanical stimulus is discussed as perception mechanism (Tichy and Loftus 1996; Steinbrecht 1998). It is not far fetched that temperature and humidity receptors of leaf-cutting ants do not adapt as well.

No other olfactory sensory neurons beside the CO_2 receptors so far have been shown to have this kind of stable response to continuous stimulation. Unfortunately, reports about neural activity of olfactory sensory neurons show recordings and analyzed data only up to a few minutes. Therefore, quantitative data concerning olfactory adaptation are rare. In most cases the tonic response adapts almost from the beginning (with a different time scale than the phasic part and less intense) which was also shown for the CO_2 receptor cells of *Cactoblastis* (Lepidoptera) (Stange *et al*. 1995). This study shows that it is not inevitable that olfactory sensory neurons adapt to continuous stimulation.

In conclusion, the described response characteristic of the CO_2 receptor cells of leaf cutting ants are well suited to monitor the absolute concentration of the stimulus. The information, necessary for microclimatic control inside the nest concerning CO_2 is available for the ants.
In addition, with the phasic response of the neurons and reduced activity after stimulation short-term fluctuations in CO_2 concentration can be perceived. Measurement of differences in CO_2 concentration over time (at each sensillum) might be used for orientation purposes.