Differential effects of ambient temperature on warm cell responses to infrared radiation in the bloodsucking bug *Rhodnius prolixus*

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Zopf L.M, Lazzari CR, Tichy H. Differential effects of ambient temperature on warm cell responses to infrared radiation in the bloodsucking bug *Rhodnius prolixus*. *J Neurophysiol* 111: 1341–1349, 2014. First published January 8, 2014; doi:10.1152/jn.00716.2013.—Thermoreceptors provide animals with background information about the thermal environment, which is at least indirectly a prerequisite for thermoregulation and assists bloodsucking insects in the search for their host. Recordings from peg-in-pit sensilla and tapered hairs on the antennae of the bug *Rhodnius prolixus* revealed two physiologically different types of warm cells. Both types responded more strongly to temperature pulses produced by switching between two air streams at different constant temperatures than to infrared radiation pulses employed in still air. In addition, both warm cells were better able to discriminate small changes in air temperature than in infrared radiation. As convective and radiant heat determines the discharge, it is impossible for a single warm cell to signal the nature of the stimulus unequivocally. Individual responses are ambiguous, not with regard to temperature change, but with regard to its source. We argue that the bugs use mechanical flow information to differentiate between pulses of convective and radiant heat. However, if pulses of radiant heat occur together with a constant temperature air stream, the mechanical cues would not allow avoiding ambiguity that convective heat introduces into radiant heat stimulation. In this situation, the warm cell in the tapered hairs produced stronger responses than those in the peg-in-pit sensilla. The reversal in the excitability of the two types of warm cells provides a criterion by which to distinguish the combination of convective and radiant heat from the stimuli presented alone.

More than 70 years ago, it was discovered that warm cells occur in the same antennal sensillum; combination of temperature and infrared stimulation; electrophysiology; performance of warm cells; warm-blooded host

Most of the information concerning the physiological properties of arthropod thermoreceptors has been obtained from studies of cold cells whose rate of discharge increases during cooling and decreases during warming. Warm cells that display an increase in the discharge rate during warming and a decrease during cooling have not been found as often as cold cells. It may be that they are fewer in number or for the most part so small that they tend to escape sampling with the usual electrophysiological technique.

The first investigation of an insect warm cell came from the peg-in-pit sensilla on the antennal tip of the mosquito *Aedes aegypti* (Davis and Sokolove 1975). Later it was demonstrated that on the antennae of the cave beetle *Speophyes lucidulus* warm cells are associated with slowly tapering hairs (Loftus and Corbière-Tichané 1981). Subsequently, warm cells were identified in long, tapering hairs on the foreleg tarsi of the tropical bont tick *Amblyomma variegatum* (Hess and Loftus 1984). Finally, in the wandering spider *Cupiennius salei*, warm cells were found in nipple-shaped sensilla inside the capsule of the tarsal organ (Ehn and Tichy 1996). Warm cells have never been reported to exist alone in a sensillum. In mosquitoes, cave beetles, and ticks, they are combined with their antagonist, the cold cell. In the spider, however, warm cells occur in the same sensillum with a pair of antagonistic hygroreceptors.

Rapid temperature changes were the optimal stimuli for eliciting the antagonist responses of the warm and cold cells allowing unambiguous identification of two thermoreceptors in the recordings. Temperature transients were produced by changing rapidly the temperature of an air stream directed onto the antennae. In contrast to the high sensitivity to changes in air temperature (*T*), Davis and Sokolove (1975) reported that warm and cold cells of mosquitoes did not respond to infrared radiation (IR). A later study, however, provided evidence that the discharge rates of the mosquito’s warm and cold cells are modulated by oscillating changes in temperature and IR (Gingl et al. 2005).

The aim of the present experiments was to investigate the effects of warm air and IR on warm cells on the antennae of the bloodsucking bug *R. prolixus*. Wigglesworth and Gillett (1934) were probably the first to suggest that the bugs use gradients in warm air in host location. Lazzari and Núñez (1989) listed good reasons that *Triatoma infestans* approaches a heat source in the absence of a warm air current and discriminates between heat sources of different intensities, independently of the source’s emitting area. By interposing filters with different IR transmittance, it was established that *T. infestans* responds to IR radiation alone. The bugs increase their locomotory activity and display characteristic antennal movements in the presence of the heat source, similar to those observed when orienting towards a live host. To exclude that the convective heat generated from the interposed infrared filters potentially elicits these motor patterns, Schmitz et al. (2000) cooled the filters slightly below ambient *T*. Their experiments on *R. prolixus* corroborate that triatomine bugs detect pure IR radiation and approach an IR source in complete darkness.

These observations suggest that bloodsucking bugs are able to extract source information of a thermal stimulus from the environmental context in which the stimulus occurs (Lazzari 2009). By analogy to odor tracking, where insects make use of flow direction information in conjunction with information derived from the odor signal, it seems likely that bugs simultaneously analyze two elements of information to assign the thermal stimulus to a radiant or convective heat source. The first and most obvious element is the occurrence of an increase in sensillum T, and the second is the flow of air carrying the thermal stimulus. Bugs may use mechanosensory deflection of...
hairs and their antennae to detect the presence (or absence) of the air flow. Thus mechanical information may reflect the source of the thermal stimulus. Perhaps the most remarkable task for the thermosensory system achieved by bloodsucking bugs is therefore to recognize an IR stimulus when the air stream temperature is changing. Previous studies do not resolve whether variation in ambient T due to convection affects the warm-cell response to IR stimulation.

A fundamental problem in T and IR neurophysiology is to understand how the intensity and intensity of the two stimuli are represented in the responses of receptor cells and how the responses are used in orientation behavior. Investigation of the representation problem is ideally based on a detailed map of the sensilla, which contain the receptor cells responsive to T and IR stimulation, coupled with detailed characterizations of the discharge evoked by those stimuli. In this study, a great number of sensilla on the antennae of *R. prolis*xus were examined for their responsiveness to T and IR pulses. Extracellular recordings revealed pairs of warm and cold cells associated with peg-in-pit sensilla and tapered hairs. The warm cells of the two morphologically distinct organelles differ quantitatively in their sensitivity to T and IR pulses, an argument for the existence of two receptor types rather than redundancy. The question posed was the resolving power of the two types of warm cells, that is, the precision with which a receptor cell can discriminate T pulses or IR pulses of different intensity.

**MATERIALS AND METHODS**

**Electrophysiological recordings.** Laboratory-reared adult *R. prolis*xus bugs were anesthetized with CO2 and fixed dorsal side-down on a closely fitting Plexiglas holder with strips of Parafilm wrapped around the holder. For unobstructed stimulation, the antenna was fastened with adhesive tape on a narrow support projecting frontally from the holder. Action potentials were recorded extracellularly with electrophysiologically sharpened tungsten electrodes. One electrode was inserted lengthwise into the tip of the antenna and the other at the base of the sensillum. Signals from the electrodes were amplified, band pass (0.1–3 kHz) filtered and displayed conventionally, passed through a CED 1401plus (Cambridge Electronic Design; 12 bit, 10 kHz) interface, and connected to a PC for online recording. The data were stored on a hard disk and analyzed offline using Spike 2 software (Cambridge Electronic Design).

**Stimulation.** T pulses were presented by way of two air streams, flowing out of 7-mm nozzles at a velocity of 2 m/s. Each air stream could be directed separately onto the antenna, which was ~2 cm from the nozzle. Adaptation for 3 min to the T of the first air stream was followed by a warming pulse. This involved switching to the second air stream at various higher T, each of which was maintained for 10 s before the return to the first air stream. A 30-s recovery period was enabled between each change. During this period the T of the second air stream was altered. Electromagnets were used for the switching. The T of the stimulating air stream was measured by a thermocouple 5-mm downstream from the sensillum.

IR pulses were presented by opening a shutter that was positioned in the path of the beam emitted by an Oriel IR element (type 6580, wavelength 1–25 μm). The temperatures of the IR source and the shutter were measured with an IR thermometer (Voltcraft, IR 800–20D). Calculation of the stimulus intensity was based on the Stefan-Boltzmann law performed with the formula (Ebert and Westhoff 2006)

\[
[D^\frac{\sigma \times A \times (T_2^4 - T_1^4)}{\pi \times D^2}]
\]

in which \(\sigma\) is the radiation constant of Stefan-Boltzmann (5.67 \(\times\) \(10^{-8}\) \(\text{W/m}^2\times\text{K}^4\)); A the radiating area (3.5 \(\times\) 3.5 mm\(^2\)); \(T_2\) the temperature of the radiating surface; \(T_1\) the temperature of the shutter which corresponds with the temperature of the small objects of the set-up immediately surrounding the preparation; and \(D\) is the distance to the antenna. Given a radiating surface temperature of 35°C and a shutter temperature of 23°C, the calculated intensity at 2 cm is 0.073 mW/cm\(^2\).

**Evaluation of the responses.** Impulse frequency (impulses per second) was calculated by impulse count for fixed 1-s periods. Since latency was not an object of this study, the peak firing frequency after stimulus onset was taken as response magnitude. Probably the most important characteristics of a receptor cell are the differential sensitivity and resolving power. With the use of the definition of differential sensitivity (gain) as the ratio of input to output or the mean change in frequency per unit change in stimulus magnitude, this value can be readily obtained from the slopes of the regression lines that approximate the relation between pulse amplitude and response. For discrimination, however, differential sensitivity is insufficient. It is also a question of reliability, of how great the difference between two stimuli must become before the larger of them can be designated on the basis of a single pair of responses.

The resolving power can be determined by the maximum number of discrete steps that the impulse frequency is capable of distinguishing within a stimulus range. To estimate the step number of a receptor cell, above and below the frequency vs. stimulus curve is another curve that shows the deviation of the responses throughout the range. Such a band reflects the degree of scatter. The stimulus steps can be drawn within the space enclosed by the deviations. Step width reflects resolving power. Resolving power was also derived directly from the experimental data. Attention was focused on a single pair of responses of a single cell. By how many percent must two stimuli differ for a single cell at average differential sensitivity to be able to identify the larger one? For a given high degree of probability, e.g., 90%? The two stimuli can be a pair of T or IR pulses. A full mathematical development of the concepts underlying the resolving power (\(\Delta x\)) was presented by Loftus and Corbrié-Tichané (1981). The equation is

\[
\Delta x = \frac{\sqrt{2\sigma^2 \Phi^{-1}(\gamma)}}{b}
\]

in which \(|b|\) is the mean absolute slope of the stimulus-response functions, \(\sigma^2\) the variance of the individual deviations of points about their respective regressions, \(\gamma\) the required probability (90%), and \(\Phi^{-1}(\gamma)\) is the inverse of the distribution function of a standardized, normally distributed, random variable. \(\Phi^{-1}(0.99) = 1.28\) (Diem and Lentner 1968, see tables, p. 28). In the case of a linear regression, \(\sigma^2\) is estimated by

\[
\sigma^2 = \frac{\sum e^2}{n-2I}
\]

where \(e\) is the deviation of each individual point from its curve, \(I\) is the number of curves, and \(n\) is the number of measurements; \(n\) is reduced by the number of degrees of freedom, which is \(2I\) for linear regressions because 2 estimators are necessary to determine each straight line (\(a\) and \(b\), \(y = a + bx\)).

This method can be applied if the following conditions are met: 1) the deviations of the individual points from their regression must be normally distributed about a mean of zero, and 2) the absolute deviations (sign ignored) must not depend on the slope. The absolute deviations of single points from their regressions did not depend on the slopes of the regressions. However, their distribution was not normal (\(\chi^2\)-test). Although bell-shaped, the flanks of the distribution curve were too steep; the points tended to be located too centrally. This type of distribution will, if anything, underestimate the resolving power. The normal distribution model was accepted for the lack of a better model.
RESULTS

Two types of thermoreceptive sensilla. The data were obtained from two morphologically distinct types of cuticular organelles. One is a peg positioned at the bottom of a pit. The wall of the pit surrounds the peg and forms a small opening so that only the tip of the peg is visible from outside (Fig. 1, A and B). Several peg-in-pit sensilla occur on each antenna; up to three are visible near the tip, at the distal region of the first flagellum, and four to five on a bare surface in its mid-region. The other type of sensillum is shaped like a hair that tapers off towards the tip and lies close to the antennal surface (Fig. 1, C). Several peg-in-pit sensilla occur on each antenna; up to three are visible near the tip, at the distal region of the first flagellum, and four to five on a bare surface in its mid-region. The other type of sensillum is shaped like a hair that tapers off towards the tip and lies close to the antennal surface (Fig. 1, A and C). Six to eight of these tapered hairs project distally from the margin of the first flagellum. Table 1 compares selected morphometric data of the two sensilla.

Both types of sensilla contain a warm cell and a cold cell that respond to constant T with a steady rate of discharge and to T changes with changes in their discharge rate. The same warming raises impulse frequency in the warm cell and lowers it in the cold cell. Corresponding contrary effects are produced by cooling. The responses of the warm and cold cell were distinguishable by the amplitude and form of their impulses, as exemplified in Fig. 2A, a typical recording from a peg-in-pit sensillum. Qualitatively, the same antagonistic responses of the warm and the cold cells can also be elicited by directing an IR source on the antenna (Fig. 2B). Occasionally, an electrode inserted at the base of both types of sensilla revealed a third cell that responded with an increase in activity when the humidity was raised by shifting from a dry air stream to a moist one at the same temperature. Such responses are characteristic for a moist cell. Definite identification, however, has proven elusive.

The form of the impulses was highly variable. Not only did their amplitude differ from one recording to the other and often tend to diminish with time during single recordings, but the ratio of their amplitude was not constant either. This variation was further compounded by the influence of T. Depending on the stimulus situation, the warm-cell impulse amplitude changed both absolutely and relatively to that of the cold cell. Even impulse polarity was affected. The cause of these changes in form is obscure. The shape of the electrode, its depth, and its position relative to the warm cell surely differed to some extent with every insertion. Based on the methods employed, one can hypothesize about impedance changes or ion accumulation in individual structures separating the excitable membrane from the electrode surface or about the degree to which they reflect transduction processes. Nonetheless, even though the cause is not clear, the effects are important. The changes in the impulse amplitude and especially in the ratio of amplitudes of the warm and cold cell repeatedly complicated automatic discrimination. Impulse amplitude and form was often decisive and had to be determined visually from impulse to impulse.

Table 1. Morphometric analysis of peg-in-pit sensillum and tapered hair; surface areas and volumes calculated by formula of circular cone

<table>
<thead>
<tr>
<th>Type of Sense Organ</th>
<th>Peg-in-Pit</th>
<th>Tapered Hair</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cone length, μm</td>
<td>1.28</td>
<td>14.65</td>
</tr>
<tr>
<td>Base diameter, μm</td>
<td>1.11</td>
<td>1.78</td>
</tr>
<tr>
<td>Surface area, μm²</td>
<td>3.41</td>
<td>42.18</td>
</tr>
<tr>
<td>Volume, μm³</td>
<td>0.41</td>
<td>11.45</td>
</tr>
<tr>
<td>Surface-to-volume ratio</td>
<td>7.7</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Fig. 2. Simultaneously recorded activity of a warm cell and a cold cell from a peg-in-pit sensillum to pulses in temperature (T) and infrared radiation (IR). A: T pulse produced by shifting from a constant-temperature air stream to another at higher constant temperature. B: IR pulse produced by opening a shutter mounted in the path of the beam; sensillum adapted to 25°C room temperature. a and b, raster plots showing the responses of the warm cell and the cold cell, respectively; c, original recordings reveal the antagonistic responses of the warm and cold cell; d, values of temperature and radiant power used for stimulation.
Two kinds of experiments involving series of T or IR pulses were performed on 45 bugs. Of the 36 peg-in-pit sensilla on which the one or the other series was tested, 12 had to be discarded for being short-lived, and of the 32 tapered hairs, 24 qualified for this study. The results presented here were obtained from two sets of 30 peg-in-pit sensilla and 20 tapered hairs; one set of each sense organ was subjected to T pulses and the other to IR pulses (Table 2).

Responses to T pulses. The warm cells in the peg-in-pit sensillum and the tapered hair were adapted for 3 min to a steady T in the 18 to 20°C range before a series of 10-s T pulses commenced, presented with interstimulus intervals of 30 s. The order of presentation of the T pulses was from low to high, and then the series was reversed progressing from high to low. The response profiles of the warm cells of a peg-in-pit sensillum and a tapered hair are reproduced in Fig. 3, A and C, respectively. The series were continuous, but only the sequences with increasing intensities are shown to present the results in a single figure. A steady, regular discharge was an invariant property of all warm cells. Figure 3 also shows that the spike trains of both warm cells such as in Fig. 2A contain information regarding the intensity of pulse T for periods beyond the first 1 s of the response. Their dynamic response, however, peaked within 1–2 s. The discharge rate then slowly decreased in the remaining 8–9 s, followed by a rapid decline during the return to the adaptation T. The responses to T pulses, graded in intensity over the 22 to 38°C range, increased in an orderly fashion with increasing stimulus intensity, and the temporal profiles of the responses showed little variability.

In Fig. 4, A and C, the peak discharge rates of both warm cells was plotted as a function of the pulse T. These two cells were typical for the sample of 12 peg-in-pit sensilla and 7 tapered hairs and show the variability of the peak responses. Peak frequency is a steadily increasing function of the pulse T. The figures also indicate that this relationship can be approximated reasonably well by linear regressions. Because the fit is good, the slope of the regressions also indicates differential sensitivity in the stimulus range. The mean slope from the pooled data is 2.02 (imp/s)/°C with a standard deviation of ± 0.15 for the warm cells in the peg-in-pit sensillum and 1.26 ± 0.18 (imp/s)/°C for those in the tapered hair (Table 2). The mean indicates that the average warm cell in the former elevates its impulse frequency by 2.02 imp/s when a given T pulse is increased by 1°C within the 22–38°C range, and the average warm cell in the latter by 1.26 imp/s. The high standard deviation relative to the mean, however, reflects the variation in the slopes of the curves more than the deviation of individual points from their characteristic curves (regressions). The reciprocals of differential sensitivity indicate that the warm pulse must be increased by on average 0.48°C to increase the impulse frequency of the warm cell of the peg-in-pit sensillum by 1 imp/s. In the warm cell of the tapered hair, the corresponding increase is 0.79°C.

A further parameter in characterizing the warm cells is their resolving power, i.e., the precision with which T pulses can be distinguished based on the firing rate. This is not a question of differential sensitivity. Rather, it is a question of reliability, of how great the difference between two T pulses must become before the larger of the two can be designated with a given probability based on a single pair of responses. The resolving power may be defined as the number of discrete stimulus steps that peak frequency is capable of distinguishing within the stimulus range. To estimate the step numbers, another curve can be plotted above and below the frequency vs. stimulus curves. The band encloses the deviations of the responses throughout the range (Fig. 4, A and C). Such a band reflects the degree of scatter. The stimulus steps can be drawn within the space enclosed by the deviations. Step width reflects the resolving power, which is ~1.5°C for the warm cell of the peg-in-pit sensillum and ~3°C for that of the tapered hair. Resolving power was also calculated as described in MATERIALS AND METHODS. According to our analysis, a pair of warm pulses must differ by 1.2°C to achieve a 90% probability that a single warm cell of the peg-in-pit sensillum (of average differential sensitivity) will cor-

### Table 2. Summary of data used to determine functional characteristics of warm cells located in two morphologically distinct sensilla

<table>
<thead>
<tr>
<th>Stimulus: convective heat</th>
<th>Peg-in-Pit</th>
<th>Tapered Hair</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range over which temperature pulses were tested, °C</td>
<td>22 to 38</td>
<td>22 to 38</td>
</tr>
<tr>
<td>Units used for regression analysis</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Mean coefficient of determination, R²</td>
<td>0.98 ± 0.01</td>
<td>0.95 ± 0.03</td>
</tr>
<tr>
<td>Mean differential sensitivity to temperature steps, (imp/s)/°C</td>
<td>2.02 ± 0.15</td>
<td>1.26 ± 0.18</td>
</tr>
<tr>
<td>Increment of temperature steps, °C, which results in an increment of 1 imp/s</td>
<td>0.48</td>
<td>0.79</td>
</tr>
<tr>
<td>Peak frequency, °C</td>
<td>1.23</td>
<td>2.08</td>
</tr>
<tr>
<td>Stimulus: radiant heat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range over which pulses in radiant power were tested, mW/cm²</td>
<td>0.5 to 5</td>
<td>0.5 to 5</td>
</tr>
<tr>
<td>Units used for regression analysis</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Mean coefficient of determination, R²</td>
<td>0.94 ± 0.04</td>
<td>0.93 ± 0.06</td>
</tr>
<tr>
<td>Mean differential sensitivity to steps in radiant power, (imp/s)/(mW/cm²)</td>
<td>13.8 ± 4.5</td>
<td>16.0 ± 3.8</td>
</tr>
<tr>
<td>Increment of steps in radiant power, (imp/s)/(mW/cm²), which results in an increment of 1 imp/s</td>
<td>0.07</td>
<td>0.06</td>
</tr>
<tr>
<td>Peak frequency, mW/cm²</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Stimulus: convective plus radiant heat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range over which temperature pulses were tested, °C</td>
<td>18 to 28</td>
<td>18 to 28</td>
</tr>
<tr>
<td>Range over which pulses in radiant power were tested, mW/cm²</td>
<td>1.5 to 5</td>
<td>1.5 to 5</td>
</tr>
<tr>
<td>Units used for regression analysis</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Mean coefficient of determination, R²</td>
<td>0.87 ± 0.02</td>
<td>0.95 ± 0.02</td>
</tr>
<tr>
<td>Mean differential sensitivity to temperature, (imp/s)/°C</td>
<td>0.96 ± 0.03</td>
<td>2.59 ± 0.5</td>
</tr>
<tr>
<td>Increment of temperature, °C, which results in an increment of 1 imp/s</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Mean differential sensitivity to steps in radiant power, (imp/s)/(mW/cm²)</td>
<td>1.9 ± 0.4</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td>Increment of steps in radiant power, (imp/s)/(mW/cm²), which results in an increment of 1 imp/s</td>
<td>0.5</td>
<td>0.2</td>
</tr>
</tbody>
</table>
rectly identify the larger of them based on a single response to each. For the warm cell of the tapered hair, the required difference is 2.08°C (Table 2).

Responses to infrared pulses. The responses of the two warm cells to radiant heat were examined in still air and at a constant ambient T in the 20–25°C range. Stimulation consisted of a series of IR pulses, with an average of 10 per series graded in intensities over the range from 0.5 to 5 mW/cm². Pulse durations and intervals varied, but they were never <30 s. All warm cells had steady discharge rates under the constant interstimulus interval conditions. They responded to these IR pulses with large onset transients lasting 1–5 s and reached a peak rate of discharge 10–20 s after the onset of stimulation; the subsequent decay phase lasted 5–12 s. Figure 3, B and D, illustrates the response profiles of the warm cells to a succession of IR pulses of graded intensity. The responses of both increased in an orderly fashion with increasing intensity, and the temporal response profiles changed very little with IR pulse intensity.

Figure 4, B and D, plots the peak discharge rates of the two warm cells as a function of the power of radiant heat. Their intensity functions (sample of 12 peg-in-pit sensilla and 7 tapered hairs) were approximately linear over the whole intensity range of 0.5 to 5 mW/cm². The slope of the regressions indicates differential sensitivity in the stimulus range. The mean slope from the pooled data is 13.8 (imp/s)/(mW/cm²) with a standard deviation of ±4.5 for the peg-in-pit sensillum and 16.0 ± 3.8 (imp/s)/(mW/cm²) for the tapered hair (Table 2). The high standard deviation relative to the mean reflects the variation in the slopes of the curves more than the deviation of individual points from their characteristic curves (regressions).

The reciprocals of differential sensitivity indicate that the pulse in IR must be increased on average by 0.07 mW/cm² to increase impulse frequency of the warm cell of the peg-in-pit sensillum by 1 imp/s. The corresponding value in the warm cell of the tapered hair is 0.06 mW/cm² (Table 2).

The resolving power was determined by drawing the maximum number of steps through the band enclosed by the deviations of the responses from the frequency vs. stimulus curves (Fig. 4, B and D). Step width reflects the resolving power, which is 0.4 mW/cm² for the warm cell of the peg-in-pit sensillum and 1.1 mW/cm² for the warm cell of the tapered hair. However, the analysis of the resolving power revealed that a pair of pulses in radiant heat must differ by 0.5 mW/cm² to achieve a 90% probability that a single warm cell of the peg-in-pit sensillum of average differential sensitivity will correctly identify the larger of them based on a single response to each. For the warm cell of the tapered hair, the required difference is also 0.5 mW/cm² (Table 2).

Responses to infrared pulses at different ambient T. A key issue in the warm-cell responses to IR was the effect of ambient T. We observed earlier that severe, long-lasting IR pulses required fairly long recovery periods before reproducible responses occurred. Warm cells withstood one series of IR pulses easily. Determining the effect of air T on the responses to IR pulses, however, required several series of IR pulses at different T levels. To cover the biological T range reasonably well, the IR pulses were kept at short durations of 15 s. Adaptation for 3 min to an air stream at a constant T in the 20 to 30°C range was then followed by a series of three IR pulses, with interstimulus intervals of 30 s.
As might be expected, the greater the amplitude of the IR pulse, the greater the magnitude of the response of both types of warm cells. This relationship is illustrated in Fig. 4, A and C, but is not the same at all T. Figure 5, A and B, for example, shows that the response to the same IR pulse increases with the T at which the IR pulse was presented. Moreover, the effect of air T on the response to IR pulses is clearly very small in the warm cell of the peg-in-pit sensillum (Fig. 5A) but pronounced in the tapered hair (Fig. 5B). To estimate the double dependence on pulse amplitude and air T, impulse frequency was plotted as a function of both parameters. Multiple regression

\[
F = a + bT + c \text{IR}
\]

showed that sensitivity to air T \((b - \text{slope})\) was 0.9 (imp/s)/°C in the warm cell of the peg-in-pit sensillum and 2.5 (imp/s)/°C in the tapered hair; sensitivity to IR pulse \((c - \text{slope})\) was 1.9 (imp/s)/(mW/cm²) in the peg-in-pit sensillum and 3.8 (imp/s)/°C in the tapered hair. Thus an increase of 1 imp/s in the warm cell of the peg-in-pit sensillum can be elicited either by an increase of 0.5 mW/cm² (at constant ambient T) or by an increase in ambient T of 1.0°C (at constant IR pulse). In the tapered hair, it takes an increase of 0.3 mW/cm² in IR to increase impulse frequency by 1 imp/s or an increase in T of 0.2°C (Table 2). The general character of the regression planes has been observed repeatedly. The responsiveness of the warm cell in the peg-in-pit sensillum to IR pulses was considerably reduced when exposed simultaneously to moving air at constant T. In contrast, the warm cell in the tapered hair responded strongly to the IR pulses when stimulated simultaneously with different ambient T. Furthermore, the higher the T level, the stronger the response to the same IR pulse. The data for six peg-in-pit sensilla and six tapered hairs are summarized in Table 2.

**DISCUSSION**

The triatomine bugs *T. infestans* and *R. prolixus* are attracted by IR radiation and do not mistake changes in IR radiation for changes in air T (Lazzari and Núñez 1989; Schmitz et al. 2000). Thus their sensory equipment is expected to differentiate between T and IR stimuli. Here, we have shown that the antennae of *R. prolixus* bear two morphologically distinct types of thermoreceptive organelles, termed peg-in-pit sensilla and tapered hairs. While a thermoreceptive function has been proposed for the former (McIver and Siemicki 1985), the latter hairs have been considered to function as proprioceptors providing information about the relative position of the antennal segments (Catalá and Schofield 1994). By means of electrophysiological recordings, however, we have shown that both types of sense organs contain a pair of antagonistically responding warm and cold cells. As is the case with the thermoreceptive cells of other insects such as mosquitoes, cockroaches, migratory locusts, and also ticks (Gingl et al. 2005; Gingl and Tichy 2001), the warm and cold cells of the peg-in-pit sensillum and the tapered hair respond to both T and IR stimuli.

**Dependence of impulse frequency on T and IR.** Impulse frequency of the warm cells of the peg-in-pit sensilla and the tapered hairs rose with the amplitude of the T and IR pulse, making it impossible for a single cell type to signal the nature of the pulse unequivocally. Thus none of these warm cells alone can discriminate between T and IR pulses, at least based on the impulse frequency integrated over 1-s periods.

One important source of information about a convective T pulse is the instantaneous observation of air flow. Many arthropods utilize the direction of air flow during odor tracking. Information regarding flow direction probably originates from mechanosensory hairs on the body surface that are stimulated by air movement but might come from joint sensors that respond to deflection caused by the flow acting on the body appendages. Similarly, we suspect that bugs might take advantage of both T and flow information to discriminate between the nature of a stimulus pulse occurring in combination with moving air or alone. The T-induced discharge of the warm cells, in the absence of a mechanical signal, would reflect the presence of an IR pulse, but if a mechanical signal is present, the warm cell discharge would be interpreted as a T pulse due to convective heat transfer. Should this kind of computation be carried out by the brain, it would get the information about the nature of stimulus pulse from a single type of warm cell: there would be no need for two different types of warm cells. In certain situations, however, the mechanical information would not allow the reliable identification of the nature of the stimulus. If the IR pulse occurs concurrently with an air stream that changes sensillum T, the mechanical signal would indicate T stimulus due to convection at the expense of the IR pulse. Importantly, the reversal of the relative excitability of the two types of warm cells provides a criterion by which to clarify this situation. In fact, the impulse frequency is lower at all amplitudes of T and IR pulses for the warm cell of the tapered hair vs. of the peg-in-pit sensillum. Conversely, the warm cell of the tapered hair responds with higher discharge rates to all amplitudes of IR pulses when they were combined with a constant T-air stream in contrast to the warm cell of the peg-in-pit sensillum. The two types of warm cells and the addi-
tapered hair.

The warm cells on the antennae of the bloodsucking bug respond much more sensitive to IR stimulation than the warm cells of the peg-in-pit sensilla on the antennal tip of the mosquito Aedes aegypti (Gingl et al. 2005) and the warm cells of the tapered hairs on the tarsi of the tick Ixodes ricinus (Gingl and Tichy 2001). Furthermore, the responses of bug’s warm cells signal IR pulses more accurately than those of the warm cells of the mosquito and the tick. These differences could arise from physical and physiological factors that increase the general level of IR sensitivity.

Physical factors of the sensillum structures, such as shape and size, or the ratio between the surface area and volume, in addition to the sensillum position and angle of insertion could be relevant. In the mosquito, the peg-in-pit sensillum is hidden in a heavily walled pit and is not visible from outside as if the sensillum surface is well shielded from IR (Gingl et al. 2005). In the bug, however, the rounded sensillum tip extends to the ring-shaped opening of the pit. This may improve contact with IR. Furthermore, the tip surface is characterized by irregular structures presumably indicating a molecular composition distinctive from the sensillum wall. The dendritic processes, typically extending into the apex of the cuticular peg, have been shown to fill the peg lumen thereby contacting the inner surface of the cuticular wall (McIver and Siemicki 1985). Compared with the peg-in-pit sensillum, our knowledge of the tapered hairs is poor. In the tick, the hairs are rigid cuticular structures projecting from the tarsus surface, an appearance that may indicate a mechanosensory rather than a thermoreceptive function (Hess and Loftus 1984). In the bug, on the other hand, the tapered hairs are delicate cuticular structures, and perhaps quite significantly, they are not hidden in a hole but raise from the antennal surface. Studies on the thickness and composition of the cuticular wall or on the diameter and extension of the dendritic processes are lacking.

In insects and ticks, therefore, warm cells are not tied down to a single sensillum type. Even the two basic forms indicate differences that will affect sensitivity. Physical relationships internal to the sense organs are complicated by the fact that the warm cells do not occur alone in the sense organs but together with a cold cell. Thus structural features may be more likely the result of the receptor cell with which the warm cell is combined rather than the result of the warm cell itself. Regardless of the question which of the dendrites is the source of the

Fig. 5. Responses of warm cells to IR pulses presented simultaneously with an air stream at constant T. A: warm cell in peg-in-pit sensillum. B: warm cell in tapered hair. Multiple regressions which utilize 3-dimensional planes \(F = a + bT + cIR\), where \(F\) is the impulse frequency and \(a\) the height of the regression plane) were calculated to determine the simultaneous effects of ambient temperature (\(b\) slope) and the IR pulse (\(c\) slope) on the response frequencies of both cell types. Frequency increases linearly with both temperature and the IR pulse, but frequency increases less in the warm cell of the peg-in-pit sensillum than the tapered hair. \(R^2\) is coefficient of determination.
warm cell activity, the dendrites of thermoreceptors may take
on different forms. Their shape can approximate fairly straight
wires or they can also be flattened and folded into lamellae
sometimes of highly complex arrangement (Ehn and Tichy
1996). The fact that the recordings contain the responses of a
warm cell and a cold cell distinguishable by differences in
action potential waveform and amplitude suggests that the
dendritic processes of the two thermoreceptors may differ in
size. An enlarged membrane area will increase the number of
transduction channels present on the dendrite and thereby increase
the signal-to-noise ratio and consequently improve sensitivity.

Although the number of components (the cuticular wall, the
receptor cells, the receptor lymph, the supporting, and sheath
cells) that are assembled to give a sense organ responding to T
and IR stimulation is small, the number of variations that
occurs among these organelles may be unlimited. Major stra-
tegic structural features of an organelle for which a function in
T and IR transfer has been established are IR absorption
properties and thermal conductivity, the position relative to the
body surface and its orientation to the IR emitting object, and
the tissue or nature of the components surrounding the sensory
cells. The question of matching structural features with phys-
iological properties of course cannot be answered with the
study of warm cells in one or two species. For the necessary
comparison, many representatives will be required from dif-
ferent biotopes. At least some of the material needed for the
comparison might be provided in the case of the bloodsuck-
ing bug. Due to the numerous variables involved in the
transfer of T and IR stimuli, one may suggest different
stimulus-response relationships. At one end of the sensitiv-
ity spectrum, there would be a warm cell with low IR
sensitivity but high T sensitivity (as in the mosquito and the
tick) and at the other end there would be a warm cell
combining high IR sensitivity with low T sensitivity (as in
the bloodsucking bug).

Comparison. Similar experiments have not been carried out
on other species so far. Therefore, there are no data available
for comparing the differential sensitivity for T and IR pulses or
the resolving power of these pulses. In the mosquito A. aegypti
(Gingl et al. 2005), stimulation consisted of slowly oscillating
changes in T and IR radiation rather than pulses used in this
study. During such slow changes in T and IR radiation, the
warm-cell differential sensitivity is 3.4 (imp/s)/°C and 0.8
(imp/s)/(mW/cm²), respectively. The warm cell of the tick I.
ricinus was also examined for its differential sensitivity to slow
changes in T and IR (Gingl and Tichy 2001); the values are 1.5
(imp/s)/°C and 0.1 (imp/s)/(mW/cm²), respectively. In the
bloodsucking bug, the warm-cell differential sensitivity of the
peg-in-pit sensillum is 2.0 (imp/s)/°C and 13.8 (imp/s)/
(mW/cm²), or 1.2 (imp/s)/°C and 16.0 (imp/s)/(mW/cm²) for
the warm cell of the tapered hair. Although quite limited, the
results obtained by the different stimulation methods allow com-
parisons to be made between the warm cells of the three species.

In the two insects as well as in the tick, the warm-cell
impulse frequency tended to be high when T or IR is rising
and low when T or IR is falling. Furthermore, impulse frequency is
a continuous increasing function of the size of the IR step, as
in the warm cells of the bloodsucking bug, or of the instantan-
eous T, as in the warm cells of the mosquito and the tick.
Even though the range of IR changes studied here is smaller
than that examined in previous studies, it is clear that the two
types of warm cells of the bloodsucking bug react much more
sensitively to changes in the power of IR than it was observed
for the mosquito and the tick. However, the IR stimuli differ in
their rate of change. While in the experiments with the mos-
quitos and the tick the rate of change was 1 mW/cm², in the
bloodsucking bug it was 10 times slower. During a stimulation
period of 30 s, a change by 4 (mW/cm²)/s corresponds to a
mean rate of change of only 0.1 (mW/cm²)/s. It is interesting in
this respect that the cold cell of the cockroach improves
sensitivity for the rate of temperature change if the rate be-
comes slow (Tichy et al. 2008). This permits a high degree of
precision at small values. A similar mechanism might be
involved in the bug’s perception of IR. To deal with such a
possibility, IR stimulation should be provided more slowly and
in an oscillating fashion.

Possible functions. Resolving power is considered to be the
difference between two IR pulses necessary for the larger of
the two responses of a single warm cell to correspond with a
given probability (e.g., 90%) to the larger IR pulse. In both
warm cells, two IR pulses must be separated by 0.5 mW/cm²
for correct identification. The literature provides only few
indications of the power of radiation emitted by a biological
object. Terashima et al. (1968), in estimating the performance
of the crotalid pit organ, calculated that a circular area of
human skin (150 cm²) at a temperature of 34°C and a surface
temperature of the walls and surrounding objects of 20°C
produced an intensity of 1.03 mW/cm² at a distance of 20 cm.
Accordingly, the intensity value of a human hand is 4.12
mW/cm² at a distance of 10 cm and 16.50 mW/cm² at a
distance of 5 cm. In view of a resolving power of 0.5 mW/cm²,
a human hand produces at a distance of 20 cm twice the
intensity being required for correct identification by a warm
cell with average differential sensitivity. When approaching
the IR source to a distance of 10 cm, the IR intensity will
increase and the responses will distinguish 6 intensity levels,
but as much as 24 intensity levels when continuing moving to
5 cm. Besides the resolving power, the number of warm cells
providing thermal information is important. Combining the
signals of all warm cells of each type probably improves the
ability to detect the IR source.

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H.T. and L.M.Z. performed experiments; H.T. and L.M.Z. analyzed data; H.T.,
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