The effect of convection on infrared detection by antennal warm cells in the bloodsucking bug *Rhodnius prolixus*

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Tichy H, Zopf LM. The effect of convection on infrared detection by antennal warm cells in the bloodsucking bug *Rhodnius prolixus*. *J Neurophysiol* 113: 2250–2261, 2015. First published January 21, 2015; doi:10.1152/jn.01047.2014.—Previous work revealed that bloodsucking bugs can discriminate between oscillating changes in infrared (IR) radiation and air temperature (T) using two types of warm cells located in peg-in-pit sensilla and tapered hairs (Zopf LM, Lazzari CR, Tichy H. *J Neurophysiol* 111: 1341–1349, 2014). These two stimuli are encoded and discriminated by the response quotient of the two warm cell types. IR radiation stimulates the warm cell in the peg-in-pit sensillum more strongly than that in the tapered hair. T stimuli evoke the reverse responses; they stimulate the latter more strongly than the former. In nature, IR and T cues are always present with certain radiation intensities and air temperatures, here referred to as background IR radiation and background T. In this article, we found that the response quotient permits the discrimination of IR and T oscillations even in the presence of different backgrounds. We show that the two warm cells respond well to IR oscillations if the background T operates by natural convection but poorly at forced convection, even if the background T is higher than at natural convection. Background IR radiation strongly affects the responses to T oscillations: the discharge rates of both warm cells are higher the higher the power of the IR background. We compared the warm cell responses with the T measured inside small model objects shaped like a cylinder, a cone, or a disc. The experiments indicate that passive thermal effects of the sense organs rather than intrinsic properties of the sensory cells are responsible for the observed results.

infrared radiation; air temperature; combinatorial code; warm and cold cells; background conditions

THE BLOOD-SUCKING BUG *Rhodnius prolixus* is attracted by the faint body heat of an animal or person. This heat must be from infrared (IR) radiation and not from convection. Behavioral experiments revealed that the bugs do not confuse radiant with convective heat, indicating that the bugs are able to differentiate between IR radiation emitted from the warm-blooded host and the warm air around them (Lazzari and Núñez 1989; Schmitz at al. 2000). A key issue is the bugs’ ability to detect the host in a highly complex environment characterized by different intensities of IR radiation emitted from surrounding objects and by different air temperatures. In this study we focus on the role of additional IR radiation and temperature (T) cues that are present simultaneously with the IR and T stimuli. These additional cues will be referred to here as “background IR radiation” and “background T.” We analyze their impact for the detection of IR and T stimuli from a physiological point of view.

The first antennal segment of *R. prolixus* bears a small number of peg-in-pit sensilla (PS) and tapered hairs (TH), which contain an antagonistically responding pair of thermoreceptive cells, a warm cell, and a cold cell (Zopf et al. 2014a,b). The warm cells in the two sensory organs differ quantitatively in their responses to IR and T stimuli. Slowly oscillating changes in IR radiation produce strong responses in the warm cells of the peg-in-pit sensilla (PSw cells) and comparatively weak responses in the warm cells of the tapered hairs (THw). Slowly oscillating changes in T evoke the reverse responses; they stimulate the latter (THw) more strongly than the former (PSw). The reversal in the relative excitability of the two warm cell types in a pair provides a criterion by which to distinguish between changes in T and IR radiation. The activity of each warm cell cannot give by itself any unequivocal information concerning IR radiation or T but is meaningful in the context of the activity of the parallel warm cell. This is a classical “combinatorial code” solution.

A key factor in combinatorial coding is the heavy dependence of both types of warm cells on the oscillation period of the IR and T stimuli (Zopf et al. 2014b). With increasing duration of the oscillation period, the rate of change of the IR and T stimuli decreases although the oscillation amplitude remains constant. This change in the stimulus rate differently affects the responses of both warm cell types to IR and T oscillations. The reversal of their responses, expressed as the response ratio or response quotient, unambiguously discriminates between IR and T oscillations although the excitation of the warm cells varies in a continuous manner from low to high. In the present experiments we examined whether the response ratio of the two warm cell types still discriminates between IR and T oscillations while the background IR radiation and the background T vary. Theoretically, the background T might be irrelevant for localizing a warm-blooded host, and the background IR irrelevant for assessing the thermal quality of a habitat, because the warm cells are adapted to the background conditions; here, the response ratio would not confuse IR and T oscillations. In another interpretation, background conditions may mask the IR stimulus, thereby reducing the response of the warm cells to an infrared source. Conversely, such background conditions might also enhance that response, indicating the presence of the warm-blooded animal, thereby “sharpening the view” for their host.

Many engineering studies have analytically and experimentally evaluated heat transfer by radiation and convection, and the mechanisms are quite well understood for simple geometric objects (Holman 2001; Theodore 2011). For more complicated geometries, such as insects, no data are available. An ectothermic insect exposed to a given ambient T will immediately take...
on this T, but the same T reading at the body or the antennae does not necessarily mean the same heat. The amount of heat that must be transferred to cause the same T change depends on the mass of the matter, the matter itself, and the T at which the matter is held. As T and heat are different measures, the heat of the slender antennae may be lower than that of the larger body even if their T is the same. The equilibrium T between the slender antennae and the environment may correspond with a different heat than the equilibrium T between the larger body and the environment. Thus an insect at T equilibrium can still have a heat gradient between the antennae and its body. Unfortunately, we know little about the heat conductance, the heat capacity, and the mass of insect antennae or bodies. These parameters certainly affect the speed of heat transfer in the sense organs and therefore the sensitivity of the warm cells to changes in IR radiation and T. To understand whether the observed warm cell responses could be accounted for by the physical properties of the sensory structures or involve more complex cellular phenomena, we subjected three model objects of different shapes but similar mass to the same experimental protocol as the warm cells. Due to their different shapes, the surface areas as well as the surface area to volume ratios of the three models differed. These two features provided a range of factors differentially determining the gain and release of heat under given thermal conditions. A comparison of the time course of the warm cell discharge rates with the time course of the T monitored inside the model objects revealed similar effects of IR and T oscillations at different IR and T background conditions. Thus physical properties of the sense organs rather than intrinsic properties of the warm cells apparently determine the responses of the two warm cell types to IR and T stimulation.

MATERIALS AND METHODS

Electrophysiological Recordings

Laboratory-reared adult *Rhodnius prolixus* were used in this study. Following anesthetization with CO₂, an animal was 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 electrolytically 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).

Convective Stimulation

T stimulation by natural convection was applied by the heat contained in the still air of the room. Air T was measured by a thermocouple 5 mm away from the antenna with a fine-wire thermocouple (wire diameter 13 μm; Type E: Cu-CR/Cu-Ni; Campbell Scientific).

Forced convective T stimuli were presented by an air stream flowing over the antenna. To this end, air from a pressure-regulated source was cleaned, dried, and split into two streams. Their flow rates were equalized by matching the rates in mass flow meters, and their temperatures were regulated by independent thermostats. After passing through electrical proportional valves (KWS 3/3; Kolvenbach), the two streams were combined to a single stream. The temperature of this stream was sinusoidally modulated by mixing the two streams in a ratio determined by the proportional valves. To hold the flow rate of the mixed air constant at 2.5 m/s, the control voltages (AD-converter, 1401 plus; Cambridge Electronic Design) of the proportional valves were phase shifted by 180°. For stimulation, this stream was directed towards the sense organ by way of a Plexiglas tube 7 mm in diameter. The sense organ was 10 mm away from the outlet of the tube. The T of the air stream was measured by a thermocouple 5 mm downstream from the antenna with the same fine-wire thermocouple described above.

**IR Stimulation**

IR stimuli were provided by opening a shutter 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). Stimulus intensity was calculated based on the Stefan-Boltzmann law using the formula (Ebert and Westhoff 2006)

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

in which \(\sigma\) is the radiation constant of Stefan-Boltzmann \(\left(5.67 \times 10^{-8} \text{ Wm}^{-2} \text{K}^{-4}\right)\); \(A\) the radiating area \(\left(3.5 \times 3.5 \text{ mm}^2\right)\); \(T_1\) the temperature of the IR beam and the air stream always met at right angles and the model objects were subjected to the same test protocol used to describe the response characteristics of the two warm cell types. The IR beam and the air stream always met at right angles and the model objects were positioned within the common point of intersection. Model T was measured with a small bead thermistor \(\left(250 \times 400 \mu\text{m}; \text{Fenwall Electronics, BC 32 L1}\right)\) positioned inside a central channel.

Evaluation of the Responses

Impulse frequency (impulses per second) was calculated from running averages of three consecutive 5-s periods. A 5-s period rather than the more common 1 s was used to keep the amount of data for long oscillation periods small.

Model Objects

Three model objects were created, a cone (radius: 1.9 mm; height: 11.5 mm), a cylinder (radius: 1.9 mm; height 5.1 mm), and a disc (radius: 2.9 mm; height: 2.1 mm), with similar volumes \((\text{cone: } 60.6 \text{ mm}^3; \text{cylinder: } 60.9 \text{ mm}^3; \text{disc: } 57.4 \text{ mm}^3)\), different surface areas \((\text{cone: } 101.9 \text{ mm}^2; \text{cylinder: } 86.4 \text{ mm}^2; \text{disc: } 93.6 \text{ mm}^2)\), and similar surface area to volume ratios \((\text{cone: } 1.6; \text{cylinder: } 1.4; \text{disc: } 1.6)\). The model objects were subjected to the same test protocol used to describe the response characteristics of the two warm cell types. The IR beam and the air stream always met at right angles and the model objects were positioned within the common point of intersection. Model T was measured with a small bead thermistor \(\left(250 \times 400 \mu\text{m}; \text{Fenwall Electronics, BC 32 L1}\right)\) positioned inside a central channel.

RESULTS

Thermoreceptors

The PS and the TH contain a pair of sensory cells that respond antagonistically to changes in T: a warm cell which is excited by rising T and a cold cell excited by falling T. The
cold cells usually produced smaller impulses in the extracellular recordings than the warm cells. The form of the impulses varied considerably in the warm and cold cells. This variation was further compounded by the influence of T. Changes in the ratio of their impulse amplitudes repeatedly ruled out discrimination. Especially, the amplitudes of the cold-cell impulses tended to vanish into the base line or to be obscured by the larger impulses of the warm cell. Investigation of the TH was therefore limited to the warm cells (THw cells). In several recordings from the PS, however, the form and the amplitude of the warm cells (PSw cells) and the cold cells (PSc cells) displayed sufficient regularity to allow a quantitative examination of their responses.

Two kinds of experiments were performed. In the first, slowly oscillating changes in the power of IR radiation were presented in combination with still air at a background T of 25°C (natural convection flow) or the IR oscillations were combined with moving air at a background T of 23 and 28°C (forced convection flow). In the second experiment, in contrast, T oscillations were tested in combination with the IR source turned off (background IR radiation off) or the T oscillations were combined with a background IR radiation of 2 and 4.8 mW/cm². Complete series of both experiments were successfully performed on 22 THw cells, 25 PSw cells, and 20 PSc cells. Experiments rarely extended beyond an hour to avoid a creeping diminution of sensitivity.

IR oscillations. When the power of IR radiation was made to rise and fall smoothly in the range of 0 to 5 mW/cm² and during periods of 300 s, the rate of change was between −0.1 and +0.1 mW·cm⁻²·s⁻¹. A test sequence always began with a series of IR oscillations in still air at a background T of 25°C (natural convection). This was followed by IR oscillations combined with moving air at a background T of 23°C (forced convection). When this series was completed, the T of the air stream was set at 28°C and allowed to stabilize for 5 min before the next series of such IR oscillations was begun.

Figure 1 shows the results of such an experiment. In all three series of IR oscillations, the impulse frequency (F) of the THw cell (Fig. 1Aa) and the PSw cell (Fig. 1Ab) tended to be higher at the higher instantaneous IR radiation values and lower at the lower instantaneous IR radiation values. The amplitude of F oscillations depended on the background T as well as on the mode of convection. Both types of warm cells responded in still air with higher F values than in moving air, and the higher the T of the air stream, the higher the discharge rate. The course of the IR radiation and F curves indicates that F and IR oscillations are not in step. There is a phase shift between them: F oscillations lag behind those of IR radiation. Apparently, parameters other than instantaneous IR radiation were driving both warm cells. The rate of IR radiation change can be excluded. As the first derivative of instantaneous IR radiation, it is necessarily in advance of the instantaneous IR radiation, not behind it. The rate with which the sensory organs warm up and cool down during IR oscillations is the obvious parameter that affects the responses. The rate of change of the T of the sense organ cannot be measured directly during IR stimulation, at least with the methods we employed. Accordingly, the effect of IR oscillations cannot be separated from the rate with which heat propagates through the sense organs. Nevertheless, the stimulating instantaneous IR radiation and its rate of change are two independent variables whose effects can be studied separately.

To estimate the extent to which the responses of the two warm cell types are governed by the instantaneous IR radiation and its rate of change, F was plotted as a function of both parameters (Fig. 1, Ba and Bb, Ca and Cb, and Da and Db). The course of the F curves reflects the strong dependence of F on the instantaneous IR radiation and its rate of change in still air at 25°C (natural convection; Fig. 1, Ba and Bb). In moving air (forced convection), the F curves demonstrate the low dependence on both IR parameters at 23°C (Fig. 1, Ca and Cb) and the moderate dependence on both parameters at 28°C (Fig. 1, Da and Db). Multiple regressions (F = \( y_0 + a \frac{dRP}{dt} + b \) RP, where F is the impulse frequency, \( y_0 \) is the height of the regression plane, and RP is the radiation power) were utilized to evaluate the effect of the three different background conditions on the responses to the instantaneous radiation power (b slope) and its rate of change (a slope). The slopes of the regression planes reveal the two properties that characterize the responses of the two warm cell types to IR oscillations at different background T: 1) the sign of the b slopes is positive, that is, an increase in instantaneous radiation power raises F of both warm cells; and 2) the sign of the a slopes is negative, that is, a decrease in the rate of radiation power increases F of both warm cells. Strikingly, sensitivity for the instantaneous IR radiation has a positive sign but a negative sign for its rate of change: F continues to increase even though the rate of change decreases.

The responses of the PSc cell were antagonistic to those of the PSw cell with respect to both IR oscillations and background conditions. As Fig. 1Ac illustrates, its discharge increased while IR radiation fell and decreased while IR radiation rose. F was low when the IR oscillations were presented in combination with still air, but moderate when the oscillations were combined with moving air at 28°C, and high when combining it with moving air at 23°C. The phase shift between F oscillations and IR oscillations was opposite to the phase shift of the PSw cell: F oscillations of the PSc cell were in advance instead of behind those of the IR oscillations.

Fig. 1. A–D: responses of a single warm cell of the tapered hair and of a single warm and cold cell of the same peg-in-pit sensillum to slowly oscillating changes in infrared (IR) radiation at different background air temperature (T). Aa–Ac: time course of impulse frequencies of warm and cold cells to IR oscillations in still air at background T of 25°C. a: Warm cell of the tapered hair (THw cell); b: warm cell of the peg-in-pit sensillum (PSw cell); and c: cold cell of the same peg-in-pit sensillum (PSc cell). Ba–c: IR oscillations in still air at background T of 25°C. Impulse frequency (F) of the 3 cells in Aa–Ac is plotted as a function of instantaneous IR and the rate of IR change. Multiple regressions, which utilize 3-dimensional planes (F = \( y_0 + a \frac{dRP}{dt} + b \) RP, where F is the impulse frequency, \( y_0 \) is the height of the regression plane, and RP is the radiation power), were calculated to determine the differential sensitivity for instantaneous RP (b slope) and the rate of RP change (a slope) on response frequency; a–c are as above. Ca–Cc: IR oscillations in moving air at background T of 23°C. F of the 3 cells in Aa–Ac is plotted as a function of instantaneous IR and the rate of IR change. Differential sensitivity for both parameters is indicated by the coefficients a and b in the equation of the regression plane, F = \( y_0 + a \frac{dRP}{dt} + b \) RP; a–c are as above. \( R^2 \), coefficient of determination.
To estimate the extent to which the instantaneous IR radiation and its rate of change affects the cold cell’s discharge rate, $F$ was plotted as a function of both parameters (Fig. 1, $B_c$, $C_c$, and $D_c$). The shape of the $F$ curves manifests the strong dependence of $F$ on the instantaneous IR radiation and its rate of change in moving air at 23°C (forced convection, Fig. 1$c$), the moderate dependence on both IR radiation parameters in moving air at 28°C (forced convection, Fig. 1$c$), and the low dependence in still air at a background $T$ of 25°C (natural convection, Fig. 1$c$). The simultaneous effect of instantaneous radiation power ($b$ slope) and its rate of change ($a$ slope) on $F$ was estimated by means of multiple regressions ($F = \ldots$).
The strong dependence of instantaneous T and its rate of change. Multiple regressions (the instantaneous T values. The oscillation amplitude of higher instantaneous T values and lower at the lower instant-
ment. In all three series of T oscillations, impulse frequen-
the height of the regression plane) were calculated to determine the differential sensitivity for instantaneous T (b slope) and its rate of change on the cold cell. Thus the warm cells and the cold cell did not behave in a reciprocal manner.

Combinatorial coding. As is evident from Figs. 1 and 2, the PSw cells and THw cells differ quantitatively in their respon-
siveness to oscillations in IR radiation and T. While the PSw cells responded strongly to T oscillations and weakly to IR oscillations, the THw cells produced weak responses to T oscillations and strong responses to IR oscillations. The reversal of the relative excitability of the two types of warm cells accounts for the neural representation of the bug's ability to discriminate between IR and T stimuli. A recent study (Zopf et al. 2014b) showed that the response quotient (Q = F_{hair}/F_{peg}) is capable of discriminating IR and T oscillations throughout a large range of oscillation periods (300–1,200 s). Nonetheless, the general formulation of the response quotient as a reliable

\[ y_0 + a \frac{dRP}{dt} + b \, RP, \text{ where } F \text{ is the impulse frequency and } y_0 \text{ is the height of the regression plane}. \] The slopes of the regression planes indicate the two properties that characterize the response of the two warm cells types to T oscillations combined with different background IR radiation: 1) the sign of the b slopes is positive, that is, an increase in the instantaneous T raises F of both warm cells; and 2) the sign of the a slopes is negative, that is, a decrease in the rate of T increases F of both cells. According to the observation that during IR oscillations F decreases at lower rates than IR radiation, the F decrease during T oscillations was also slower than that of the air T.

The responses of the PSc cell to T oscillations at different IR backgrounds are shown in Fig. 2Ac. F rose while T decreased and F fell while T increased. The F curves are similar, indicating that the background IR radiation has no great effect on the responses to T oscillations. Thus the PSc cell does not mirror the PSw cell. However, the phase shift between F oscillations and T oscillations is opposite to the phase shift observed for the warm cell: F oscillations of the PSc cell are in advance of the IR oscillation. As already argued, the rate of T change of the sensillum is slower than that of the stimulating air stream. To estimate the effects of instantaneous T and its rate of change on the cold cell, F was plotted as a function of both parameters (Fig. 2, Bc, Cc, and Dc). The F curves indicate a strong dependence of F on the instantaneous T and its rate of change when the background IR radiation was turned off, but the dependence was also strong when the background IR radiation was set at 2 or 4.8 mW/cm². Multiple regressions (F = y_0 + a \frac{dRP}{dt} + b \, RP, where F is the impulse frequency and y_0 is the height of the regression plane) were used to evaluate the simultaneous effect of instantaneous T (b slope) and its rate of change (a slope) on F obtained at the three IR backgrounds. The slopes demonstrate the two properties that characterize the cold cells: 1) the sign of the b slopes is negative, that is, a decrease in instantaneous T raises F of the cold cell; and 2) also the sign of the a slopes is negative, that is, a decrease in the rate of T increases F of the cold cell. Thus the warm cells and the cold cell did not behave in a reciprocal manner.

Temperature oscillations. Understanding the phase difference between F oscillations and IR oscillations requires knowing the first differential T component in the responses of the warm and cold cells. To this end, T of an air stream flowing over the antenna was modulated at rates between −0.2 and + 0.2°C/s and at an amplitude of roughly 15°C in the 15 to 30°C range. The T-oscillations of both parameters (Fig. 2, Ba and Bb, Ca and Cb, and Da and Db). The F oscillations approached closed curves, indicating a phase difference of the two warm cell types on the instan-
taneous T. Nonetheless, F did not depend exclusively on the instantaneous T. F oscillations lag behind those of T. The receptive structures apparently change their T slower than the air stream. To estimate the effect of T and its rate of change, F of the two warm cell types was plotted as a function of both parameters (Fig. 2, Ba and Bb, Ca and Cb, and Da and Db). The F oscillations approached closed curves, indicating a strong dependence of F of the two warm cells on the instan-
taneous T and its rate of change. Multiple regressions (F = y_0 + a \frac{dT}{dt} + b \, T, where F is the impulse frequency and y_0 is the height of the regression plane) were calculated to determine the simultaneous effect of instantaneous T (b slope) and its rate of T change (a slope) on F. The slopes demonstrate the two properties that characterize the response of the two warm cell types to T oscillations with the IR source turned off and set at 2 and 4.8 mW/cm²: a: warm cell of the tapered hair (THw cell); b: warm cell of the peg-in-pit sensillum (PSw cell); and c: cold cell of the same peg-in-pit sensillum (PSc cell). Ba–Bc: T oscillations with the background IR radiation turned off. F of the 3 cells in Aa–c is plotted as a function of instantaneous T and the rate of T change. Multiple regressions, which utilize 3-dimensional planes (F = y_0 + a \frac{dT}{dt} + b \, T, where F is the impulse frequency and y_0 is the height of the regression plane), were calculated to determine the differential sensitivity for instantaneous T (b slope) and the rate of T change (a slope) on the response frequency, a–c are as above. Ca–Cc: T oscillations at background IR radiation of 2 mW/cm². F of the 3 cells in Aa–Ac is plotted as a function of instantaneous T and the rate of T change. Differential sensitivity for both parameters is indicated by the coefficients a and b in the equation of the regression plane, F = y_0 + a \frac{dT}{dt} + b \, T; a–c are as above.

Fig. 2. A–D: responses of a single warm cell of a tapered hair and of a single warm and cold cell of the same peg-in-pit sensillum to slowly oscillating changes in T combined with different background IR radiation. Aa–Ac: time course of impulse frequencies of warm and cold cells to T oscillations with the IR source turned off and set at 2 and 4.8 mW/cm²: a: Warm cell of the tapered hair (THw cell); b: warm cell of the peg-in-pit sensillum (PSw cell); and c: cold cell of the same peg-in-pit sensillum (PSc cell). Ba–Bc: T oscillations with the background IR radiation turned off. F of the 3 cells in Aa–c is plotted as a function of instantaneous T and the rate of T change. Multiple regressions, which utilize 3-dimensional planes (F = y_0 + a \frac{dT}{dt} + b \, T, where F is the impulse frequency and y_0 is the height of the regression plane), were calculated to determine the differential sensitivity for instantaneous T (b slope) and the rate of T change (a slope) on the response frequency, a–c are as above. Ca–Cc: T oscillations at background IR radiation of 2 mW/cm². F of the 3 cells in Aa–Ac is plotted as a function of instantaneous T and the rate of T change. Differential sensitivity for both parameters is indicated by the coefficients a and b in the equation of the regression plane, F = y_0 + a \frac{dT}{dt} + b \, T; a–c are as above.

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neural code for IR stimuli must consider the effects of the background T.

To examine the validity of the response quotient as a discriminator between IR and T oscillations, the \( F \) values of the THw cell were plotted against the \( F \) values of the PSw cell for a given oscillation period at corresponding points in time. The implication is not that the responses of one warm cell type depend on those of the other type. Rather, both types depend on a third parameter, which is a change in T or IR radiation at a given instantaneous level of T or IR radiation, respectively. The plots in Fig. 3 indicate closed curves for each oscillation that are separated into groups of points: those from

**stimulus: T oscillations at different IR background**

**Aa** tapered hair warm cell

**Ab** peg-in-pit sensillum warm cell

**Ac** peg-in-pit sensillum cold cell

**IR source OFF**

**Ba**

**Bb**

**Bc**

**background IR radiation: 2 mW/cm²**

**Ca**

**Cb**

**Cc**

**background IR radiation: 4.8 mW/cm²**

**Da**

**Db**

**Dc**
IR oscillations combined with different background T (Fig. 3A) and those from T oscillations combined with different background IR radiation (Fig. 3B). A line drawn through the origin can serve as a boundary between them. Such a boundary has the slope $Q = 1; Q = F_{\text{hair}}/F_{\text{peg}}$ for IR oscillations is larger than $Q = 1$ for any background T to the left and above the boundary, and $Q = F_{\text{hair}}/F_{\text{peg}}$ for T oscillations is smaller than $Q = 1$ for any background T to the right and below the boundary. While there are no overlapping Q values for T oscillations combined with different IR backgrounds (Fig. 3B), slightly overlapping Q ranges are generated for IR oscillations when combined with moving air at 23°C (Fig. 3A). Here, Q values do not permit identification of IR changes at 23°C background T. These Q values, however, are formed by low-frequency values close to the excitation threshold.

Model Objects

The T within the sense organs cannot be measured directly, at least with the methods employed here. It is therefore difficult to define the lag of the F oscillations behind the IR and T oscillations as an adaptation of the warm cells to ambient conditions or simply as fatigue. Instead of intrinsic properties of the warm cells, however, the lag in F may reflect the rate with which the sense organs change their T. The organs' rate of T change could be slower than the rate of IR or T change provided by the oscillating stimuli. This issue was studied by describing the phase relationships between the oscillating T of different model objects, the oscillations in IR radiation at different background T, and the oscillations in T at different background IR radiation.

Three model objects of different geometry were subjected to the same experimental protocol used to describe the response characteristics of the two warm cell types (Figs. 1 and 2). The model T was measured with a small thermistor fixed inside a central channel.

IR oscillations. In the experiment shown in Fig. 4A, the IR source was located at the broad side of the model objects and the air stream was flowing along the small side. Figure 4A, A and B, top trace represents the time course of IR oscillations, and Fig. 4A and B, bottom three traces show the time course of the model T in still air at a background T of 25°C and in moving air at a background T of 28 and 23°C. Three effects clearly emerged. One is that in the three model objects the amplitude of the T oscillations was large in still air at 25°C but small in moving air at both 28 and 23°C. The second effect is that the T maxima assumed the highest values in still air at 25°C and in moving air at a background T of 25°C. The third effect is that the T oscillations of the model objects lagged behind the IR oscillations. Such a negative phase shift was observed for different background T and for both natural and forced convection.

In Fig. 4B, the IR source was located at the small side of the model objects and the air stream flowing along the broad side. Here, the model T was oscillating less pronouncedly than in the first series of IR oscillations. As in the first series, the T maxima were highest in still air at 25°C, lowest in moving air
at 28°C, and intermediary in moving air at 23°C. Furthermore, the oscillating model T always lagged behind the IR oscillations, regardless of the background T and the mode of convection. The experiments indicate that the oscillating T of three model objects did not match well with the oscillating IR radiation. If indeed the model objects change their T at lower rates than the IR oscillations, one would certainly expect a similar low rate of T change when the model T is changed by oscillations in air T at constant levels of background IR radiation.

**DISCUSSION**

Our results confirm that the ratio of the responses of two types of warm cells is an unambiguous discriminator between IR and T oscillations, as postulated earlier by Zopf et al. (2014b). We demonstrate that the message sent by the two warm cell types can be reconstructed based on the ratio of their responses even at different IR and T backgrounds. This result is particularly important for understanding the sensory codes for slow and continuous changes in IR radiation and T. Three main topics are discussed: 1) the warm cell responses to IR and T oscillations at different backgrounds, 2) the T change of the model objects measured for the same experimental protocol as the warm cells, and 3) the information provided by the warm cells about slow and continuous changes in IR radiation and ambient T.

**Thermoreceptors**

**IR oscillations at different background T.** A comparison of the response profiles of the two warm cell types to IR oscillations reveals the same relative effectiveness of the background T and the mode of convection. Still air (natural convection) at 25°C is most effective, followed by moving air (forced convection) at 28 and 23°C. However, IR oscillations always produce stronger responses in the PSw cell than in the THw cell, regardless of the background. The continuous flow of a constant T air stream over the surface of the sense organs appears to mask the stimulating effect of IR oscillations. The lower the air stream T, the stronger is the masking effect and
T oscillations at different background IR radiation

The rank order of the response profiles observed for the PSc cell is oppositely to that of the two warm cells. Therefore, still air at 25°C is least effective, and moving air is slightly more effective at 28°C but most effective at 23°C. In general, the higher the air stream T, the stronger is the masking effect and the weaker are the responses. Furthermore, the positive phase difference is also oppositely to that found in the PSw cell.

Model Objects

The model objects were used to evaluate how well physical factors explain the responses of the two types of warm cells to IR and T oscillations at different backgrounds. A major strategic function of the structural elements comprising the PS and TH (Zopf et al. 2014a) is that of mechanical support. At the same time, the theory and practice of thermodynamics tell us that structural features such as size, shape, and position affect the gain or loss of heat by convection and radiation. In an attempt to determine how the sense organs themselves affect heat transfer, we created three model objects shaped like a cone, a cylinder, and a disc. These models have almost the same volume but different surface areas and therefore different surface-to-volume ratios, which are determining factors of heat exchange.

IR oscillations at different background T. In the three model objects, the range of the T oscillations is highest when the IR source is turned off and less effective at the highest IR background.
oscillations occur in still air (natural convection). It is somewhat lower during forced convection, even if the background T is higher than in natural convection. Moving air apparently dampens the thermal effect of IR oscillations. Independent of the convection mode, the oscillating T lags behind the oscillating IR.

\textbf{T oscillations at different background IR radiation.} Oscillations in air T cause distinct T oscillations in the three model objects, which depend on the IR background. The higher the IR background, the higher is the T oscillation range. The lowest range is attained by turning the IR source off. The IR background apparently enhances the thermal effect of T oscillations. Independent of the IR background, the oscillating T of the three objects is not in phase with the oscillating air T; the model T lags behind air T.

\textbf{Similarity Between Warm Cell Responses and Model Temperatures}

There are two potential reasons why differences in the response magnitudes of the two warm cell types to T and IR oscillations could arise from physical factors inherent in the sense organs rather than from intrinsic warm cell properties. The first concerns the identical rank order of the F profiles of the warm cells and the T profiles of the model objects. Even the reverse sensitivity of the two warm cell types to IR and T oscillation can reflect physical properties. In the PS and the TH, heat gain and release by IR and T may differ under the same thermal conditions. Heat transfer depends on various thermal characteristics of the sense organs such as heat conductance, heat capacity, and mass. While data on intrinsic structures are available for the PS (McIver and Siemicki 1985), nothing is known about the dendritic processes of the warm cell in the TH, their diameter, or whether they terminate at the base of the hair or extend into the hair shaft.

The second potential reason is the negative phase difference that occurred not only between the F oscillations and the IR and T oscillations but also between the T oscillations of the model objects and the same IR and T oscillations. The results indicate that the rates at which the model objects change their T are slower than the rates produced by IR and T oscillations. The phase lag can therefore be explained by physical properties of the sense organs.

\textbf{Some Comparisons with Other Receptors}

This is the first time that IR-sensitive cells have been studied for the combined effect of convection and IR radiation. The basic ideas underlying this approach are not specific for \textit{Rhodnius} but may also be applicable to IR detection in other species. The number of species that are demonstrably attracted by IR radiation is quite small and smaller still is the number in which IR-sensitive cells have been examined electrophysiologically. Moreover, different methods of stimulation and evaluation complicate comparison.

To mimic natural stimulation, we used slow and continuous changes in IR radiation and T instead of rapid, step-like changes. The obvious advantages are the possibility to correlate F with the instantaneous T of the sensory cells during T changes and also with quite accurate values of the rate of T change. The possibility of assigning instantaneous T values of the air stream to the sense organ exists only when the T of the air stream is changing so slowly that the difference in T between the sense organ and the air stream is insignificant. During low rates of T change, the air stream moves across the sense organ at a velocity of more than 10,000 sense-organ diameters per second. Thus the T of the receptive site can be considered as locked to that of the air stream and can be used to determine the T of the receptive site. During rapid T changes, when the T wave front is steep, instantaneous values of T and its rate of change cannot be determined at the receptive site. In most electrophysiological studies, however, IR and T stimuli were changed at fast rates. Nevertheless, some characteristics of the pit organs of snakes and buprestid beetles will be discussed.

The facial pits of crotaлиne snakes (Bullock and Diecke 1956; Goris and Nomoto 1967; Campbell et al. 2002) enable their possessors to strike successfully at small warm objects in the dark. These organs consist of an innervated membrane <15-\(\mu\)m thick. The membrane is supplied with branches of the trigeminal nerve and suspended over an air-filled cavity. Electrophysiological recordings from single fibers revealed a high sensitivity to step-like changes in T and IR radiation. Direct measurements of the time course of T during the first few milliseconds of exposure to IR radiation were not possible. Bullock and Diecke (1956) calculated 0.02°C within the membrane or a heat flux of 1 mW/cm\(^2\) of irradiated membrane surface. By using the amplitude of the generator potential as an indicator of sensitivity, Terashima et al. (1968) were able to lower this estimation to \(\sim\)100 \(\mu\)W/cm\(^2\). Bullock and Diecke (1956) let warm water at constant T flow across the membrane and then observed an increase in impulse frequency during the first 60 ms of transition to a higher water T. During this period the water T increased by only 0.003°C. Suggesting a similar rise time for the pit membrane, this value was referred to as the minimum rise in T needed to stimulate the sensory cells. It could be, however, that the rate of T change rather than the T difference is the significant stimulus parameter. The T rate is almost impossible to determine for rapid T changes, except indirectly by way of inference. However, the pit membrane has been shown to respond to its own change in T brought about by IR radiation.

There is another IR sense organ with a function resembling that of the facial pits of vipers, the pit organ of the buprestid beetle \textit{Melanophila} (Evans 1964, 1966; Schmitz et al. 1997; Campbell et al. 2002). These beetles detect IR radiation emitted by forest fires as far as 60–100 miles away. While the trees are still burning, the male and female beetles mate and the females lay their eggs in the bark of burnt trees. The IR organ consists a field of dome-shaped sensilla (diameter: 12–18 \(\mu\)m) located in a depression at the edge of both the right and the left middle coxal cavities (Schmitz and Bleckmann 1998). Each sensillum is innervated by a single sensory cell, which displays a distal structure resembling a mechanoreceptor. Electrophysiological recordings revealed that brief IR flashes of an intensity of 5 mW/cm\(^2\) evoke bursts of 2–3 action potentials (Schmitz et al. 1997; Schmitz and Bleckmann 1998). Linear extrapolation indicates that an intensity of 500 \(\mu\)W/cm\(^2\) might suffice for eliciting one action potential. These beetles appear to be an interesting example of an animal’s adaptation to a limited biotope by means of specialized receptor cells. However, there seems to have been no electrophysi-
ological investigation of the relationship between temperature changes of the sensilla and the discharge of the IR-sensitive cells.

**Function of the Warm Cells**

The existence of two types of sensory cells for detecting IR and T stimuli appears to be essential to prevent radiation from being confused by convection and thus to ensure successful host location in nature. Nonetheless, the two cell types respond to both IR radiation and air T. This is not an inadequacy of their function. On the contrary, it is essential for combinatorial coding, which requires that more than one sensory cell responds to each stimulus. These sensory cells have been termed warm cells and divided into two types according their relative excitability to IR and T stimuli.

The range of the $F$ scale that both warm cells assume depends on the combination of the kind of oscillating stimulus and background, as visualized in Fig. 6. During constant-amplitude IR oscillations, strong responses are elicited in still air (Fig. 6Aa), whereas medium responses occur in moving air at high background T (Fig. 6Ac) and low responses in moving air at low background T (Fig. 6Ab). Thus the effect of IR oscillations on the warm cell responses is masked by moving air, and the amount of masking increases with decreasing background T. Moving air seems to be a constraint for the receiver of the IR radiation, but a benefit for the hosts, enabling them to “hide” in a complex physical environment. As with acoustic perception, tone cannot be detected when there is too much noise around; masking by moving air at a low T impedes the detection of IR stimuli. When the bug is searching for a warm-blooded host in sylvatic or peridomestic shelters, or in human dwellings, the $F$ of the warm cells will tend to fall off in low-T moving air as the outside is approached. Inside the shelter or the dwelling, where there is no forced convection, moving in a direction...
producing higher $F$ values would take the bug nearer to the IR source.

During constant-amplitude T oscillations of an air stream flowing over the antennae, strong responses of the warm cells are elicited at high background IR radiation (Fig. 6Bc), whereas medium responses occur at medium background IR radiation (Fig. 6Ab) and low responses when the IR source was turned off (Fig. 6Ac). Thus the attractiveness of convective T stimuli increases with rising background IR radiation. As with visual perception, in which a color becomes attractive at the background of another color, background IR radiation enhances the detection of air-borne T stimuli. Background IR radiation is “sharpening the view” for the thermal quality of a location in which warm-blooded hosts likely occur. When searching in moving air for such a location, $F$ of the warm cells will tend to increase as the background IR radiation rises. To differentiate between locations of strong and low radiation intensities, the bug should move in a direction which results in higher $F$.

To date, the ability to detect IR and T stimuli has only been explored with respect to the intensity or the extent of the stimulus change affecting the sensory cells. We emphasize that the background conditions are also important when considering the detectability of IR and T stimuli. As described in this study, the background T may hide an IR source and the background IR may help to detect a thermally suitable location. Large changes in IR radiation or in ambient T have been generally considered to provide high detectability. When combined with forced convection at low T, however, IR radiation will not trigger a given intensity-dependence in warm-cell responses. With a background IR radiation value that enhances the responses to a T stimulus, slight T changes will provide a high detectability. Thus the background IR radiation and the background T determine which intensities of the IR and T stimuli are detectable and which ones are below the threshold. Since background IR radiation and background T change the response to T and IR stimuli, respectively, future studies must consider background effects when attempting to evaluate the responses of IR- and T-sensitive cells in natural situations.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

H.T. and L.M.Z. conception and design of research; H.T. and L.M.Z. performed experiments; H.T. and L.M.Z. analyzed data; H.T. and L.M.Z. interpreted results of experiments; L.M.Z. prepared figures; H.T. drafted manuscript; H.T. edited and revised manuscript; H.T. approved final version of manuscript.

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