Infrared detection without specialized infrared receptors in the bloodsucking bug *Rhodnius prolixus*

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Zopf LM, Lazzari CR, Tichy H. Infrared detection without specialized infrared receptors in the bloodsucking bug *Rhodnius prolixus*. J Neurophysiol 112: 1606–1615, 2014. First published June 18, 2014; doi:10.1152/jn.00317.2014.—Bloodsucking bugs use infrared radiation (IR) for locating warm-blooded hosts and are able to differentiate between infrared and temperature (T) stimuli. This paper is concerned with the neuronal coding of IR in the bug *Rhodnius prolixus*. Data obtained are from the warm cells in the peg-in-pit sensilla (PSw cells) and in the tapered hairs (THw cells). Both warm cells responded to oscillating changes in air T and IR with oscillations in their discharge rates. The PSw cells produced stronger responses to T oscillations than the THw cells. Oscillations in IR did the reverse: they stimulated the latter more strongly than the former. The reversal in the relative excitability of the two warm cell types provides a criterion to distinguish between changes in T and IR. The existence of strongly responsive warm cells for one or the other stimulus in a paired comparison is the distinguishing feature of a “combinatory coding” mechanism. This mechanism enables the information provided by the difference or the ratio between the response magnitudes of both cell types to be utilized by the nervous system in the neural code for T and IR. These two coding parameters remained constant, although response strength changed when the oscillation period was altered. To discriminate between changes in T and IR, two things are important: which sensory cell responded to either stimulus and how strong was the response. The label warm or infrared cell may indicate its classification, but the functions are only given in the context of activity produced in parallel sensory cells.

combinatorial code; discrimination between temperature and infrared radiation; electrophysiological recording; localization of warm-blooded host; two types of thermoreceptors

AN INSECT THAT DISTINGUISHES an increase in air temperature (T) from an increase in infrared radiation (IR) can do so because its sensory organs provide it with the necessary information. This implies that the neural response evoked by the two stimuli shows some consistent difference that is detectable by the insect’s central nervous system (CNS). It is difficult to determine exactly how the CNS decodes the incoming neural signals in a specific case, i.e., which feature is actually extracted from the spatiotemporal pattern of incoming neural activity. Nonetheless, studying the afferent sensory information may reveal whether or not a proposed encoding scheme can account for a given behavioral discriminative capability.

A relatively acute discriminating ability of the T and IR was demonstrated in bloodsucking bugs. *Triatoma infestans* and *Rhodnius prolixus* are attracted by an IR source and do not mistake changes in the power of IR for changes in air T (Lazzari and Núñez 1989; Schmitz et al. 2000). We identified two types of warm cells in peg-in-pit sensilla (PS) and tapered hairs (TH) on the antennae of *R. prolixus* (Zopf et al. 2014). The number of these sensory organs is small, and their position easily identifiable. Three PS are visible at the distal region of the first flagellar segment, and four to five in its midregion. Each peg is located at the bottom of a pit. The wall of the pit surrounds the peg and extends up the length of the peg. There it forms a small opening so that only the tip of the peg is exposed to the outside. Six to eight TH project from the distal margin of the segment. They lie close to the cuticular surface without touching it. The warm cells in the PS (PSw cells) and in the TH (THw cells) have been shown to respond more strongly to T pulses produced by switching between two air streams at different constant T than to IR pulses provided in still air (Zopf et al. 2014). None of these warm cells alone, however, is able to discriminate between T and IR pulses. This is because impulse frequency depends on the intensity as well as the nature of the pulse, making it impossible for a single warm cell to signal the stimulus unequivocally. This situation would be sorted out by some additional information such as the deflection of hairs and the movements of the antenna by mechanosensory cells that respond to the physical forces of airflow. From this information, the air pulse associated with the T stimulus will be identified and distinguished from the still air IR pulse by multimodal convergence. This, of course, requires integrating the excitation of the warm cells with those of the mechanoreceptors.

Localizing a warm-blooded host in a natural environment based on a series of interrupted samples of rapid changes in IR is challenging. This is because the altered frequency and intensity of discrete IR pulses may not solely be due to changes in the position of the host or the IR sensor. Intermittent stimulation may reflect physical hazards, obstacles, or competitors, and these factors vary with the type of habitat or host. IR, however, offers an advantage over olfaction or CO₂ in providing directional information about the host (Lazzari 2009). By definition, IR propagates radially. Once detected, therefore, the IR source must be in an unobstructed line of sight. A continuously, slowly rising or falling IR signal would mean that the IR sensor is moving straight toward or away from the IR source or, vice versa, that the IR source is moving straight toward or away from the IR sensor. Thus the IR sense is advantageous when used in host location because it offers a high spatial precision and little interference of environmental noise.

In this study, we examined the response characteristics of the PSw cells and the THw cells to slowly oscillating changes in both air T and IR power. Electrophysiological recordings
revealed the existence of small but clear-cut differences between the responses of the two types of warm cells to T and IR oscillations. This would appear to be the classic situation for a “combinatorial code” solution in which the activity in a sensory cell or type of cell does not signal a separate message, but its meaning is only given by a particular combination of the activity of other sensory cells. In itself, however, the term combinatorial code is somewhat vague. The difference and the ratio between the response magnitudes of the two warm cell types seem to be a more specific application of the principle.

Here, both coding parameters enable discrimination between oscillations in air T and in IR power. The basic ideas of this study are not limited to IR or T reception but may also be applicable to neural coding and processing in other sensory systems.

MATERIALS AND METHODS

Electrophysiological recordings. Laboratory-reared adult R. prolixus 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 electrolytically sharpened tungsten electrodes. One electrode was inserted lengthwise into the tip of the antennae, and the other at the base of the sensillum. Signals from the electrodes were amplified, band-pass (0.1–3 kHz) filtered, displayed conventionally, passed through a CED 1401plus (12 bit, 10 kHz; Cambridge Electronic Design) interface, and connected to a personal computer for online recording. The data were stored on a hard disk and analyzed offline using Spike2 software (Cambridge Electronic Design).

Stimulation. T stimuli were produced by an air stream continuously flowing over the antenna. Compressed air was cleaned, dried, and split into two streams. Their flow rates were equalized by matching the rates in mass flow meters, and their T was regulated by independent thermostats. After passing through electrical proportional valves (KWS 3/3; Kolvenbach), the two streams were combined to a single stream. The T 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 (analog-to-digital converter; CED 1401plus) of the proportional valves were phase-shifted by 180°. For stimulation, this stream was directed toward 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 a fine-wire thermocouple (wire diameter 13 µm; Type E: Cu-Cr/Cu-Ni; Campbell Scientific).

IR stimuli were provided by opening a shutter positioned in the path of the beam emitted by an Oriel Instruments 6580 Infrared Element (wavelength 1–25 µm). The T of the IR source and the shutter were measured with an IR thermometer (Volkert IR 800-20D). Stimulus intensity was calculated based on the Stefan-Boltzmann law using the formula (Ebert and Westhoff 2006):

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

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

Oscillating changes in radiation power were presented by varying the voltage to the Oriel Instruments 6580 Infrared Element. IR radiation was measured in the area radiated and close to the antenna by a T-calibrated IR thermocouple (Omega OS36). The output signal of the IR thermocouple was substituted for T in the formula of Stefan-Boltzmann to calculate radiation power.

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 so that the amount of data for long oscillation periods was kept small.

RESULTS

Identification. Most recordings from the PS and the TH revealed the activity of two antagonistically responding thermoreceptive cells, a warm cell and a cold cell. Both cells discharged at a quite steady rate as long as the T of an air stream blowing over the antenna did not change. They also exhibited a continuous discharge in still air to constant IR. The warm cells were identified by their responses to slowly oscillating changes in T or IR. When either the T of the air stream or the power of IR was made to rise and fall smoothly at varying rates, the discharge rate of the warm cells increased when T or IR rose and decreased as T or IR fell (Fig. 1A and B).

All warm cells examined responded the same way. They produced very regular discharge even during long oscillation periods. For this reason and because the capability to detect slight changes in T and IR are important for short-range orientation of a bug seeking a warm-blooded host, we studied the responses of the warm cells to trains of slowly oscillating changes in T and in the power of IR.

T oscillations. An effort was made to produce sinusoidal T changes. The obvious advantages were the repetition of measurements under nearly identical stimulus conditions and the fact that the instantaneous T and its rate of change vary differently with the oscillation period. By examining the response of a warm cell to T oscillations with different periods, it should be possible to determine the relative degree to which these two components of the T stimulus are contained in the discharge rates. The oscillation periods were 300 s (5 min), 600 s (10 min), and 1,200 s (20 min). The rate of the T change varied between −0.2 and +0.2°C/s during the 300-s oscillation periods and between −0.05 and +0.05°C/s during the 1,200-s periods. The amplitude of the T oscillations was ~15°C between 20 and 35°C. Of the 35 warm cells on which T oscillations were tested, only 20 qualified for this study, i.e., those for which the firing rate continued undiminished after at least 2 trains with different oscillation periods. These were from 10 PS and 10 TH. Figure 2A, a−c, shows the results of such an experiment.

Impulse frequency of both warm cell types displayed one clear maximum per T maximum and one clear minimum per T minimum. The ratio of frequency oscillations to T oscillations was always 1:1 even though the duration of the oscillation periods varied considerably and the rates of T change were permitted to assume many different values (Fig. 2A). The discharge rates of both cells took a parallel course to each other, with the THw cell above the PSw cell. In addition, the impulse frequency of both types tended to rise with increasing duration of the oscillation period.

In the examples shown in Fig. 2A, a−c, impulse frequency of the PSw cell oscillated between 1 and 10 per second for oscillation periods of 300 s and between 1 and 15 per second for the 1,200-s periods. In the THw cell, impulse frequency
varied between 2 and 16 per second for oscillation periods of 300 s and between 2 and 20 per second for the 1,200-s periods. This frequency increase with the increasing oscillation period indicates that parameters other than ambient T were driving impulse frequency up and down in this range. The rate of T change is the obvious candidate. It declines with increasing oscillation period. To estimate the simultaneous effect of T and its rate of change, impulse frequency of the two types of warm cells was plotted as a function of both parameters. Figure 2, B and C, shows that the frequency oscillations approached closed curves. They indicate a strong dependence of impulse frequency of the two warm cell types on the rate of T change and a lower dependence on instantaneous T. Multiple regressions (F = \( y_0 + a \frac{dT}{dt} + b \), where F is the impulse frequency, and \( y_0 \) the height of the regression plane) were calculated to determine the simultaneous effect of the rate of T change (a slope) and instantaneous T (b slope) on the impulse frequency for different oscillation periods. The slopes demonstrate the three properties that characterize the response of the two types of warm cells to T oscillations: 1) the sign of the a slope and the b slope is positive, that is, an increase in both instantaneous T and its rate of change raises the impulse frequency; 2) the b slopes of the two cells types are similar, that is, changes in instantaneous T have similar effects in the PSw cell and in the THw cell; and 3) the a slopes are steeper for the THw cell than for the PSw cell, that is, variations in the rate of T change have stronger effects on the former than on the latter. Note that the gradual change in sensitivity of the two types is not caused by fatigue because sensitivity did not decrease but increased with the duration of the oscillation period.

**Infrared oscillations.** A different sample of PSw cells and THw cells was subjected to slowly oscillating changes in the power of IR with periods of 300, 600, and 1,200 s. The range of IR rates varied between \(-0.1\) and \(+0.1\) mW·cm\(^{-2}\)·s\(^{-1}\) during the 300-s oscillation periods and between \(-0.01\) and \(+0.01\) mW·cm\(^{-2}\)·s\(^{-1}\) during the 1,200-s periods. The IR range covered in these experiments was \(-5\) mW·cm\(^{-2}\). Figure 3A gives an example of the response curves of the two cell types to a set of IR oscillations. Ten PSw cells and ten THw cells were tested with similar results.

During slowly oscillating changes in IR, impulse frequency of the PSw cell produced one clear maximum per IR maximum and one clear minimum per IR minimum. Furthermore, the discharge rate of the PSw cell increased with oscillation duration (Fig. 3A, a–c). The THw cell not only displayed lower discharge rates than the PSw cell, but also activity decreased with increasing oscillation period. In the THw cell, the 1:1 ratio between frequency oscillations and IR oscillations was clearly visible for oscillation periods of 300 and 600 s but not for 1,200-s periods. In Fig. 3A, a–c, impulse frequency of the PSw cell oscillated between 5 and 30 per second during oscillation periods of 300 s and between 5 and 45 during 1,200-s periods. For the THw cell, impulse frequency varied between 1 and 15 per second during oscillation periods of 300 s and between 1 and 10 per second during 1,200-s periods. The variation in the frequency curves indicates that the two cell types do not depend exclusively on the change in IR. The rate of change, which varies with oscillation duration, may also affect impulse frequency. To estimate the simultaneous effect of IR and its rate of change, impulse frequency was plotted as a function of both parameters. Figure 3, B and C, shows that the frequency oscillations approached closed curves. The course of the curves indicates a strong dependence of the PSw cell on the instantaneous radiation power and its rate of change but only a slight dependence of the THw cell on these two parameters. Multiple regressions (F = \( y_0 + a \frac{dRP}{dt} + b \))
were utilized to evaluate the simultaneous effect of instantaneous radiation power ($b$ slope) and its rate of change ($a$ slope) on the impulse frequency for the three different oscillation periods. The slopes demonstrate the two properties that characterize the two types of warm cells to IR oscillations: 1) the sign of the $b$ slopes is positive, that is, an increase in the instantaneous radiation power raises the impulse frequency in both warm cells; and 2) the sign of the $a$ slopes is negative, that is, a decrease in the rate of radiation power increases the impulse frequency of both warm cells. Because the THw cell responds less vigorously to IR oscillation than the PSw cell, the sensitivity for the instantaneous radiation power and its rate of change is less pronounced.

Fig. 2. A–C: responses of single warm cells of the peg-in-pit sensillum and the tapered hair to slowly oscillating changes in $T$. A: time course of impulse frequency of both cell types to $T$ oscillations with different periods. a: Oscillation period, 300 s. b: 600 s. c: 1,200 s. B: impulse frequency of the warm cell of the peg-in-pit sensillum during the oscillation periods shown in A, a–c, plotted as a function of instantaneous $T$ and the rate of $T$ change ($\Delta T/\Delta t$). Multiple regressions that use 3-dimensional planes ($F = Y_0 + a \Delta T/\Delta t + b T$, where $Y_0$ 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. C: impulse frequency of the warm cell of the tapered hair during the oscillation periods shown in A, a–c, plotted as a function of instantaneous $T$ and its rate of change. The differential sensitivity for instantaneous $T$ and its rate of change are indicated by the coefficients $b$ and $a$ in the equation of the regression plane, $F = Y_0 + a \Delta T/\Delta t + b T$. 

$\Delta T/\Delta t$.
It is striking that sensitivity of the two types of warm cells for the instantaneous radiation power has a positive sign and the rate with which the radiation power changes a negative sign. One would expect the combination of two positive signs, that is, the effect of IR is reinforced by the rate of change. In this case, impulse frequency of the two types would be high when the radiation power is high and higher still when the radiation power is also rising. However, the recordings in Fig. 3A reveal that impulse frequency continues to rise even though the rate of change decreases. Thus the frequency maxima are not in step with the IR maxima but rather lag behind. The reason could be that the reduction of the radiation power does not cause a corresponding

Fig. 3. A–C: responses of single warm cells of the peg-in-pit sensillum and the tapered hair to slowly oscillating changes in IR. A: time course of impulse frequency of both cell types to IR oscillations with different periods. a: Oscillation period, 300 s. b: 600 s. c: 1200 s. B: impulse frequency of the peg-in-pit sensillum during the oscillation periods shown in A, a–c, plotted as function of instantaneous RP and the rate of RP change. Multiple regressions that use 3-dimensional planes (F = y0 + a dRP/dt + b RP) were calculated to determine the differential sensitivity for instantaneous RP (b slope) and the rate of RP change (a slope) on the response frequency. C: impulse frequency of the warm cell of the tapered hair during the oscillation periods shown in A, a–c, plotted as function of instantaneous RP and its rate of change. The differential sensitivity for instantaneous RP and its rate of change are indicated by the coefficients b and a in the equation of the regression plane, F = y0 + a dRP/dt + b RP.
decrease in T of the sensory organs. In effect, the faster the power of radiation is falling, the slower the warm cells reach equilibrium T with instantaneous IR.

**Combinatorial coding.** The results in Figs. 3A and 4A indicate that the two types of warm cells have the potential for combinatorial coding of IR and T. Combinatorial coding occurs when the relevant sensory information is not encoded by the activity of a single sensory cell or a single cell type but in the relationship between the responses of different cells or types. This relationship must have certain features that permit identifying the stimulus. Combining the responses by a simple additive or averaging process will not eliminate the ambiguity introduced by the dependence of the two warm cell types on the stimulus amplitude. Two coding parameters are worth considering here, namely the difference between the absolute response magnitudes of the two warm cells and their ratio. In as far as the coding parameter for changes in T and IR are disjoint, the two stimuli should be distinguishable based on the combinatorial response.

**Response difference.** Difference curves were calculated from the frequency values of the two warm cell types at corresponding points in time during the same period of T or IR oscillations. These curves were examined for their ability to discriminate oscillations in T and IR throughout the three oscillation periods. When the responses of the PSw cells are subtracted from those of the THw cells, the difference between the frequency values ($\Delta F = F_{\text{hair}} - F_{\text{peg}}$) is then either positive for T oscillations or negative for IR oscillations (Fig. 4A); conversely, subtracting the THw cells responses from those of the PSw cells ($\Delta F = F_{\text{peg}} - F_{\text{hair}}$) yields a difference that is either negative for T oscillations or positive for IR oscillations (Fig. 4B). The value $\Delta F = 0$ becomes a “dividing line” between the difference values for T and IR oscillations. Note that the difference curves for lengthy IR oscillations slightly overlap the dividing line (Fig. 4, A and B). In these regions, slow changes in IR power are not discriminable from slow T changes. However, these overlaps were generated by the very low discharge rates of the two types of warm cells, which were difficult to confirm as a significant change.

The advantage of treating each warm cell individually and pairing them selectively is that the scatter of points about the characteristic curves is very small (Figs. 2 and 3). The scatter, however, must be greater when dealing with mean differences from lumped data. We considered this possibility extensively. To avoid redundancy, the mean difference curves we show are formed by subtracting the responses of the PSw cells from those of THw cells ($\Delta F = F_{\text{hair}} - F_{\text{peg}}$) but not the reverse differences ($\Delta F = F_{\text{peg}} - F_{\text{hair}}$). Even with this restriction, two main approaches remain, both of which compare frequency curves for the three oscillation periods. The first approach is to obtain an average difference curve from all individual difference curves that can be formed by pairing each PSw cell with each THw cell (Fig. 5). The second approach, not shown here, is to average the output of each warm cell type and then form the difference curves of the averages. Both methods corroborate the conclusions drawn from single pairs of warm cells (Fig. 4). Throughout the whole range of T and IR oscillations, the two cell types yield average difference curves that differ in sign for T and IR oscillations. As shown for the single pair of warm cells, the mean difference curves slightly overlap the dividing line ($\Delta F = 0$) when the rate of IR change is very low (Fig. 5). In these situations, the discharge rates of the two cells are very low, probably close to the level of excitation. The lumped data indicate that slowly oscillating T changes can be discriminated from slowly oscillating IR changes simply by forming the differences between the responses of the PSw cell and those of the THw cell.

**Response quotient.** The division of T and IR oscillations into two groups, one generating positive differences ($+\Delta F$) between the responses of the two warm cell types and the other...
The period, $F_{\text{hair}}$ as a function of $F_{\text{peg}}$ (Fig. 6) or, conversely, $F_{\text{peg}}$ as a function of $F_{\text{hair}}$, can be illustrated by plotting, for each $T$ and IR oscillation from $T$ changes generating $Q$. The slope of the line, $F_{\text{hair}} / F_{\text{peg}}$ area) and IR changes (gray area).

The implication is not that the responses of one warm cell type depend on those of the other type. Rather, both depend in each frequency value on a third parameter, which is a change in $T$ or IR power at a given instantaneous $T$ or IR level, respectively. As is evident from Figs. 2 and 3, the plots in Fig. 6 indicate closed curves for each oscillation period that are separated into groups of points: those from $T$ oscillations and those from IR oscillations. 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 $T$ oscillations is larger than $Q = 1$ for any oscillation period to the left and above the boundary, and $Q = F_{\text{hair}} / F_{\text{peg}}$ for IR oscillations is smaller than $Q = 1$ for any oscillation period to the right and below the boundary. Although there are no overlapping $Q$ values for $T$ oscillation, slightly overlapping $Q$ ranges are generated in the low IR range for slow IR changes. Here, $Q$ values do not permit identification of IR changes. However, these $Q$ values are formed by low frequency values close to the excitation threshold.

Mean $Q$ values were determined by two approaches. The first approach was to obtain an average quotient curve from all individual quotient curves that can be formed by pairing each PSw cell with each THw cell. The second approach was to average the output of each warm cell type and then form the quotient curves of the averages, as illustrated in Fig. 7. For corresponding points in time of given periods of $T$ and IR oscillations, the mean frequency values and standard deviations of the 10 THw cells were plotted as a function of the mean frequency values (and standard deviations) of the 10 PSw cells. The course of the mean quotient values produce closed curves, and the 4 standard deviations (2 from the THw cells and 2 from the PSw cells) indicate for each time point the range where 68% of the $Q$ values for a given oscillation period are found. In the case of $T$ oscillations, the standard deviations of the THw cells came close to the boundary $Q = 1$ or even overlapped slightly in the range of very low rates of change. This is because low rates of $T$ or IR change elicit low responses in the two warm cells, and at low response levels either the numerator or the denominator can assume values that are quite large in relation to the other. A cutoff response level for the two cell types would probably be essential to keep the $Q$ range for $T$ and IR oscillations from becoming very wide and thus reintroducing the ambiguity $Q$ was supposed to eliminate.

The cumulative evidence from the pooled data in Fig. 7 shows that the general discrimination of $T$ and IR oscillations...
DISCUSSION

An interesting characteristic of the antennal thermoreceptive system of the bloodsucking bug *R. prolixus* is the presence of two morphologically distinct types of sensory organs, the PS and the TH (Zopf et al. 2014). Each sensory organ houses a cell pair consisting of a warm cell and a cold cell responding antagonistically to changes in air T. As in other arthropods such as the tick *Ixodes ricinus* (Gingl and Tichy 2001) and the mosquito *Aedes aegypti* (Gingl et al. 2005), the two types of warm cells of the bug’s antenna are excited not only by increasing air T, but also by increasing IR power. However, well-designed behavioral experiments have convincingly shown that bloodsucking bugs are able to discriminate between T and IR stimuli (Lazzari and Núñez 1989; Schmitz et al. 2000).

Combinatorial coding. Here, we have shown that information about T and IR stimuli is encoded by the relative amount of activity elicited in the two warm cell types associated with the PS and the TH. Slow changes in T produce stronger responses in the PSw cells than in the THw cells, whereas slow changes in IR produce stronger responses in THw cells than in the PSw cells. The existence of strongly responsive warm cells for one or the other stimulus in a paired comparison is the distinguishing feature of a “combinatory coding” mechanism. This mechanism enables the information provided by the difference or the ratio between the response magnitudes of both cell types to be utilized by the nervous system in the neural code for T and IR. These two coding parameters remained constant, although response strength changed when the oscillation period was altered.

The discrimination between T and IR oscillations by means of combinatorial coding implies parallel processing. This requires early divergence to yield two sets of axonal branches, one for determining changes in T and the other for changes in IR power. Accordingly, the discharge rates in two different sets of interneurons would be a function of changes in either T or IR power. Note, however, that the two processes the bug’s brain might use to combine the sensory inputs of simultaneously responding warm cells, the response differences and the response quotients, are logically simple and estimated based on the actual recorded action potentials. More sophisticated coding parameters cannot be excluded. The impulse frequency of the two cell types is linearly related to the instantaneous T and its rate of change as well as to the instantaneous IR and its rate of change. Of course linear functions facilitate the formation of response differences or response quotients.

Responses to T and IR pulses. The two types of warm cells respond not only to oscillating changes in the T of an air stream moving at constant velocity over the antenna, but also when, in still air, an air stream at constant T higher than ambient is directed rapidly onto the antenna. Furthermore, the warm cells are excited not only by oscillating IR changes, but also by rapidly opening a shutter positioned in the path of the IR beam. Such rapid, pulselike changes in air T or IR cause the discharge rate of the two cells to increase rapidly. The activity increase of the PSw cells to both T and IR pulses was always larger than that of the THw cells (Zopf et al. 2014). Thus, at different stimulus amplitudes, T and IR pulses evoke robust responses in the former and smaller responses in the latter. During slowly oscillating changes in T, however, the THw cells produce stronger responses than the PSw cells. The lower sensitivity of the THw cells during T pulses might be explained by the slower rise time of T within the TH than indicated by the thermocouple. The T of the receptive site within the TH may lag behind that of the air stream stimulus due to mixing with the still air around the antenna. The possibility of assigning instantaneous T values of the thermocouple to the receptive site exists only when the T wave front is not steep but when T changes at low rates. Similar arguments, however, cannot be applied to the PS. Thus the reversal of the T pulse sensitivity can have two explanations: 1) differences in the physiological properties of the two warm cell types; and 2) differences in the design of the PS vs. TH. Such design differences may reflect physiological characteristics of the sensory organs and the behavior of the animal where they are found.

Comparison. Similar experiments have been conducted only on the warm cells of the PS on the antennal tip of the mosquito.
A. aegypti (Gingl et al. 2005) and on the warm cells of the hairlike sensilla on the tarsi of the tick I. ricinus (Gingl and Tichy 2001). These cells display very similar responses to T oscillations as the bug’s warm cells. In the tick, the differential sensitivity is 1.5 impulses per second per degrees Celsius for periods of 1,000 s, and in the mosquito, 3.4 impulses per second per degrees Celsius for periods of 2,000 s. In the bug, the corresponding value is 0.9 impulses per second per degrees Celsius for 1,200-s periods. However, the bug responds more strongly to IR oscillations than the tick and mosquito; differential sensitivity in the tick is 0.1 impulses·s⁻¹·mW⁻¹·cm⁻² for periods of 100 s and in the mosquito 0.8 impulses·s⁻¹·mW⁻¹·cm⁻² for similar periods. In the bug, the PS values lie between 2.2 and 3.1 impulses·s⁻¹·mW⁻¹·cm⁻² for periods between 300 and 1,200 s, and the TH values between 5.3 and 7.1 impulses·s⁻¹·mW⁻¹·cm⁻² for the same periods. Moreover, the bug’s absolute response magnitude is higher than that of the tick and mosquito, but the range of radiation power covered by IR oscillation is lower. This relatively higher sensitivity for IR oscillations seems to be an adaptation for detecting warm-blooded host, and the different sensitivity for T and IR oscillations an adaptation for discriminating between changes in T and IR power. The key to these properties involves physical and physiological factors that increase the general level of IR sensitivity, as outlined in a recent study of the bug’s warm cell responses to pulselike changes in T and IR power (Zopf et al. 2014).

Dependence on IR oscillations: shift in sign. As might be expected, impulse frequency of the two warm cells increases with instantaneous radiation power (Fig. 3, B and C). Here, the sign of the differential sensitivity is positive (b slope). However, the sign of the differential sensitivity to the rate of IR change is negative (a slope). As the regression planes obtained for the three oscillation periods indicate, the higher the instantaneous radiation power, the higher the impulse frequency (b slope). Impulse frequency, however, is higher still when radiation power is also falling (a slope). Accordingly, there may be some low rate of change that would no longer have a detectable effect. Here, impulse frequency would vary only with instantaneous radiation power. It can be argued that the positive dependence of impulse frequency on the instantaneous IR and the negative dependence on the rate of IR change are defined by the series of events leading from IR stimulation to excitation. For example, warming up the sensory organs during the increase in the instantaneous IR values may be faster than cooling down during dropping IR values. Thus the thermal effect of IR on the receptive sites within the sensory organs may not be in phase with the oscillating radiation power. The thermal effect may lag behind the IR oscillation, and the lag may be longer during the falling than the rising phase of radiation power. Such a phase shift has not been observed in the tick (Gingl and Tichy 2001) or mosquito (Gingl et al. 2005). It may well reflect differences in the mechanisms underlying IR sensitivity of the bug’s warm cells. This clearly calls for further experimentation.

Possible functions. IR emitted from the surface of a warm-blooded host radiates in straight paths in all directions like visible light. The stimulus field of a discrete radiant source has definite directions, so that an increase and decrease in the radiation power can be distinguished based on the relative angular orientation of the radiating surface and the receiver. As the distance to the host decreases, the radiation power increases (Stefan-Boltzmann law; see above): the distance to the heat source can be derived easily (Lazzari 2009). Another advantage of perceiving and distinguishing both air T and IR is that these two heat-exchange mechanisms behave differently and follow different laws. Air T gradients allow a higher exchange of thermal energy by conduction, increasing the ability to detect weak signals, but they are perturbed by air turbulence, including ascending convection currents produced by differences in air T. Moreover, the amount of energy transported by the air depends on its humidity. IR contains relatively less energy but is independent of turbulences and allows better spatial definition of the properties of the source such as size or borders (Lazzari 2009). The presence of obstacles between the insect and the radiant object can also be detected. Finally, warm cells assist in the search for warm-blooded hosts by providing background information on ambient T, the direction and rate at which ambient T is changing. Such information is important both generally and specifically, e.g., finding specialized ecological niches and distinguishing between warmed and shaded sites. The present analyses demonstrate that data on changes in both air T and IR power can be extracted by simply comparing the discharge rates of two types of warm cells, one located in PS and the other in TH.

Optimal sensitivity range or specificity. The description of single sensory cells in terms of distinct types is an expedient first step for the discovery of peripheral coding mechanisms and the explanation of sensory function in general. A quantitative best-stimulus classification would be misleading for sensory cells that participate in the encoding of IR and T stimuli like the warm cells in the TH and PS. The function of the two warm cells would be given in the relationship of their activity to changes in IR and T. The present study shows that specific tuning of individual sensory cells is not a general necessity to provide fine discrimination between IR and T changes. Broadly tuned sensory cells are helpful if not essential for the economic representation of information of IR in the peripheral nervous system. Only by employing a range of IR and T stimuli and by distinguishing carefully between evidence for specifically or broadly tuned warm cells will it be possible to resolve the issue of IR detection.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

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