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DISSERTATION

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Electrophysiological studies of infrared detection in the bloodsucking bug *Rhodnius prolixus*

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1. Introduction

Preface

Ever since Bullock and Diecke (1956) observed surprisingly high temperature sensitivity in the infrared pit organs of crotaline vipers, the problem of what information is coded, and how it is coded have been topics for lively discussions in the field of infrared reception. If the nerve endings innervating the pit organs respond to temperature changes, what sets so-called infrared receptors apart? Evidence for specialized infrared sense organs have been provided in different animals, including *Crotaline* and *Boid* snakes, vampire bats, ticks and insects (for review see Campbell et al. 2002).

While some investigators have preferred to present only infrared stimuli, other have been less parsimonious. In fact, all infrared sense organs that have been examined for temperature sensitivity, showed to develop respectable rates of discharge to slight temperature changes of the surrounding medium. Thus there are two kinds of information confounded. As an informal guess one would suggest, that the brain obviously has at its disposal perfectly good, unadulterated information about temperature in the form of the discharge of those units which are more selective than the ones we are now discussing. Measuring temperature could be left over to those receptors which are only slightly affected by infrared radiation and measuring infrared radiation to those receptors which are only slightly affected by temperature. This matter has been made a key issue in thermoreception. Those leaning towards the theory of "patterns" like to point towards mixed temperature- and infrared units as disproving any concept of strict specificity. Those who think in "labeled lines" prefer to dismiss this as insignificant imperfection.

Ultimately the question cannot be resolved by weighing possibilities but must be asked from the receptor cell itself, for what seems the best method of information processing may not be the choice of the evolutionary process. The question remains, whether the nervous systems uses separate transmission lines for encoding temperature and infrared radiation, which makes the mixing of diverse data unnecessary. The alternative position is that the activity in a single receptor cell or type of receptor cell is not signaling a separate message, but that its meaning is only given in its participation in a pattern of activities across a set of receptor cells or classes of receptor cells.

Most studies aiming at the labeled line and pattern position were not specifically devised to distinguish between pure thermoreceptors and pure infrared receptors, and the data may be interpreted in terms of either position. Since the temperature dimension in infrared reception

is very hard to define and manipulate in a quantitative way, other studies may have been somewhat hampered by addressing directly the coding of infrared radiation. This applies especially to intensity differences between the temperature and the infrared quality, a parameter that would be most amenable to sensory analysis. Since the responses of temperature and infrared receptors can be modulated by stimulus qualities and its intensity as well, the response of any particular sensory cell is typically ambiguous with respect to either parameter. If temperature and infrared radiation are coded by the pattern of activity across a set of receptor cells, then intensity and quality would not be confounded since the pattern elicited at different intensities would be similar in shape, varying primarily in amplitude. According to this pattern model, temperature and infrared radiation should exhibit different patterns of activity across a set of receptor cells. Thus I studied the capabilities of discriminating temperature and infrared radiation at various intensities in the antennal thermoreceptors of the bloodsucking bug *Rhodnius prolixus* (Fig.1).

I was led to this study by the considerations outlined below. One was the concept of quality distinction in the sense organs of the skin, which emerged as a cornerstone in the coding of information of the immediate environment. Below the epidermis, in the dermal connective tissue, lie bare or free nerve endings in addition to morphologically specialized sensory structures of myelinated nerve endings. Temperature or pain are sensed by free nerve endings, and there are others which have mechanoreceptive properties, related to complex sensation such as itch and tickle but also serving touch. In addition to specialized temperature receptors some sensory receptors respond to both mechanical stimulation and temperature. These bimodal receptors show a fairly high sensitivity to temperature changes with a temperature-dependent baseline discharge. They may contribute to different kinds of sensation. Another topic was the facial pit organ of snakes which do not fit with the predictions of infrared specificity. These sense organs enable their possessors to strike successfully at warm-blooded pray in the dark (Goris and Terashima, 1976). The receptors are free nerve endings terminating in the ultra-thin (< 25 μm) pit membrane that acts as the heat sensing surface, capable of detecting a 0.003°C change in the temperature of water flowing across the pit membrane.

Principals of neural coding: Specificity versus pattern theory

The specificity theory was confounded by Johannes Müller in 1837, who concluded that different modalities of sensation are the result of the stimulation of specific nervous pathways, which he called “labeled lines” (Belmonte and Viana, 2008; Craig, 2003 and Perl, 2007). This theory is based on the assumption that sensory receptors are maximally sensitive to a specific form of a stimulus (e.g., pain, heat, cold, etc.) but it is not the stimulus which determines the perception but the neural pathway and its projection site in the brain. This theory was soon supported by the discovery of specific fiber types that respond preferentially to touch or to cold and warm temperatures (Hunt and McIntre, 1960).

The pattern theory on the other hand states that receptors are polymodal and can respond to a wide range of stimuli. The definite perception is determined only through the pattern of activity from several nerve fibers. An example of polymodal receptors are the non-selective cationic TRP channels (Transient Receptor Potential). Of particular interest for multimodal thermoreceptors are the vanilloid receptors (TRPV) (Caterina et al., 1997; Jordt and Julius, 2002).

Pit organs of snakes: Responding to IR radiation and temperature

The facial pit organs, which rattlesnakes and their relatives share with the family Boidea, consist of a rich innervated membrane in places less than 25 μm thick. The membrane is supplied by all three branches of the trigeminus nerve. Individual fibers undergo repeated branching (Bullock and Fox, 1957) and form a trigeminal nerve mass about 10 μm thick and 40 mm in diameter (Terashima et al., 1970).

In crotaline vipers the membrane lies at the bottom of a pit on either side of the head above the upper lip and between the eye and nostril. It is suspended along its perimeter. Beneath it is an air chamber with an opening to the surface. Thus insulated from other tissue, this thin membrane can be quickly influenced by the temperature of close objects, either warming up slightly when presented by an object warmer than its surrounding, or losing heat when directed at cooler objects. Due to its position at the bottom of a pit, it is somewhat shielded from fluctuating temperatures of air currents. This location also provides directionality.

Although the infrared radiation, corresponding to the temperature the membrane detects, is 1 to 20 μm and therefore ten or more times greater than that of visible light, it is still short enough, to cast a fairly sharp heat shadow across the membrane, when the heat source is sufficiently off center. Presumably the directionality is enhanced by deepening the pit and

thus increasing the ratio of its depth to the diameter of the membrane. Goris and Terashima (1976) described experiments in which the scales covering the snake's eyes were blackened. Thus blinded, the snake strikes successfully at fixed and moving warm objects. This behavior corresponds to the snake's nocturnal hunting habits and fits in with recordings from single units in the optic tectum where neurons were found with overlapping fields of 50° or 60°.

That such an organ is quite sensitive to warm and cool objects in its surroundings is clear enough, but to determine the threshold of individual neurons is not easy. The membrane can be irritated by a calibrated incandescent heat source whose distance from the pit can be varied arbitrarily. But because the thickness of the membrane can be less than 15 μm and the distance from its outer surface to the receptors much less, direct measurements of the temperature course within the membrane during the first few critical milliseconds of exposure are hardly possible and assumptions have to be made regarding heat absorption and transfer during this period. Bullock and Diecke (1956) made a conservative calculation of 0.02°C within the membrane, or a heat flux slightly over 1 mW/cm² of irradiated surface. Using generator potential as an indicator, Terashima et al. (1968) were able to reduce this estimate to 100 μW/cm². Another approach made by Bullock and Diecke (1956) was to let warm water at constant temperature flow gently across the membrane and then, after the flow as such has ceased to stimulate, to warm the water by about 0.1 °C and observe the response during the transition. Impulse frequency rose 50% during the first 60 msec. As indicated by a small thermocouple in the stream, water temperature during this period increased by only 0.003°C. The high heat capacity of water suggests a similar rise time for both thermocouple and membrane under these conditions and thus provides an estimate of 0.003°C as the minimum rise in temperature (ΔT) needed to stimulate the organ clearly. It could be however, that the rate of temperature change (dT/dt) rather than the temperature difference (ΔT) is the significant stimulus parameter. Unfortunately its value is almost impossible to determine for fast temperature changes with sufficient accuracy, since the time course of temperature recorded is that of the thermocouple, not of the organ, except indirect by way of inference.

Due to the results of the above experiments, it can be reasonably assumed that to what the membrane is really responding, is its own change in temperature, as brought about by infrared radiation from the object it faces. There is an alternative hypothesis, however. The slight changes in temperature might be only a concomitant effect and the low energy infrared photons might trigger photomechanical processes in the membrane analogous to those in photoreceptors. Either hypothesis is beset with the difficulty of explaining the detection of very small changes, whether in temperature or in infrared radiation as such. But regardless of

the nature of their primary processes, the pit organs are obviously stimulated by warm objects, as the behavior of snakes demonstrated, and the response of the organs bears a clear relationship to temperature changes in the membrane in any case. Consequently it can be classed as thermal.

Hensel (1974, 1976) found that there is more in determining the response of the facial pits of Boas than only the magnitude of the temperature jump (ΔT). Beginning at 20°C, he stimulated the organ with a series of rapid rises in temperature, each of 5°C. The response magnitude not only depended on the sudden rise in temperature, but also on the temperature level from which the temperature rise was initiated. The consequences of these experiments are important. First, they place these receptors in a framework not very different from that of other temperature receptors, since the response of other temperature receptors to rapid temperature change is also dependent on initial temperature – cold fibers in the lingual nerve of the cat, for example, observed by Hensel and Zotterman as early as 1951. Second, they indicate that a given response is ambiguous. The same temperature jump produced different responses at different initial temperatures. Thus, the same responses could be produced by a variety of combination of temperature jumps and initial temperatures. Such a situation has been demonstrated for insect cold cells as well (Gingl et al., 2005). In the case of pit organs, the ambiguity may not be very important. When individual receptors in the membrane are suddenly confronted with an object several degrees warmer than its surroundings, their impulse frequency shoots to high values capable of indicating the presence of a warmer object to the central nervous system. But how necessary or even advantageous it would be for the snake to be able to determine that an object was very close to the surface temperature of a mouse and not merely warmer than its surrounding may be questionable. The position of the object together with its direction and manner of motion might offer a predatory animal better clues regarding suitability than a precise measurement of its temperature, particularly in the dark. But how spatial and temporal resolution of these organs or their ability to discriminate temperatures is correlated with the hunting habits of their possessors remains to be examined.

Pit organs of pyrophilous beetles: Responding to IR, but no attempts of testing complementary temperature stimuli

There appears to be another infrared sense organ with a function closely resembling that of facial pit organs of vipers. Evans (1964, 1966a,b) describes it in the case of the bupestrid beetle, *Melanophila accuminata*. Active during the heat of the day, bupestrids are attracted to tree trunks warmed by the sun. But *Melanophila accuminata* even flies to the scene of forest fires. The larvae of the beetles depend on the bast and the wood of recently burnt trees as a food source. The beetles lay their eggs near the root level in trunks of trees which fresh fire damage has exposed to attack (Palm, 1962). The organ responsible is a pair of sensory pits located directly behind the mesothoracic legs. Each pit has 50 – 70 hemispherical sensilla, ranging in size from about 15 to 20 microns in diameter, and each having a small, centrally located indentation. Next to each sensillum is an associated wax gland, which secrets a fibrous waxy matrix covering the pit. There is no exocuticle in the pit area, and only a very thin layer of mesocuticle covers the sensillum's sphere. It is thought that these sensilla are derived from sensilla trichodea, and it appears that signal transduction is essentially mechanical in nature. Infrared energy may cause fluid inside the sphere to expand slightly, thereby compressing the distal tip of the receptor cell. In a general sense, the sensilla are fluidic converters of infrared radiation into an increase in the pressure inside the sphere which then stimulates the mechanoreceptor. By means of electrophysiological recordings it was shown that brief infrared flashes at a radiation power of 24 mW/cm² elicited phasic responses with up to 7 spikes. Further experiments in which gentle movements of the recording electrode were used as mechanical stimuli have led Schmitz and Bleckmann (1998) to the conclusion that the infrared receptors function by means of a photomechanic process. A model for this infrared sensor is the Golay sensor, which is filled with a liquid instead of a gas (Klocke et al., 2011). Unfortunately, testing the effect of temperature stimuli has been neglected so far. Therefore it remains an open question whether an increase in ambient temperature may elicit similar responses as an increase in the power of infrared radiation so that infrared stimuli are confounded with temperature stimuli, or more precisely, whether the sensilla do fit well with the predictions of specificity theory or its opponent, the pattern theory.

Infrared sensing in bloodsucking bugs

The bloodsucking bugs *Triatoma infestans* (Klug) and *Rhodnius prolixus* Stàl (Fig.1) act as the most important vectors of Chagas disease, a protozoan infection of man and other mammals caused by *Trypanosoma cruzi* (Chagas). This trypanosomiasis constitutes not only one of the most serious sanitary problems in Latin America but has an important social and economic impact on the region (Dias and Schofield, 1999). About 8 million people are infected with this disease and millions more are at risk of becoming infected (WHO, 2014). The haematophagous bugs, commonly called kissing bugs, live in many different natural habitats in contact with birds and mammals. They have been found in bird nests, rock piles, hollow trees, rodent dens and caves in which bats roost, but are adapted to human dwellings like cracks and crevices in walls made of dried mud thatched roofs, and peridomestical habitats like chicken coops, guinea-pig runs, and goat corrals. Chagas disease, a deadly disease that may result in irreversible damage to the nervous system, the skeletal muscle tissue and the heart, is transmitted to humans via the feces of the kissing bug. The bug acquires the flagellated trypanosome from an infected mammal and retains it for life. After the bug defecates or after feeding, the parasite may penetrate the wound left by the feeding insect. Bug disease vectors use different sensory modalities to locate a blood source such as thermo-, hygro-, mechano- and olfactory chemoreception. I will review in the chapters that follow studies concerning the effects of host-emitted odors and heat.

Nùñez (1982) found that *R. prolixus* is able to orientate towards odors emitted by a human arm or a mouse, but also towards odors released by a cage previously occupied by a mouse, or to CO_2. Likewise, Taneja and Guerin (1995) observed upwind anemotaxis in *R. prolixus* and *T. infestans* towards airstreams transporting CO_2, mouse odors or volatiles from rabbit urine. Barrozo and Lazzari (2004) investigated the behavioral responses of *T. infestans* to CO_2 and reported threshold values between 300 and 400 ppm over the ambient level. The authors observed that L-lactic acid was not attractive to *T. infestans* but when L-lactic acid was offered together with CO_2, a synergetic effect occurred between these two odors, resulting in an attractive response.

The pioneering study of Wigglesworth and Gillett (1934) on the searching behavior of *R. prolixus* revealed up and down movements of the antennae in the presence of heat sources. In choice experiments two heat sources (test tubes) were used which were kept at the same surface temperature. One tube was clean and polished; the other was covered with lampblack. *R. prolixus* did not prefer one of the two tubes. Thus the authors concluded that the bugs were not able to perceive IR radiation. However, this conclusion was based on the assumption that

the emissivity of the blackened tube was many times greater than that of the clean one. But Schmitz et al. (2000) pointed out that this assumption is not correct. The emissivity of glass is in the long-wave IR range ~0.98 (Stahl and Miosga, 1986) and that of a surface covered with lampblack is nearly identical, namely ~0.95 (Glückert, 1992). Therefore, the experiments of Wigglesworth and Gillett (1934) could not exclude IR sensitivity in *R. prolixus*.

By means of a different experimental approach, Lazzari and Nùñez (1989) were able to demonstrate IR sensitivity in *T. infestans*. Their experiments revealed that the locomotory activity and the antennal movements of the bugs are significantly increased in the presence of an IR stimulus. In some cases which were, however, statistically not significant, the locomotory activity was oriented towards the IR source. To exclude the stimulating effect of convective heat by warm air, a polyethylene window was positioned between the IR source and the bugs. However, Schmitz et al. (2000) noted that polyethylene absorbs IR radiation very efficiently at ~7 µm and between 13 and 14 µm. Therefore a slight warming of the polyethylene window by a few hundredth of a degree cannot be excluded. Although the temperature of the polyethylene window was measured before and after the experimental trials with an accuracy of 0.1°C it is possible that warm air escaped from the polyethylene window. The only chance to overcome this problem is to cool the polyethylene window below ambient temperature.

Schmitz et al. (2000) set up a pure IR stimulus by using a sapphire window that transmits electromagnetic radiation from the visible range of the spectrum up to a wavelength of 7 µm. Human skin at 32°C has its emission maximum between 9 and 10 µm, but emissivity already starts at 4 µm (Hardy and Muschenheim, 1934). In the experiments with pure IR stimulation, *R. prolixus* is attracted by wavelengths shorter than 7 µm. It was also observed that *R. prolixus* walked with a low speed of 1 to 2 cm s^{-1} when approaching the IR source (Schmitz et al., 2000). But when disturbed, the bugs ran quickly with a speed of ~10 cm s^{-1}. This agrees with the searching behavior of *T. infestans* where running phases alternate with standing phases (Taneja and Guerin, 1995; Flores and Lazzari, 1996). In conclusion, Schmitz et al. (2000) provided evidence that R. prolixus is not only able to sense IR radiation but also to approach an IR source in complete darkness. However, IR receptors have not yet been convincingly identified in bloodsucking bugs. Specialized IR receptors have been described only in buprestid beetles of the genus Melanophila (Evans, 1964; Schmitz et al., 1997; Schmitz and Bleckmann, 1997, 1998) but in bloodsucking bugs it is even not clear whether ordinary thermoreceptors may serve the function of IR receptors by responding to the warming effect that occurs during the absorption of IR radiation.

Fig.1. The bloodsucking bugs *Triatoma* (left) and *Rhodnius* (© by L.M. Zopf)

Fine structure of antennal sensilla in bloodsucking bugs

In spite of the considerable medical importance of triatomine bugs, little is known about the sensory equipment of the antennae. There are some morphological observations on the antennal sensilla which are either limited to light microscopy (Wigglesworth and Gillett, 1934), superficial transmission electron microscopy (Chaika, 1980), or are yielding a SEM investigation of hair-like sensilla (Bernard, 1974) or trichobothria for taxonomic purposes (Lent and Wygodzinsky, 1979). By using both SEM and EM techniques, McIver and Siemicki (1984, 1985) provided a detailed description of the antennal sensilla of *R. prolixus*. In addition to numerous mechanoreceptive sensilla, the authors described various types of sensilla characterized by a perforated wall and five sensilla with a nonporous wall (np-sensilla). While the wall-pore sensilla were suggested to act as olfactory receptors, the np-sensilla were assumed to be thermo-hygroreceptive (Fig.2). The np-sensillum appears as short, rounded peg set into a pit surrounded by a depression, and it is innervated by three sensory cells with unbranched dendrites. Two dendrites extend to the tip of the peg and contain a large number of microtubules. The third dendrite terminates near the base of the peg and partially wraps around the other two dendrites. In an extensive morphological and electrophysiological investigation of np-sensilla in different insects, Altner and Loftus (1983) revealed structural features adapted for hygro- and thermoreception. Usually np-sensilla contain a triad of sensory cells displaying excitatory responses to cold air, moist air and dry air. Bernard (1974) reported from electrophysiological studies on *T. infestans* that three sensory cells are present in peg-shaped sensilla: one increases it's discharge rate with increasing humidity, the second with decreasing humidity, and the third with decreasing

temperature. It seems probable that the three sensory cells in the np-sensilla of *R. prolixus* (McIver and Siemicki, 1985) have similar sensitivities.

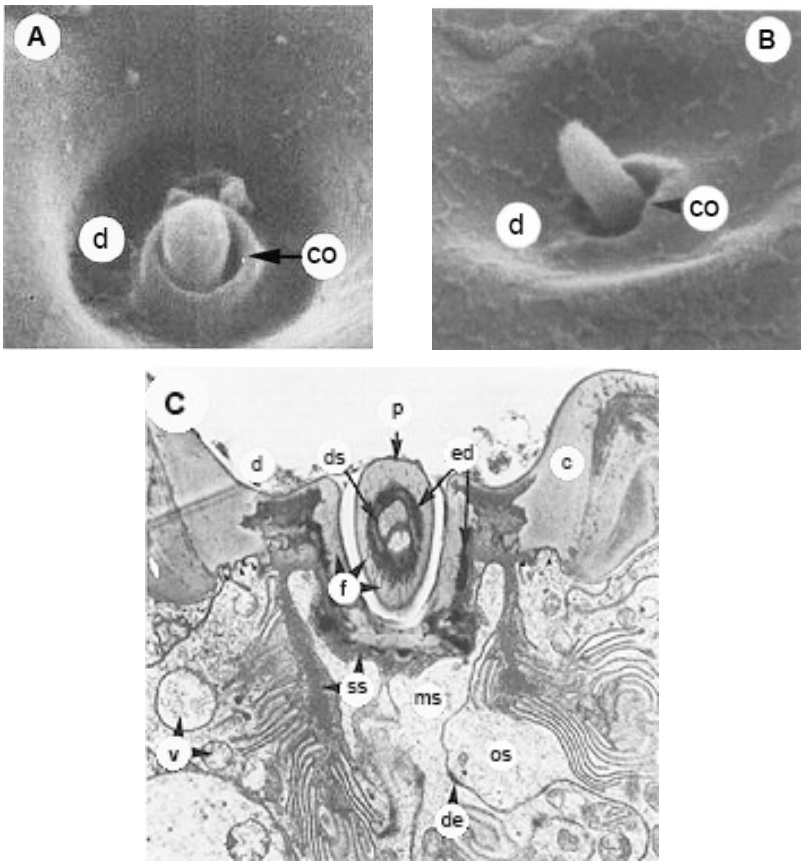

Fig.2. A-C: Non pore, peg-in-pit sensilla on the first flagellar segment of *Rhodnius prolixus*. **A**: REM of peg-in-pit sensillum located in a deep depression (d); the collar (co) of the pit extends up the length of the sensillum (x 11300). **B**: REM of peg-in-pit sensillum that located in a shallow depression (d); the collar (co) of the pit is low (x 9100). **C**: SEM of oblique section of peg-in-pit sensillum located in a depression (d) of the antennal cuticle (c). The pore-less cuticle of the peg (p) encloses the unbranched dendrites of two sensory cells which contain many microtubules. Filaments (f) filled with electron-dense material (ed) radiate into the peg cuticle. A dendritic sheath (ds) surrounds the dendrites. The middle sheath cell (ms) and the outer sheath cell (os) are visible, also vacuoles (v) and the sensory sinus (ss) (x 20700) (from McIver and Siemicki 1985[1]).

[1] used with kind permission of Susan McIver, 2014

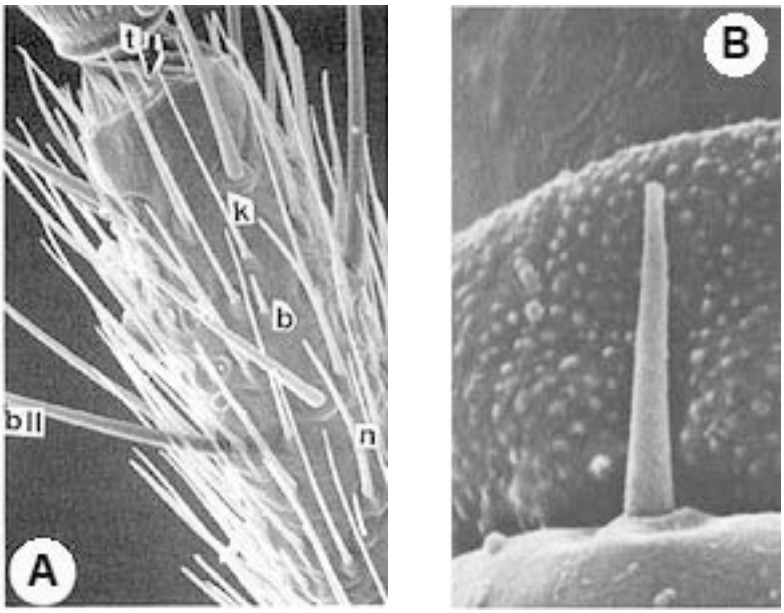

Fig. 3. A,B: SEM of tapered hairs on the first flagellar segment of *Rhodnius prolixus*. **A**: Small tempered hair (t) located on the distal margin of the flagellar segment. Visible are thin (n) and thick-walled (k) sensilla trichoidea and basiconica (b), as well as various bristles (b II) (500) (from Catala and Schofield, 1994)[2]. **B**: Closer view of a tapered hair (x 4560) (from McIver and Siemicki 1984[3]).

Catala and Schofield (1994) provided a REM study of the distribution of various sensilla types on the antennae of ten species of *Rhodnius* and found the thermo-hygroreceptive np-sensilla in all species examined– three at the distal margin of the second flagellar segment and several more on the first. Furthermore, the authors investigated the topography and morphology of several hair-like sensilla including the small tapered hairs which are located on the distal margin of the first flagellar segment (Fig.3A,B).

[2] used with kind permission of Silvia Catalá, 2014.
[3] used with kind permission of Susan McIver, 2014

Identification of two types of IR-sensitive thermoreceptors in the bloodsucking bug *Rhodnius prolixus*

The first challenge that had to be mastered was the discovery and identification of a receptor that would respond to a change in temperature. Several earlier works describe peg-in-pit sensilla on the antennae of the bug *Rhodnius prolixus*, which were suspected to be thermo-hygroreceptors (Altner and Loftus, 1985) but so far no one could successfully prove this general assumption by means of electrophysiological recording techniques.

By using the techniques described below (Material and Methods) I stimulated several types of receptors on the bug's antennae by switching two airstreams of constant temperature. I didn't extend recordings on the proboscis, because SEM pictures didn't reveal any sensilla structures indicating thermoreceptor function and moreover by removing the antennae bugs were completely disorientated with regard to thermal sources (Wiggelsworth and Gillet, 1934).
I was successful in identifying two morphologically different types of thermoreceptors:
The peg-in-pit-sensilla and the tapered hairs, which are located at the distal end of the first flagellum.
The peg-in-pit-sensillum revealed a warm cell, it's antagonistic a cold cell and a moist cell, which could be easily distinguished by the size of their amplitude (Fig.4). In the recording of the tapered hairs, only a warm cell was present (Fig.5).

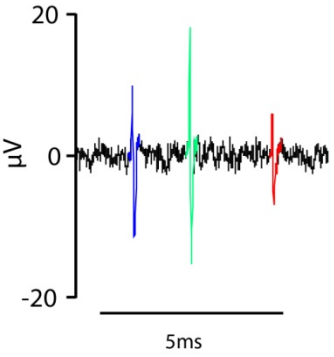

Fig.4: Original recording from a peg-in-pit sensillum. Blue: action potential of a cold cell. Green: action potential of a warm cell. Red: action potential of a moist cell.

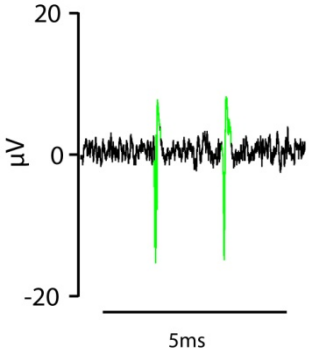

Fig.5: Original recording from a peg-in-pit sensillum. Green: action potentials of a warm cell.

By switching from a cold airstream to a warm airstream, the impulsfrequency of the peg-in-pit's warm cell rose significantly, while the activity of the cold cell decreased (Fig.6A). For

the identification of the moist cell, two airstreams with different relative humidity, but the same temperature were blowing alternately over the antenna. By switching from a dry airstream to humid air, an increase in the impulsfrequency of the moist cell was shown. The activity of the cold cell was reduced by a small amount and the warm cell's activity showed a little increase (Fig.6B). The warm cell of the tapered hair showed a similar reaction to an increase in temperature like the warm cell of a peg-in-pit (Fig.7).

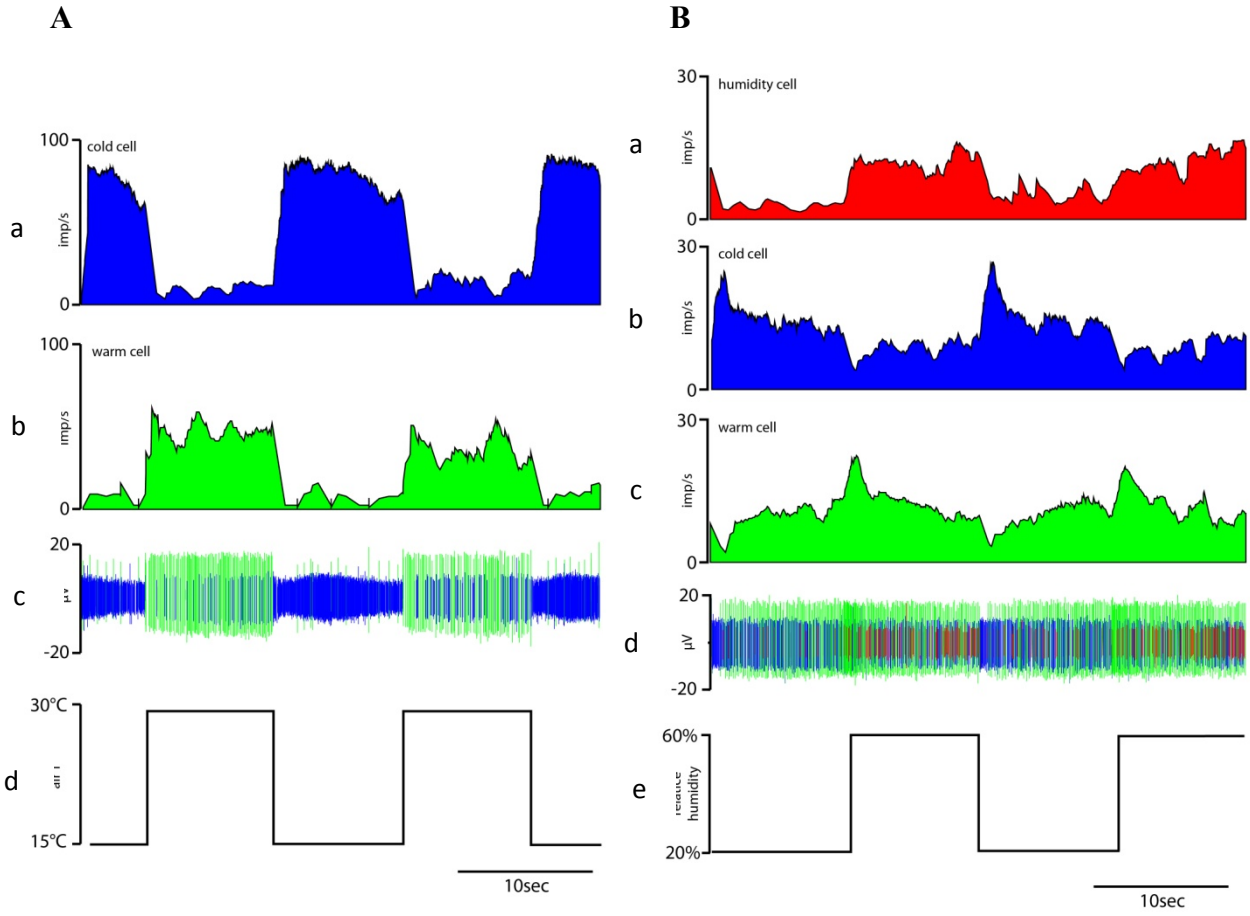

Fig.6.A,B: **A**: Recording from a peg-in-pit-sensillum during step-like temperature changes. **a:** Discharge rate of the cold cell **b:** Discharge rate of the warm cell **c:** Simultaneously recorded activity of a warm cell (green) and a cold cell (blue) **d**: Temperature pulses provided by shifting between two air streams at 10seconds interval. Water vapor pressure of both airstreams was 3.7 mbar. **B:** Recording from a peg-in-pit-sensillum during step-like humidity changes. **a:** Discharge rate of the moist cell **b:** Discharge rate of the cold cell **c:** Discharge rate of the warm cell **d**: Simultaneously recorded activity of a warm cell (green), a cold cell (blue) and a moist cell (red) **e:** Humidity pulses provided by shifting between two airstreams at 10 seconds interval. Temperature of both airstreams: 18°C.

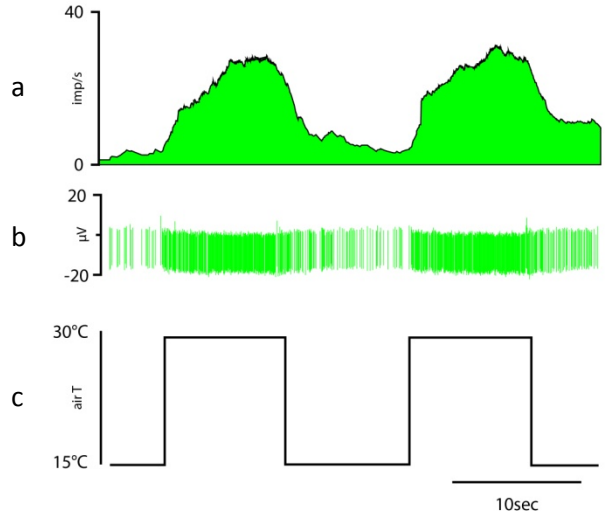

Fig.7: Recording from tapered hair during step-like temperature changes. **a:** Discharge rate of the warm cell **b:** Activity of a warm cell (green) **c:** Temperature pulses provided by shifting between two air streams at 10 seconds interval. Water vapor pressure of both airstreams was 3.7 mbar.

2. Material and Methods

Temperature and infrared stimulation

In order to identify thermo- and hygroreceptive sensory cells I utilized the methods described by Tichy (2007). Temperature and humidity stimuli were presented by way of three airstreams, flowing over the antenna. Two of the streams (*a* and *b*) had the same partial pressure of water vapor (Pw) but different temperatures (T), and two (*b* and *c*) had the same T but different Pw. Each airstream could be directed separately onto the preparation, which was about 20 mm away from each nozzle. Rapid step-like changes in temperature or humidity were produced by switching from a conditioning air stream at steady conditions of temperature and humidity to another at different steady temperature or humidity. Because it is extremely difficult to measure the humidity of an airstream precisely without changing or disrupting the flow, Pw was not be monitored during the experiment but rather adjusted to psychrometrically calibrated values. Temperature was measured by a thermocouple positioned within the air stream. It should be noted that the temperatures measured by the thermocouple apply directly to the airstream in which it is located and less directly to the sensillum or it's receptive site. The possibility of assigning instantaneous temperature values to the receptive site exists when temperature is changing only if the rate of temperature change during the experiments is slowly. Under these conditions the difference in temperature between the sensillum and the air stream will be insignificant. Thus, during slow and continuous temperature changes, the temperature of the air stream stands for that of the receptive site.

Slowly oscillating temperature changes were provided by a single uninterrupted air stream at rates in the range ±0.02°C s^{-1}. 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 temperatures were regulated by independent thermostats. After passing through electrical proportional valves (KWS 3/3, Kolvenbach KG) 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 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 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)

$$\frac{\sigma * \mathrm{A} * (\mathrm{T}_2{}^4 - T_1{}^4)}{\tau * D^2} = W/cm^2$$

in which σ is the radiation constant of Stefan-Boltzmann ($5.67 * 10^{-8}$ W/m^2 * K^4); A the radiating area (3.5 * 3.5 mm^2); T2 the temperature of the radiating surface; T1 the temperature of the shutter, which corresponds to 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/cm². Oscillating changes in radiation power were presented by varying the voltage to the Oriel IR element. IR radiation was measured in the area radiated and close to the antenna by a temperature-calibrated IR thermocouple (Omega OS 36). The output signal of the IR thermocouple was substituted for T in the formula of Stefan-Boltzmann to calculate radiation power.

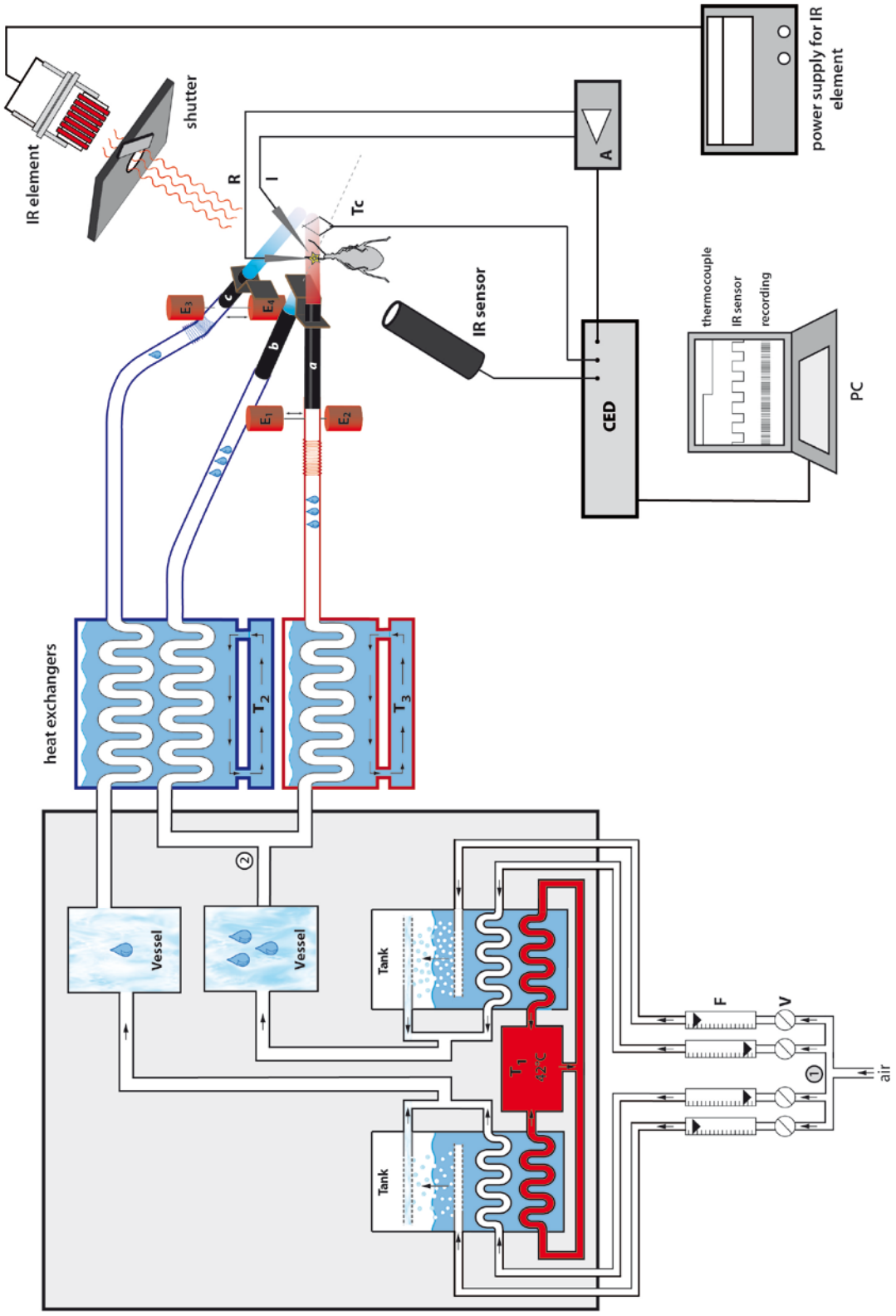
IR element
shutter
R
I
Tc
A
power supply for IR element
IR sensor
CED
thermocouple
IR sensor
recording
PC
heat exchangers
T_2
T_3
E_1
E_2
E_3
E_4
a
b
c
Vessel
Vessel
Tank
Tank
T_1
42°C
F
V
air
1
2

Fig. 8. Delivery system for rapid changes in temperature and infrared radiation used in the study of the bug's peg-in-pit sensilla and tapered hairs. Flow diagram illustrating the production of three air streams, two (*a, b*) at the same partial pressure of water vapor but at different temperatures, and two (*b, c*) at the same temperature but different partial pressures of water vapor. Compressed air was reduced by needle vales, dried, cleaned and divided at ① into two air streams to be set at different values of water vapor pressure. Each stream was further split into two substreams and their flow rates adjusted by needle valves (*V*) and monitored continuously by flow meters (*F*). One substream bubbled out through many holes in a polyethylene tubing firmly anchored in a tank of ion-exchange water at constant 42°C. Temperature was controlled by *thermostat 1*. The second substream was conducted through the spiral tube in the same tank but remains dry when it was also warmed to 42°C. After emerging from the tank, the two substreams were combined in a single stream variable in water-vapor content from dry to almost saturated. Homogeneity of mixture was enhanced by a 2 liter series connected vessel. By dividing one mixed air stream (at ②) and sending it through two separate heat exchanges that were controlled by *thermostats 2* and *3*, rapid shifts in temperature can be made at a common but variable water vapor pressure. To produce sudden changes in water vapor at the same temperature, the air stream of the second subunit passed through a heat exchanger controlled by *thermostat 2*. Air stream from any of the 3 jets (*a*, *b* or *c*) can be brought to bear on a common point of intersection (*star*) where the antenna is located. Stream *b* is always directed at the preparation (*star*), but is deflected (not interrupted) by gate attached to *a* when electromagnet (E_1) is deactivated and snaps into position shown. Similarly when upper electromagnet (E_3) is deactivated, so that stream *c* is directed at the preparation, gate attached to *c* deflects stream *b*. Only when both upper electromagnets (E_1 and E_3) are activated, stream *b* is not deflected. Infrared stimuli were provided by an Oriel IR element (type 6580, wavelength of 1 to 25 μm). A rapid, step-like increase in IR-radiation was produced by opening a camera shutter fixed in the path of the beam. IR-radiation was measured within ± 2 % by a temperature calibrated IR-thermocouple (Omega OS 36) inserted into the spot near the preparation. The output signal of IR-thermocouple was substituted for temperature in the formula of Stefan-Bolzmann to calculate radiation power (mW/cm²). *A* amplifier, $E_{1,2,3,4}$ electromagnets, *F* flowmeter, *I* indifferent electrode, *R* recording or different electrode, $T_{1,2,3}$ thermostats, *Tc* thermocouple, *V* needle valve.

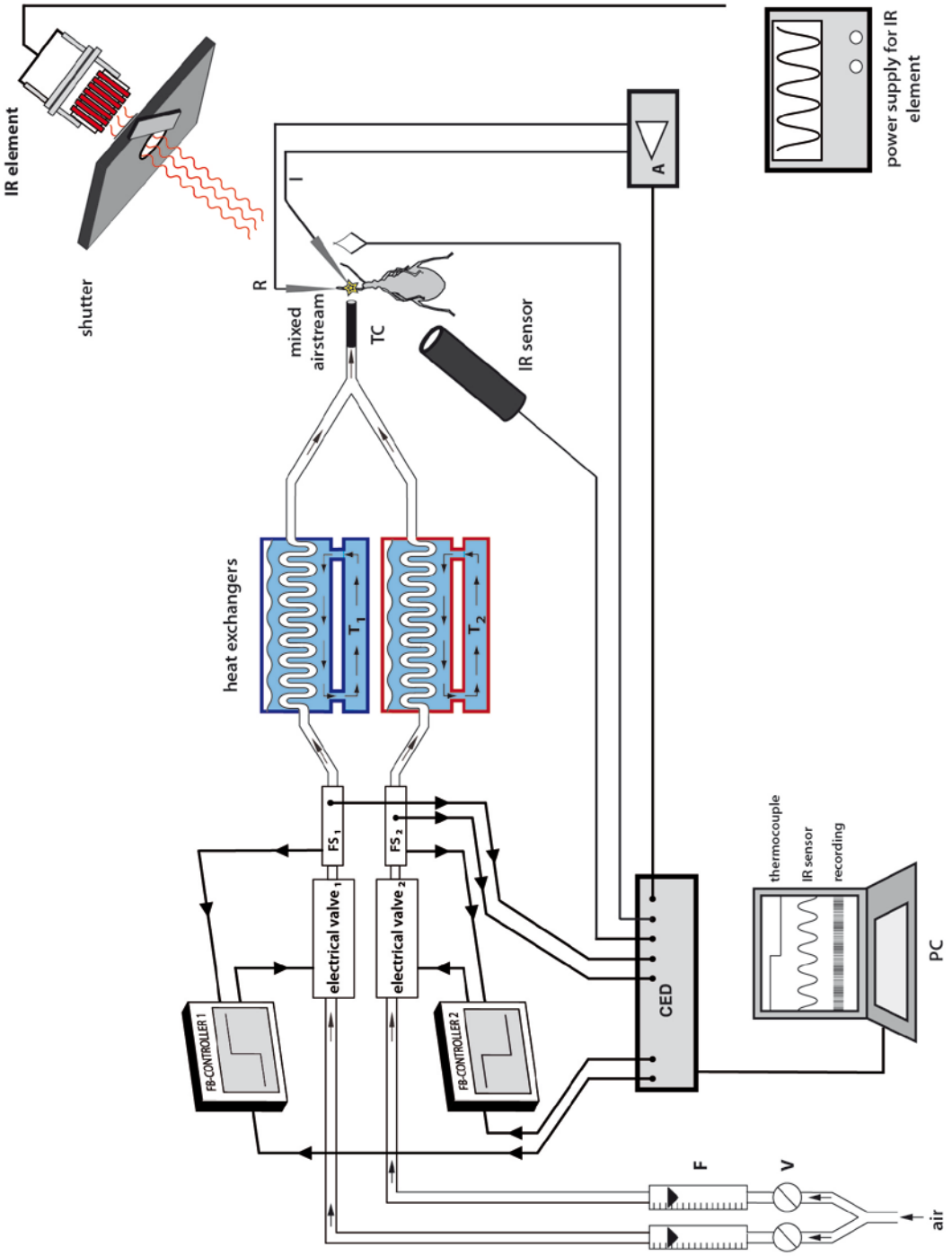
IR element
shutter
R
I
A
power supply for IR element
mixed airstream
TC
IR sensor
heat exchangers
T1
T2
FS1
FS2
electrical valve 1
electrical valve 2
FB-CONTROLLER 1
FB-CONTROLLER 2
CED
thermocouple
IR sensor
recording
PC
F
V
air

Fig. 9. Delivery system for slowly oscillating changes in temperature and infrared radiation used in the study of the bug's peg-in-pit sensilla and tapered hairs. Flow rate of two air steams was kept constant by needle valves (*V*) and flowmeters (*F*). Each air stream was passing through an electrical proportional valve, an air flow sensor ($FS_{1,2}$) and led though two separate heat exchanger that were controlled by *thermostats 1* and *2*. The two streams were then united to a single air stream. The temperature of this air stream was modulated by mixing the 2 airstreams in a ratio determined by the proportional valves by means of the output sequencer function of the data acquisition software (spike 2, Cambridge Electronic Design, CED), using a self-written sequencer script. The flow rate of the mixed airstream was held constant be shifting the phase of the control voltages (A-D converter, 1401plus, CED) of the proportional valves by 180°. A feedback linearization, which integrated the voltages used to control the proportional valve with those received from the flowmeters, counteracted any deviations of the flow rate set by the output sequencer. Slowly oscillating changes in radiation power were provided by varying the voltage to an Oriel IR element (type 6580, wavelength of 1 to 25 µm). IR-radiation was measured within ± 2 % by a temperature calibrated IR-thermocouple (Omega OS 36) inserted into the spot near the preparation. The output signal of IR-thermocouple was substituted for temperature in the formula of Stefan-Bolzmann to calculate radiation power (mW/cm²). *A* amplifier, *CED* data acquisition interface, $electric\ valve_{1,2}$ proportional valves, *F* flowmeter, $FB\text{-}controller_{1,2}$ feedback controller of proportional valves, $FS_{1,2}$ flow sensors, *I* indifferent electrode, *R* recording or different electrode, $T_{1,2}$ thermostats, *Tc* thermocouple, *V* needle valve.

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

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Zopf LM, 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.

antennal sensilla; 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 Nùñez (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 at 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

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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 identity 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. prolixus* 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. prolixus* bugs were anesthetized with CO_2 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 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)

$$\left[\sigma \times A \times \left(T_2^4 - T_1^4\right)\right] / \left(\pi \times D^2\right)$$

in which σ is the radiation constant of Stefan-Boltzmann (5.67×10^{-8} W/m^2 $\times$ K^4); A the radiating area (3.5×3.5 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 of them with 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 (Δx) was presented by Loftus and Corbiére-Tichané (1981). The equation is

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

in which $|b|$ is the mean absolute slope of the stimulus-response functions, σ^2 the variance of the individual deviations of points about their respective regressions, γ the required probability (90%), and $\Phi^{-1}{}_{(\gamma)}$ is the inverse of the distribution function of a standardized, normally distributed, random variable. $\Phi^{-1}{}_{(0.9)} = 1.28$ (Diem and Lentner 1968, see tables, p. 28). In the case of a linear regression, σ^2 is estimated by

$$\sigma^2 = \frac{\sum \varepsilon^2}{n - 2I}$$

where ε 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 (χ^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.

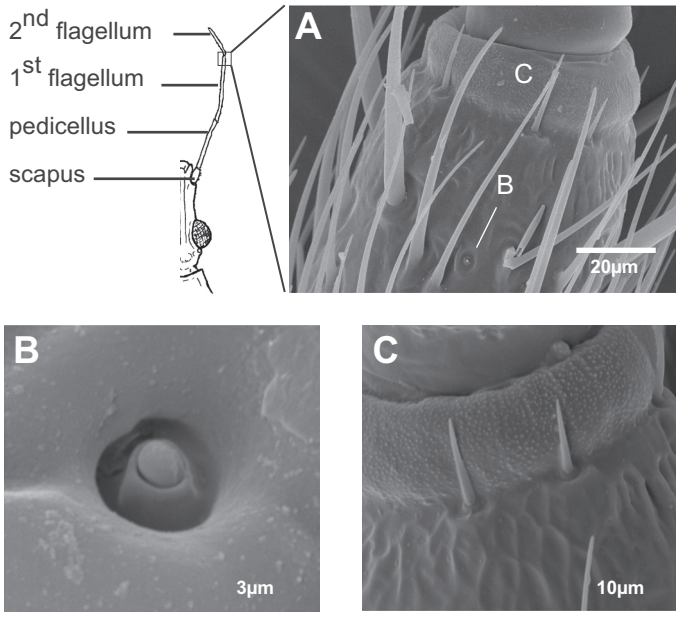

Fig. 1. *A*: scanning electron micrograph of flagellar segment 1 showing the peg-in-pit sensillum in the mid-region and the tapered hair near the distal margin. *B*: peg is set deeply in the pit and the collar of the pit extends the length of the peg. *C*: conical shaft of the tapered hair points to the antennal tip; the hair base is surrounded by a ring of cuticle.

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, *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. 2*A*, 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. 2*B*). 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.

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

Type of Sense Organ	Peg-in-Pit	Tapered Hair
Cone length, m	1.28	14.65
Base diameter, m	1.11	1.78
Surface area, m^2	3.41	42.18
Volume, m^3	0.41	11.45
Surface-to-volume ratio	7.7	3.6

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.

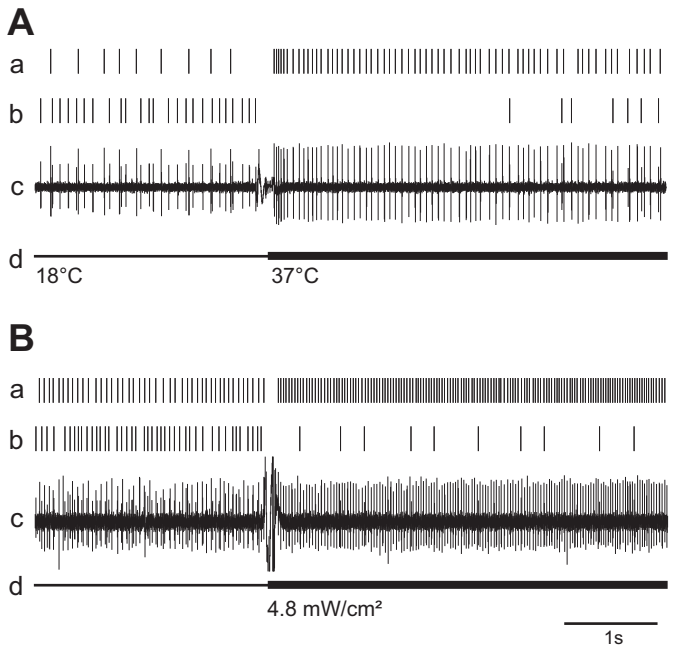

Fig. 2. Simultaneously recorded activity of a warm cell and a cold cell from a peg-in-pit sensillum to pulses in temperature (T) and infrared radiation (IR). *A*: T pulse produced by shifting from a constant-temperature air stream to another at higher constant temperature. *B*: IR pulse produced by opening 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*A* also shows that the spike trains of both warm cells such as in Fig. 2*A* 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*

Type of Sensillum	Peg-in-Pit	Tapered Hair
Stimulus: convective heat		
Range over which temperature pulses were tested, °C	22 to 38	22 to 38
Units used for regression analysis	12	7
Mean coefficient of determination, R^2	0.98 ± 0.01	0.95 ± 0.03
Mean differential sensitivity to temperature steps, (imp/s)/°C	2.02 ± 0.15	1.26 ± 0.18
Increment of temperature steps, °C, which results in an increment of 1 imp/s	0.48	0.79
Mean resolving power, °C	1.23	2.08
Stimulus: radiant heat		
Range over which pulses in radiant power were tested, mW/cm^2	0.5 to 5	0.5 to 5
Units used for regression analysis	12	7
Mean coefficient of determination, R^2	0.94 ± 0.04	0.93 ± 0.06
Mean differential sensitivity to steps in radiant power, (imp/s)/(mW/cm^2)	13.8 ± 4.5	16.0 ± 3.8
Increment of steps in radiant power, (imp/s)/(mW/cm^2), which results in an increment of 1 imp/s	0.07	0.06
Mean resolving power, mW/cm^2	0.5	0.5
Stimulus: convective plus radiant heat		
Range over which temperature pulses were tested, °C	18 to 28	18 to 28
Range over which pulses in radiant power were tested, mW/cm^2	1.5 to 5	1.5 to 5
Units used for regression analysis	6	6
Mean coefficient of determination, R^2	0.87 ± 0.02	0.95 ± 0.02
Mean differential sensitivity to temperature, (imp/s)/°C	0.96 ± 0.03	2.59 ± 0.5
Increment of temperature, °C, which results in an increment of 1 imp/s	1.0	0.3
Mean differential sensitivity to steps in radiant power, (imp/s)/(mW/cm^2)	1.9 ± 0.4	3.8 ± 0.3
Increment of steps in radiant power, (imp/s)/(mW/cm^2), which results in an increment of 1 imp/s	0.5	0.2

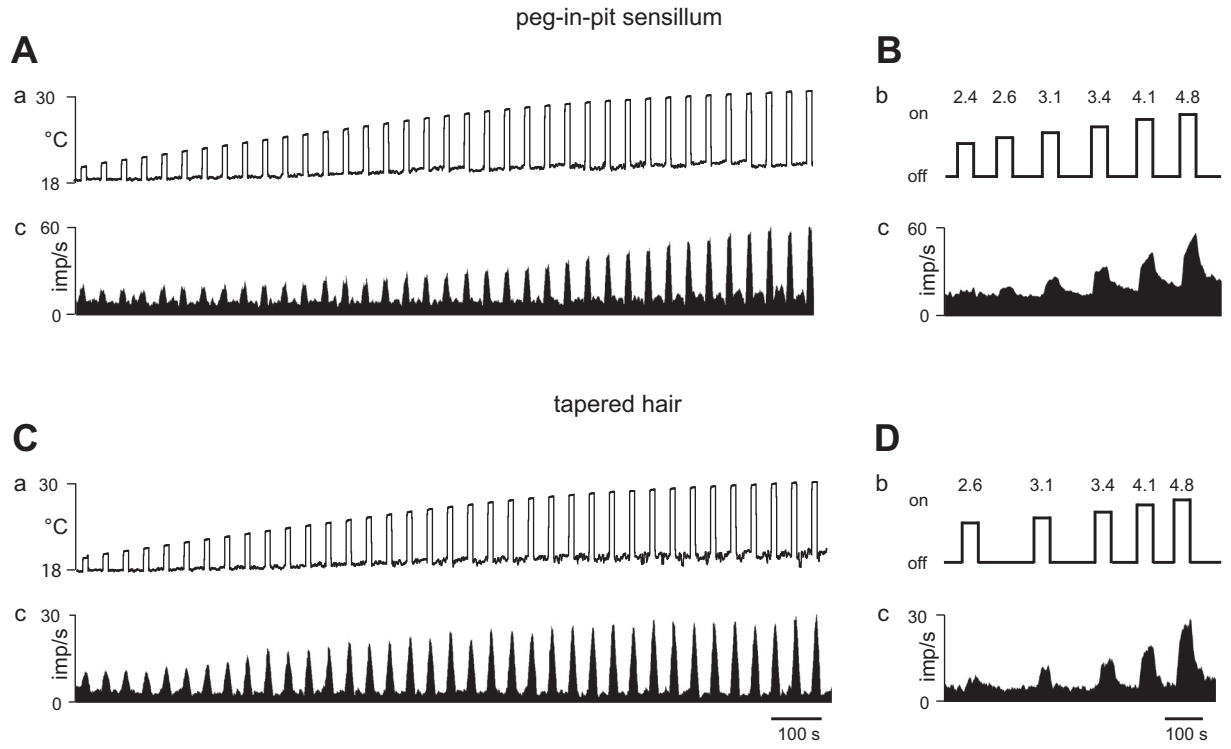

Fig. 3. Discharge rates of warm cells to graded series of T and IR pulses. For descriptive purposes, only the sequences with increasing intensities are shown. *A* and *B*: warm cells from peg-in-pit sensilla. *C* and *D*: warm cells from tapered hairs. *a*, temperature pulses provided by shifting between two air streams at different temperatures. *b*, IR pulses provided by opening a camera shutter; numbers indicate power of radiant heat in mW/cm^2 used for stimulation. *c*, response rates determined for 1-s periods.

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/cm^2. 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/cm^2. 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/cm^2) with a standard deviation of ± 4.5 for the peg-in-pit sensillum and 16.0 ± 3.8 (imp/s)/(mW/cm^2) 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/cm^2 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/cm^2 (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/cm^2 for the warm cell of the peg-in-pit sensillum and ~1.1 mW/cm^2 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/cm^2 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/cm^2 (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.

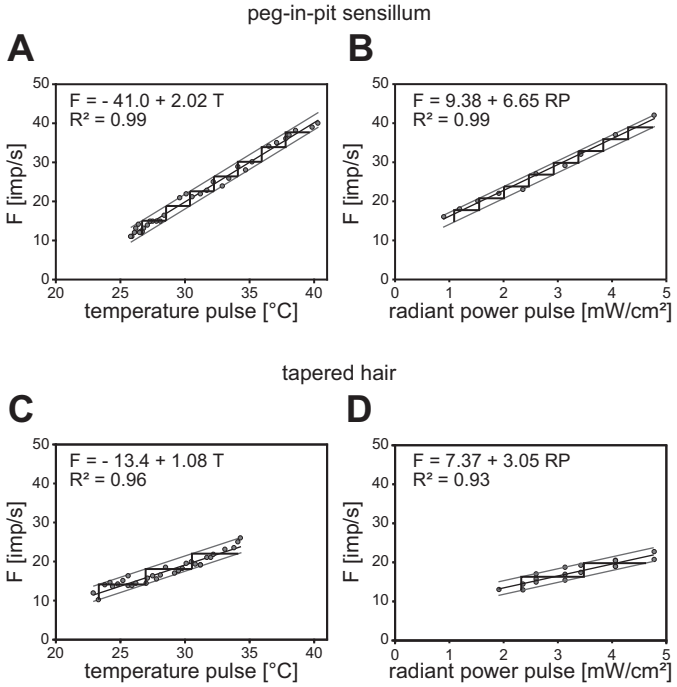

Fig. 4. Intensity functions of warm cells for T and IR pulses. *A* and *B*: warm cells from peg-in-pit sensilla. *C* and *D*: warm cells from tapered hairs. A linear relationship was recorded in each case. Band enclosing the response distribution of all pulses is used to draw the maximum number of stimulus steps the impulse frequency can distinguish within the stimulus range. R^2 coefficient of determination.

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. 5*A*) but pronounced in the tapered hair (Fig. 5*B*). To estimate the double dependence on pulse amplitude and air T, impulse frequency was plotted as a function of both parameters. Multiple regression ($F = a + b\mathrm{T} + c\mathrm{IR}$) showed that sensitivity to air T (b − 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 − slope) was 1.9 (imp/s)/(mW/cm^2) 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/cm^2 (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/cm^2 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 at 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-

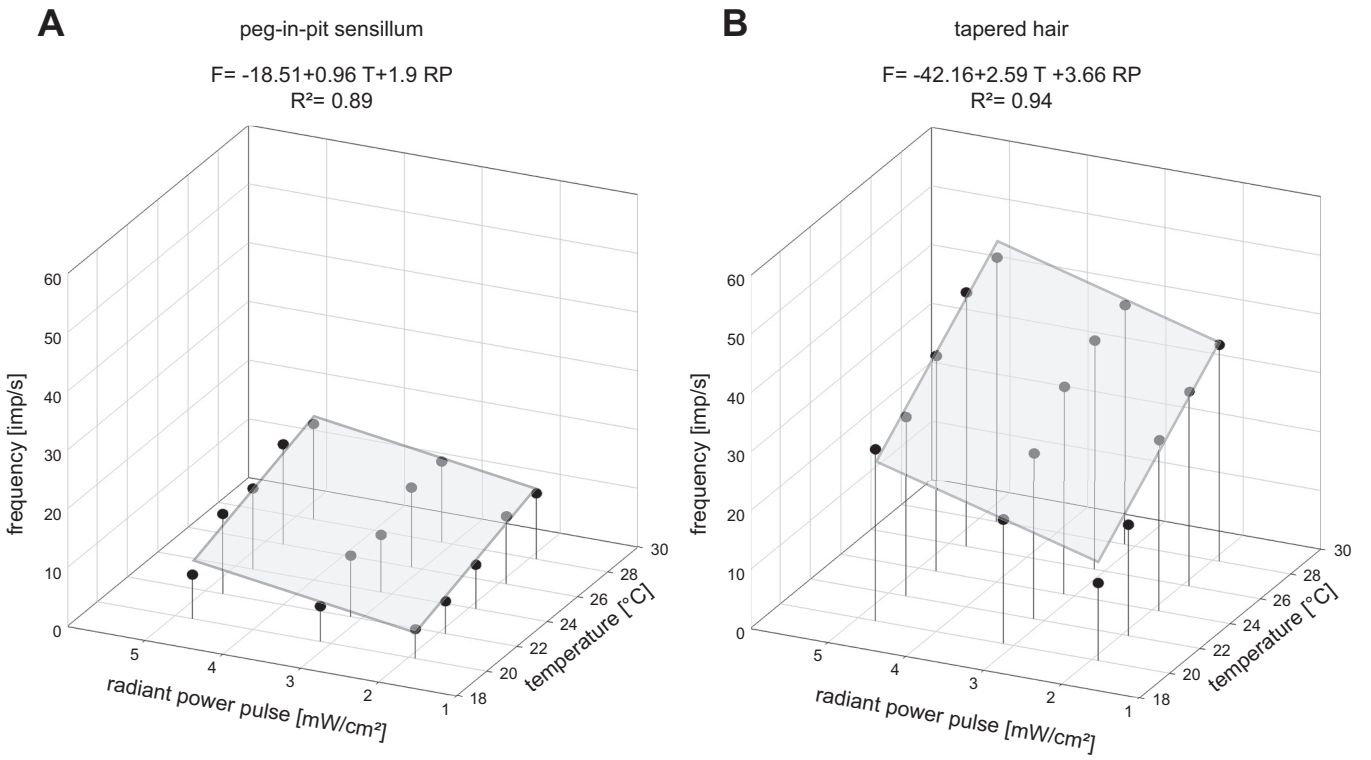

Fig. 5. Reponses of warm cells to IR pulses presented simultaneously with an air stream at constant T. *A*: warm cell in peg-in-pit sensillum. *B*: warm cell in tapered hair. Multiple regressions which utilize 3-dimensional planes ($F = a + b\mathrm{T} + c\mathrm{IR}$, where F is the impulse frequency 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.

tional mechanical information due to a moving air stream enable discriminating not only T and IR pulses but also the effect of combining T and IR pulses from T and IR pulses presented alone.

Turbulences may set limits in the discriminating abilities of the two warm cells. Note that the peg-in-pit sensilla and the tapered hairs are ~35 μm apart, meaning that the temperature stimuli must take the form of patches or filaments <35 μm in diameter to achieve stimulation of only one or the other warm cell. Because of their close location, it will be reasonably assumed that the two warm cells share functionally the same receptive field. It seems that in the temperature system fine discrimination is made possible by conveying thermal information in two subsystems of warm cells and by combining it with the mechanosensory system. Parallel channels that integrate thermal and mechanosensory information have been proposed already as a mechanism for evaluating the apparent size of thermal sources by this bug (Lazzari 2009).

Sensillum structure. 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 sensilla, 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

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 strategic 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 physiological 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 different biotopes. At least some of the material needed for the comparison might be provided in the case of the bloodsucking 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 sensitivity 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/cm^2), 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/cm^2), 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/cm^2), or 1.2 (imp/s)/°C and 16.0 (imp/s)/(mW/cm^2) for the warm cell of the tapered hair. Although quite limited, the results obtained by the different stimulation methods allow comparisons 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 instantaneous 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 mosquito and the tick the rate of change was 1 mW/cm^2, in the bloodsucking bug it was 10 times slower. During a stimulation period of 30 s, a change by 4 (mW/cm^2)/s corresponds to a mean rate of change of only 0.1 (mW/cm^2)/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 becomes 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/cm^2 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 cm^2) 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/cm^2 at a distance of 20 cm. Accordingly, the intensity value of a human hand is 4.12 mW/cm^2 at a distance of 10 cm and 16.50 mW/cm^2 at a distance of 5 cm. In view of a resolving power of 0.5 mW/cm^2, 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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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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

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

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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 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 CO_2 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

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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 CO_2 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 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 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 (Voltcraft IR 800-20D). Stimulus intensity was calculated based on the Stefan-Boltzmann law using the formula (Ebert and Westhoff 2006):

$$\left[\sigma \times A \times \left(T_2^4 - T_1^4\right)\right] / \left(\pi \times D^2\right),$$

in which σ is the radiation constant of Stefan-Boltzmann (5.67×10^{-8} W/m^2 × K^4); A the radiating area (3.5×3.5 mm^2); T_2 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. 1, *A* 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 2*A*, *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. 2*A*). 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. 2*A*, *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

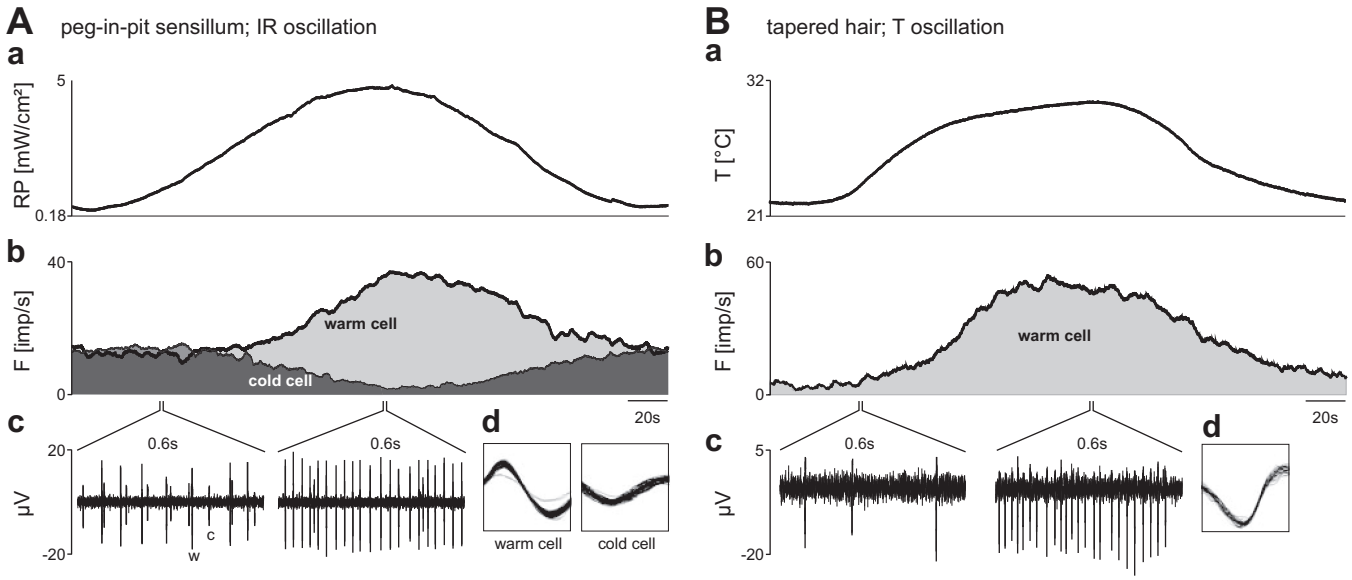

Fig. 1. *A* and *B*: extracellular recorded activity from a warm cell and a cold cell of a peg-in-pit sensillum and a warm cell of a tapered hair on the antennae of the bloodsucking bug to slowly oscillating changes in infrared radiation (IR) and temperature (T). *A*: responses of a pair of warm and cold cells of a peg-in-pit sensillum during 1 IR oscillation period of 600-s duration. *a*: Time course of instantaneous radiation power (RP). *b*: Time course of instantaneous impulse (imp) frequency (F) of warm cell and cold cell. *c*: Expanded views of original recorded action potentials. Large-amplitude action potentials are from warm cell (w), and small-amplitude action potentials from the cold cell (c). *d*: Template window showing the template boundaries of the spike waveforms from warm and cold cells. *B*: responses of a warm cell of a tapered hair during 1 T oscillation period of 600-s duration. *a*: Time course of T. *b*: Time course of instantaneous impulse frequency of the warm cell. *c*: Expanded views of original recorded action potentials from the warm cell. *d*: Template window showing the template boundaries of the spike waveforms from the warm cell.

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 ($F = y_0 + a\ dT/dt + b\ T$, 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 $\sim$5 mW·cm^{-2}. Figure 3*A* 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. 3*A*, *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. 3*A*, *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 THw cell on these two parameters. Multiple regressions ($F = y_0 + a\ dRP/dt + b\ RP$)

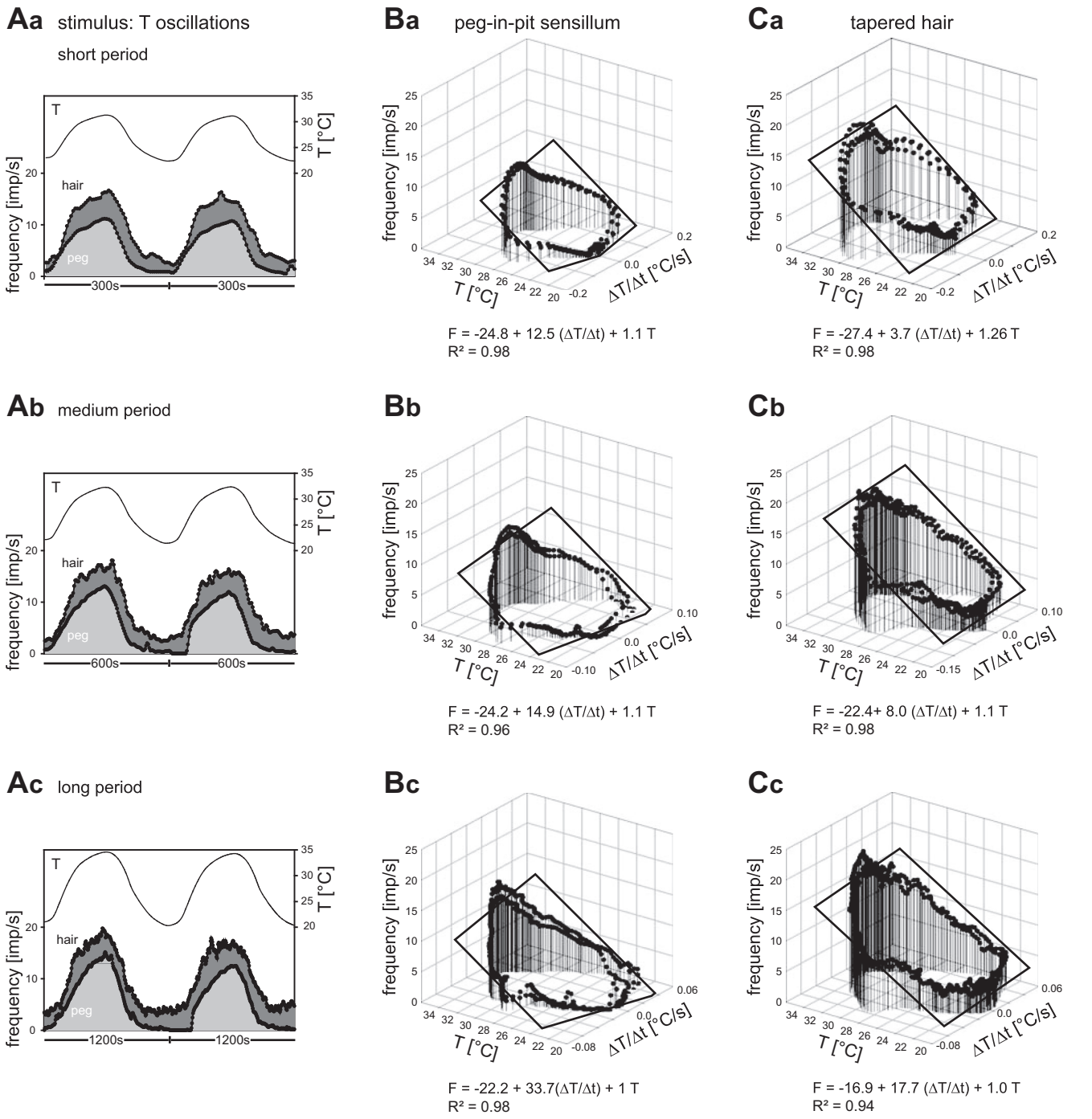

Fig. 2. *A–C*: responses of single warm cells of the peg-in-pit sensillum and the tapered hair to slowly oscillating changes in T. *A*: time course of impulse frequency of both cell types to T oscillations with different periods. *a*: Oscillation period, 300 s. *b*: 600 s. *c*: 1,200 s. *B*: impulse 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 ($F = y_0 + a\ dT/dt + b\ T$, 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, $F = y_0 + a\ dT/dt + b\ T$.

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.

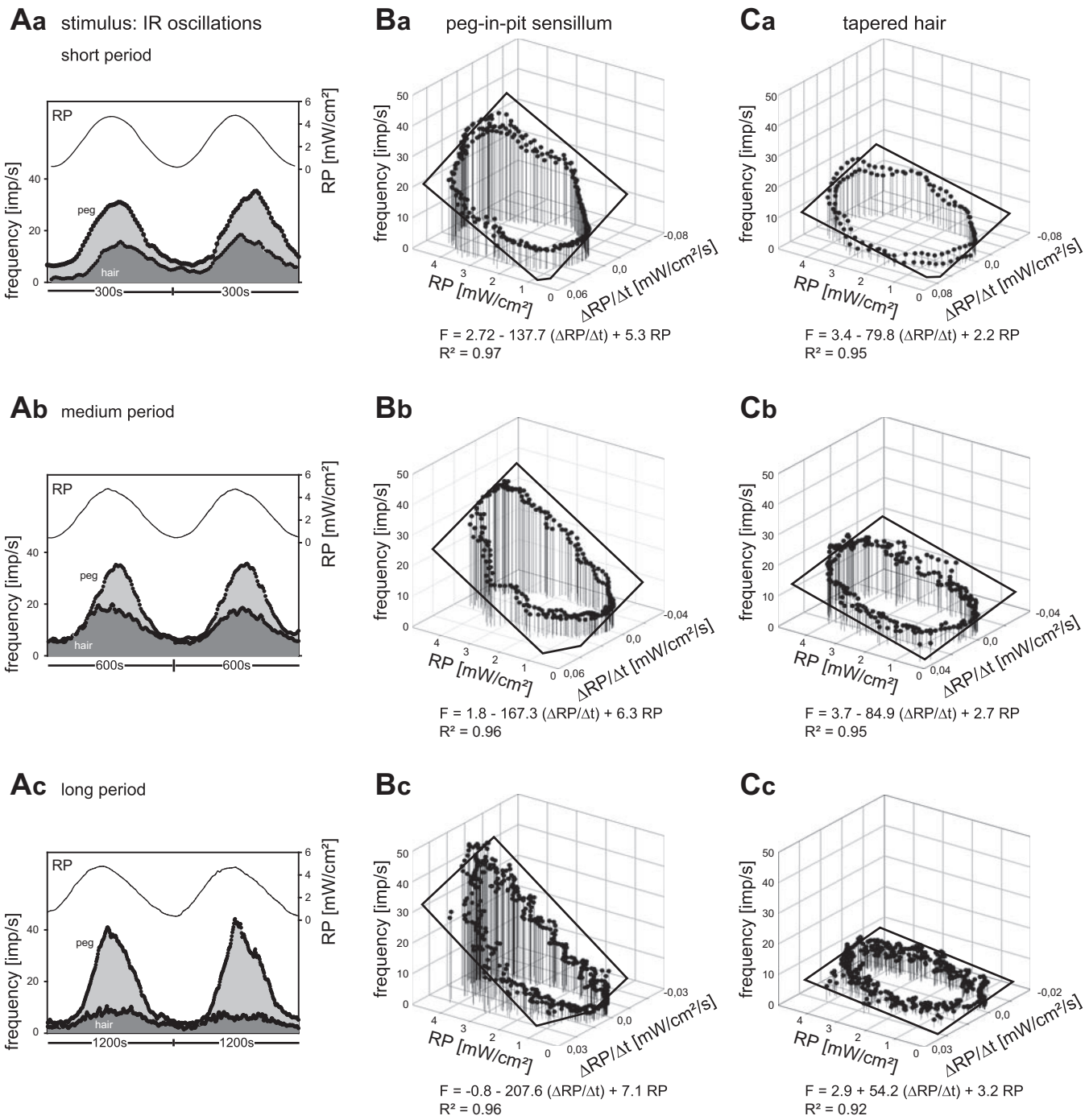

Fig. 3. *A–C*: responses of single warm cells of the peg-in-pit sensillum and the tapered hair to slowly oscillating changes in IR. *A*: time course of impulse frequency of both cell types to IR oscillations with different periods. *a*: Oscillation period, 300 s. *b*: 600 s. *c*: 1,200 s. *B*: impulse 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 ($F = y_0 + a\ dRP/dt + b\ RP$) 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, $F = y_0 + a\ dRP/dt + b\ RP$.

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. 3*A* 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

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. 3*A* and 4*A* 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 combinatory 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 ($\Delta F = F_{hair} - F_{peg}$) is then either positive for T oscillations or negative for IR oscillations (Fig. 4*A*); conversely, subtracting the THw cells responses from those of the PSw cells ($\Delta F = F_{peg} - F_{hair}$) yields a difference that is either negative for T oscillations or positive for IR oscillations (Fig. 4*B*). 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 ($\Delta F = F_{hair} - F_{peg}$) but not the reverse differences ($\Delta F = F_{peg} - F_{hair}$). 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

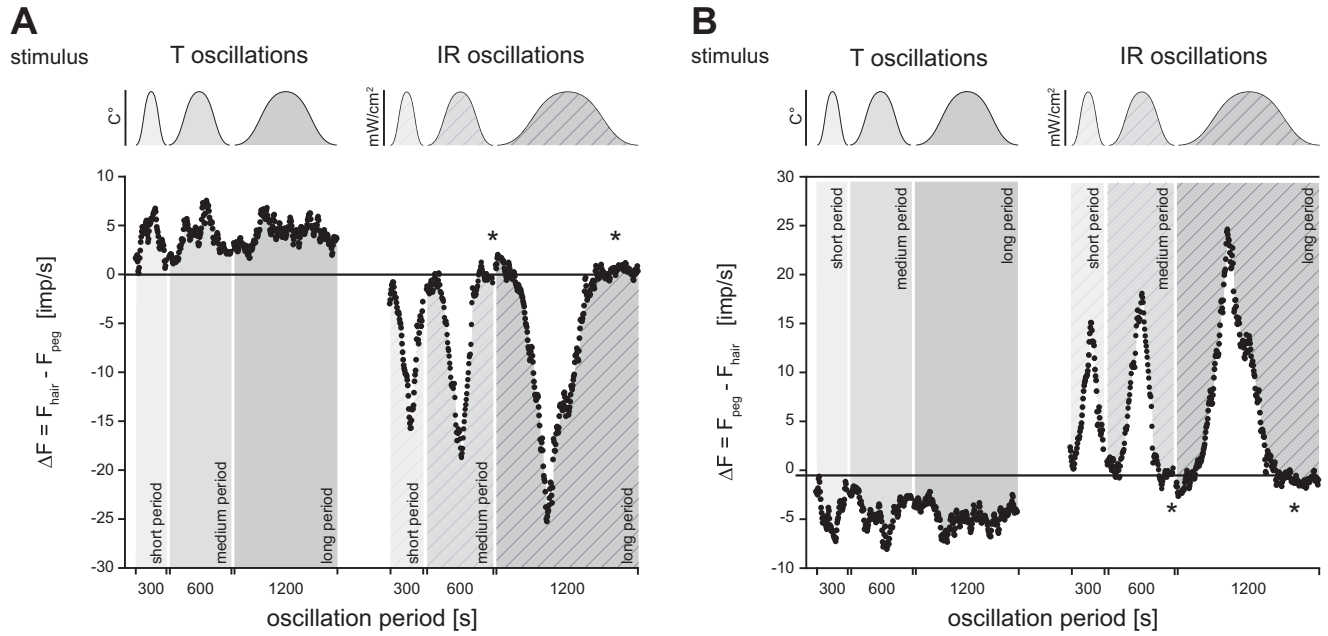

Fig. 4. *A* and *B*: differences in impulse frequencies (ΔF) of the 2 warm cell types in Figs. 2 and 3 during slowly oscillating changes in T and IR plotted as a function of the oscillation period. *A*: warm cell impulse frequency of tapered hair (F_{hair}) minus impulse frequency of peg-in-pit sensillum (F_{peg}). *B*: warm cell F_{peg} minus F_{hair}. Zero lines are border lines between positive and negative ΔF values elicited by T or IR stimulation. ΔF serves as discriminator between T and IR oscillations throughout the range of oscillation periods. Asterisks indicate partial overlapping of difference ranges for long-period IR oscillations.

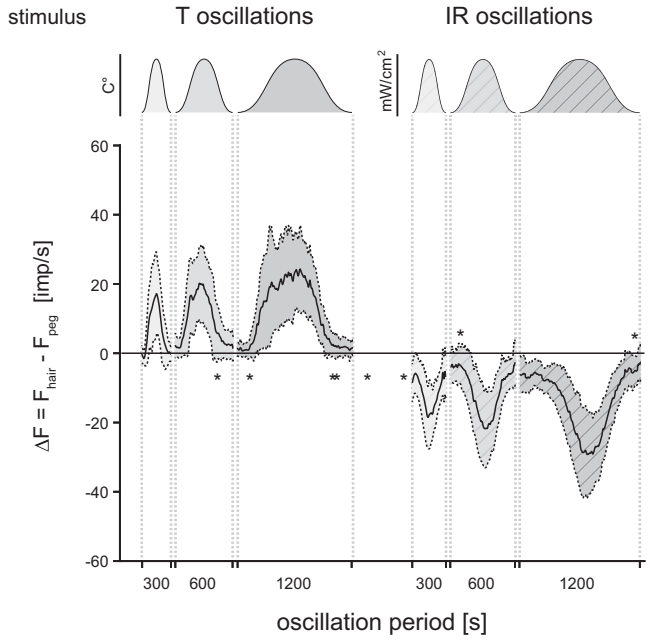

Fig. 5. Mean ΔF of the 2 warm cell types during 3 periods of slowly oscillating changes in T and IR plotted as a function of the oscillation period. Mean difference curves (solid lines) with their standard deviations (hatched lines) derived from pairing all tapered hair with all peg-in-pit sensilla cells. Zero line is a border line between positive and negative ΔF values elicited by T or IR oscillations, indicating that ΔF serves as discriminator between T and IR oscillations throughout the range of oscillation periods. Asterisks indicate partial overlapping of standard deviation of differences ranges.

negative differences ($-\Delta F$), of course can be expressed in terms of the response quotient (Q) as being either greater or smaller than 1. When for given oscillation periods at corresponding points in time the frequency values of the THw cell were divided by the values of the PSw cell, then $Q = F_{hair} / F_{peg} > 1$ can be used to discriminate T changes throughout the range from IR changes generating $Q = F_{hair} / F_{peg} < 1$. In contrast, when, for the same oscillation periods at corresponding time points, the frequency values from the PSw cell were divided by those from the THw cell, then $Q = F_{peg} / F_{hair} < 1$ can be used to discriminate IR changes throughout the range from T changes generating $Q = F_{peg} / F_{hair} > 1$. This approach can be illustrated by plotting, for each T and IR oscillation period, F_{hair} as a function of F_{peg} (Fig. 6) or, conversely, by plotting F_{peg} as a function of F_{hair} (data not shown). 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 $Q = 1$; $Q = F_{hair} / F_{peg}$ for T oscillations is larger than $Q = 1$ for any oscillation period to the left and above the boundary, and $Q = F_{hair} / F_{peg}$ 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

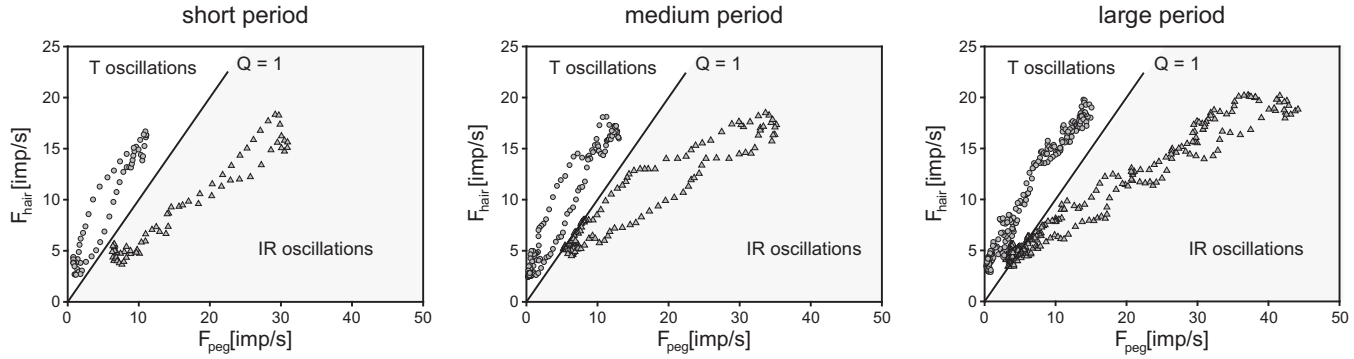

Fig. 6. Quotients (Q) in impulse frequencies of the 2 warm cell types in Figs. 2 and 3 for slowly oscillating changes in T and IR. Quotients formed by plotting F_{hair} as a function of F_{peg} for different oscillation periods. Response quotients for T oscillations (●) are separated from those for IR oscillations (○) by the solid line through origin. The slope of the line, $F_{hair} / F_{peg} = 1$, is a value of Q that could serve as a boundary between the response quotients for T changes (white area) and IR changes (gray area).

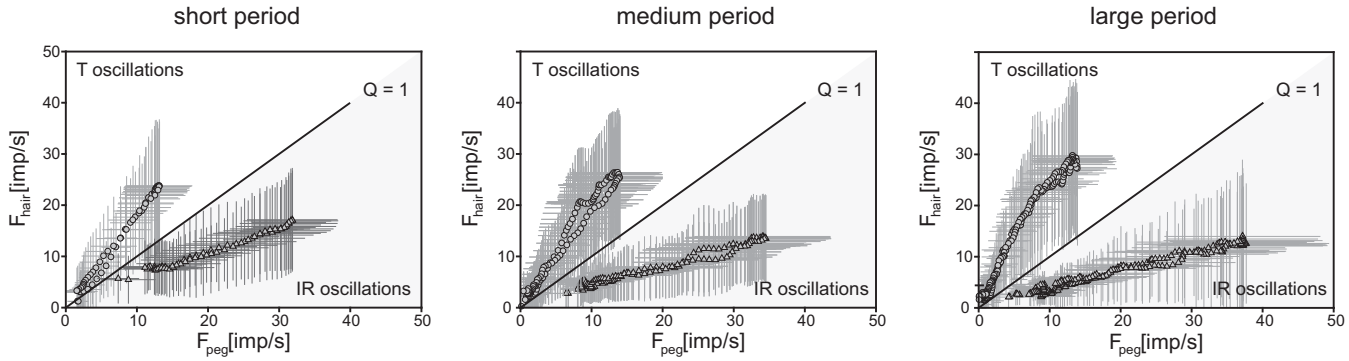

Fig. 7. Mean quotients in impulse frequencies of the 2 warm cell types for slowly oscillating changes in T and IR. Mean quotients formed by plotting the mean impulse frequency of 10 warm cells of tapered hairs (F_{hair}) as function of the mean impulse frequency of 10 warm cells of peg-in-pit sensilla (F_{peg}) for different oscillation periods. Responses of the 2 types of warm cells intersect at their means. Horizontal and vertical bars are standard deviations of their means. Mean quotients and standard deviations for T oscillations (Œ) are separated from those for IR oscillations (o) by solid line through origin. The slope of the line, $F_{hair} / F_{peg} = 1$, is a value of Q that could serve as a boundary between the response quotients for T changes (white area) and IR changes (gray area).

based on Q can be applied throughout the range of tested oscillations periods to both cell types. A drawback to the pooling, large standard deviations about the means, is also shown in Fig. 7. These deviations, however, do not result only from the deviations of the points from characteristic curves proper to individual warm cells. They are also the product of the variance in the slopes of the regression planes, i.e., of variance in differential sensitivity.

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$\cdot s^{-1}\cdot mW^{-1}\cdot cm^{-2}$ for periods of 100 s and in the mosquito 0.8 impulses$\cdot s^{-1}\cdot mW^{-1}\cdot cm^{-2}$ for similar periods. In the bug, the PS values lie between 2.2 and 3.1 impulses$\cdot s^{-1}\cdot mW^{-1}\cdot cm^{-2}$ for periods between 300 and 1,200 s, and the TH values between 5.3 and 7.1 impulses$\cdot s^{-1}\cdot mW^{-1}\cdot cm^{-2}$ 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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No conflicts of interest, financial or otherwise, are declared by the author(s).

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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5. Discussion and Outlook

The search for reliable coding mechanisms for infrared radiation (IR) is complicated by the fact that warm cells, whose rate of discharge is a function of the power of IR, display an additional high sensitivity to changes in ambient temperature (T). Since impulse frequency is caused by radiant or convective heat, a single warm cell cannot discriminate the source of the T increase. The response of any particular warm cell is ambiguous, with respect to either mode of heat transfer. Encoding of IR and T must be a cooperative venture among different types of warm cells characterized by different sensitivities for IR and T. Inferences about such a "combinatorial code" are often made from color vision where appear to be three types of photopigments, each responsive to a visual spectrum but peaking at one of three points along the wavelength continuum. Color vision is a function of the relative responsiveness of all three receptor types, no one is sufficient by itself to code unambiguously both wavelength and intensity. A similar coding mechanism could apply to discriminate between IR and T at different intensities. One purpose of my thesis was to identify, by means of electrophysiological recording methods, distinct types of warm cells which do not respond equally well to IR and T. If the two stimuli evoke equally robust responses in different types of warm cells, then their responses could not be indicative of one stimulus or the other. Instead, if one stimulus evokes strong responses exclusively in one type of warm cell, and the second stimulus produces strong responses in a different type of warm cell, a pair-wise comparison of the responses is the distinguishing feature of the coding mechanism.

As outlined in my thesis, I was successful in identifying 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). These two types of warm cells differ quantitatively in their responses to temperature and infrared stimuli. Slowly oscillating changes in temperature produced strong responses in the warm cells of the hairs (THw-cells) and comparatively weak responses in the warm cells of the peg-in-pits (PSw-cells). Slowly oscillating changes in IR evoked the reverse responses; they stimulated the latter more strongly than the former. The reversal in the relative excitability of the two warm cell types responding in a pair provides a criterion to distinguish between changes in T and IR. This suggests that the nervous system is reading the inputs from two separate warm channels simultaneously, so that the determination of the source of the temperature change is unambiguous. The notion of "specialisation" of sensory cells for IR was released in favour of the "combinatorial code" mechanism. The study revealed that the difference or the ratio between the response magnitudes of both warm cell types can 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.

Combinatorial coding of IR is unique to date. Other blood-sucking insects have still to be examined in this regard. Also unique on a single antenna is the finding of two types of IR-responsive warm cells in morphologically distinct sensilla, the peg-in-pit sensilla (PS) and tapered hairs (TH). Each of these sensilla has been found in different insects and ticks, but only alone and not in combination with the other one. Clearly, a major challenge in understanding IR detection is the description of a sample of warm cells in terms of distinct types. This will be an expedient step for the discovery of peripheral encoding mechanisms and explanation of sensory function. We are just beginning to learn about discriminating IR and T by reading the inputs from separate warm channels simultaneously. The implication is parallel processing. For this purpose early divergence would be required to yield two sets of axonal branches, one for determining IR and the other for T. In each set the output of several warm cells would be pooled. Then an excitation level would be generated in an interneuron on which they converge which would be a function of IR and it's intensity parameter that influences the response. Similarly, the excitation level of another interneuron, receiving the second output, would be a function of T and its intensity. The existence of such a neuronal network should manifest itself both anatomically and physiologically. Not only should convergence be encountered, but also an early divergence. Further, interneurons should be detected, whose activity level depend on IR. In addition, other interneurons should be found whose level of activity is affected by T. A key question that these studies should answer is, how warm-cell responses, (which are necessary ambiguous) account for an unambiguous behavioral response to an IR source.

I hope my thesis will stimulate further research in the area of IR detection, not only at the peripheral level but also in the central nervous system.

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7. Author Contributions

Zopf, L.M., Lazzari, C.R., Tichy H. (2014).Differential effects of ambient temperature on warm cell responses to infrared radiation in the bloodsucking bug *Rhodnius prolixus*. J. Neurophysiol. 111(6): 1341-1349

Original Idea: LMZ, HT,

Experimental Setup: LMZ, HT

Data Collection and Data Analysis: LMZ, HT

Preparing of the Figures: LMZ

Writing of the Manuscript: LMZ, HT

Supervision and Commenting on the Manuscript: HT, CRL

Zopf, L.M., Lazzari, C.R., Tichy H. (2014). Infrared detection without specialized infrared receptors in the bloodsucking bug *Rhodnius prolixus*. J.Neurophysiol. 112(7):1606-1615

Original Idea: LMZ,

HT Experimental Setup: LMZ, HT

Data Collection and Data Analysis: LMZ, HT

Preparing of the Figures: LMZ

Writing of the Manuscript: LMZ, HT

Supervision and Commenting on the Manuscript: HT, CRL

8. Summary

The bug *Rhodnius prolixus* is an obligatory blood feeder which uses infrared radiation (IR) as a means of locating warm-blooded hosts. Although IR receptors have been described in many animals (including rattlesnakes, vampire bats, ticks and insects), broad generalizations of their mode of action have been extremely difficult because of an exquisite sensitivity to temperature (T) stimulation. But if IR receptors respond to changes in both IR and T, what sets IR receptors apart from thermoreceptors? Are there receptors which allow differentiating between IR and T stimuli at all? Bloodsucking bugs, however, are the only insects which have been examined for the ability to differentiate between IR emitted from warm-blooded hosts and warm air around the host. Behavioral experiments have actually established that bloodsucking bugs do not confuse radiant with convective heat. The neural mechanism underlying this exceptional ability remains elusive. Since the information available to the bug regarding IR and T stimuli is restricted to the information encoded in the neural responses of sensory receptors, the answer must lie in some mechanisms operating in the receptor cells. What is special about the bug's IR receptors? Thus a necessary step towards understanding IR detection is to analyze the information content of individual sensory cells on the bug's antennae to behaviorally relevant IR stimuli.

The results of my studies have been published in two papers. The first paper deals with the identification of specialized receptors responsive to IR and their response characteristics to a range of IR as well as T stimuli (J. Neurophysiol. 111(6): 1341-1349). The second paper deals with the neural code for IR which accounts for the bug's ability to discriminate between IR and T (J. Neurophysiol. 112(7): 1606-1615).

In my first study, I identified two types of warm-sensitive cells responsible for the detection of IR. They are located in morphologically different antennal sense organs, in peg-in-pit sensilla (PS) and tapered hairs (TH). The two warm cells produce stronger responses to T pulses produced by switching between two air streams at different constant T than to IR pulses employed in still air. In addition, both warm cells are better able to discriminate small changes in air T than in IR. As convective as well as 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 T change, but with regard to it's source. We argue that the bugs use mechanical flow information to differentiate between pulses of convective and radiant heat.

For an insect moving towards a warmblooded host, IR offers an advantage over odor cues or CO_2 – IR stimuli are inherently directional. IR emitted from the surface of a warmblooded host radiates in straight paths in all directions like visible light. Odors and CO_2 reveal many discontinuous patches of local eddies, thus creating local concentration maxima that can mislead a gradient following strategy. IR is independent of turbulences and allows detection of obstacles between the insect and the radiant object. In order to mimic natural conditions more closely, I decided to employ slowly oscillating changes in the power of IR rather than pulses of IR.

My second study is concerned with the neuronal discriminator of slow and continuous changes in IR and T. Analysis is based on the responses of the warm cells in the peg-in-pit sensilla (PSw-cells) and in the tapered hairs (THw-cells). When IR or air T are made to rise and fall smoothly at varying rates, both types of warm cells display oscillating changes in their discharge rates. The PSw-cells produce stronger responses to IR oscillations than the THw-cells. Oscillations in T do the reverse: they stimulate 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 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 “combinatorial 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 procedures and results of this study appear relevant not only for bloodsucking bugs but also applicable to IR and T coding in general. Putting emphasis on a single cell’s activity seems a logical consequence of our most powerful tool for the evaluation of the mechanisms underlying the encoding of sensory “qualities”: the single-unit microelectrode recording technique. The “combinatory code”, on the other hand, seems almost self-evident, if one realizes that the CNS is inevitably confronted with changing patterns of neural activity, coming from different types of sensory cells.

9. Zusammenfassung

Die blutsaugende Wanze *Rhodnius prolixus* benutzt die Wärmestrahlung (IR) von warmblütigen Tieren um ihre Nahrungsquelle zu finden. Die Wahrnehmung von IR wurde im Tierreich schon öfter beschrieben (zB. bei Klapperschlangen, Gemeinen Vampir-Fledermäusen, Zecken und bei Insekten), jedoch war eine generelle Beschreibung ihrer Funktionsweise eher schwierig, da sie eine sehr hohe Empfindlichkeit auf Temperaturänderung (T) aufweisen. Doch was unterscheidet IR Rezeptoren von Thermorezeptoren, wenn sie auf beide Reize reagieren? Gibt es Rezeptoren, die tatsächlich zwischen diesen beiden Reizen unterscheiden können?

Tatsächlich besitzen blutsaugende Wanzen die Fähigkeit, die Wärmestrahlung eines Körpers von der warmen Luft, die ihn umgibt, zu unterscheiden.
Welche neuronalen Prozesse dieser Fähigkeit zugrunde liegen, ist noch unbekannt. Jede Information, die die Wanze von der Umwelt empfängt, ist in der Frequenz der Aktionspotentiale der Sinneszelle enthalten. Um die Funktion eines IR Rezeptors zu verstehen, muss die Aktivität dieser Sinneszelle elektrophysiologisch untersucht werden.

Die Ergebnisse meiner Forschung wurden in zwei Publikationen veröffentlicht.
In meiner ersten Arbeit behandle ich die Identifikation von IR Rezeptoren und deren Antwortverhalten in der Impulsfrequenz bezüglich IR und T Reize (J. Neurophysiol. 111(6): 1341-1349). Es war mir möglich, zwei Arten von wärmeempfindlichen Rezeptorzellen zu lokalisieren, die sich in morphologisch unterschiedlichen Typen von Sinnesorganen auf der Antenne befinden. Die sogenannten „peg-in-pit sensilla“ (PS) und die „tapered hairs“ (TH). Beide Typen reagieren auf schnelle Temperaturänderungen der Luft mit einer stärkeren Entladungsrate als auf rapide Änderungen von IR Strahlung. Daraus ergibt sich für beide Rezeptorzellen ein besseres Auflösungsvermögen für Temperaturreize, als für Strahlungsreize. Da beide Reize einen Einfluss auf die Impulsfrequenz haben, ist es unmöglich für eine einzelne Rezeptorzelle zwischen IR Strahlung und Temperaturänderung der Umgebungsluft zu unterscheiden. Aufgrund dieser Ergebnisse war anzunehmen, dass zusätzliche Informationen, wie die Bewegung der Luft als mechanischer Reiz, in die Verarbeitung miteinfließen und eine Unterscheidung ermöglichen.

In der zweiten Arbeit behandle ich die Verarbeitungsmechanismen, die der Unterscheidung von IR und T Reizen zugrunde liegen (J. Neurophysiol. 112(7): 1606-1615).
Für diese Untersuchungen wurden sinusförmige Reize präsentiert, da sie die graduelle Veränderungen simulieren, die eine Wanze erfährt, wenn sie sich einer Wärmequelle nähert oder sich von ihr entfernt. Hier zeigen sich deutliche Unterschiede im Antwortverhalten der Wärmezellen der „peg-in-pit sensilla“ (PSw) und jener der „tapered hairs“ (THw). Beide Typen folgen mit ihrer Impulsfrequenz dem gegebenen Reizverlauf, jedoch liegt die Impulsrate der THw-Zellen bei T Änderungen deutlich über derjenigen der PSw-Zellen. Umgekehrt verhält es sich bei sinusförmigen IR Reizen: hier liegt die Impulsrate der PSw-Zellen über der der THw-Zellen.
Durch Vergleich der Impulsraten der beiden Wärmezellen- entsprechend dem Prinzip „Kodierung durch Kombination“ kann das ZNS Infrarotreize von Temperaturreizen unterscheiden.

Die Ergebnisse meiner Arbeit sind nicht nur für die blutsaugende Wanzen von Bedeutung, sondern für die Erforschung von IR Wahrnehmung im Allgemeinen. Elektrophysiologische Ableitungen von einzelnen Sinneszellen liefern ausreichend Informationen über die Qualität der Rezeptoren, jedoch liegt die wahre Herausforderung des Zentralnervensystems in der Verarbeitung des sich ständig ändernden Musters der neuronalen Aktivität unterschiedlichster Sinneszellen.

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Curriculum Vitae

Mag. Lydia Zopf

Personal Information

Place of Birth	Vienna, Austria
Citizenship	Austria

Education

2011 - present	**PhD-Student** at the Department of Neurobiology, University of Vienna, Supervisor: Prof. Harald Tichy
Oct 01 - April 09	**Studies in Biology** (**first diploma** examination), University of Vienna
1993 - 2001	**High school** GRG Auf der Schmelz

Employment

Teaching Fellow at the University of Vienna for:

March 10- Aug 13	Animal Physiology Laboratory 2 - Nerve, Muscle, Sense
Oct 10 - Feb. 14	Neurobiology: Visual Systems

Teaching Assistant at the University of Vienna for:

Oct 07 – Feb 10	Organ und Kommunikationssysteme
March 08 – July 10	Animal Physiology Laboratory 2 - Nerve, Muscle, Sense

Scholarships

Oct 11 - Sep 14	FWF fellowship

Scientific Publications

Zopf, L.M., Lazzari, C.R., Tichy, H. (2014) Infrared detection without specialized infrared receptors in the bloodsucking bug *Rhodnius prolixus*., *J Neurophysiol*, **112**:1606-1615

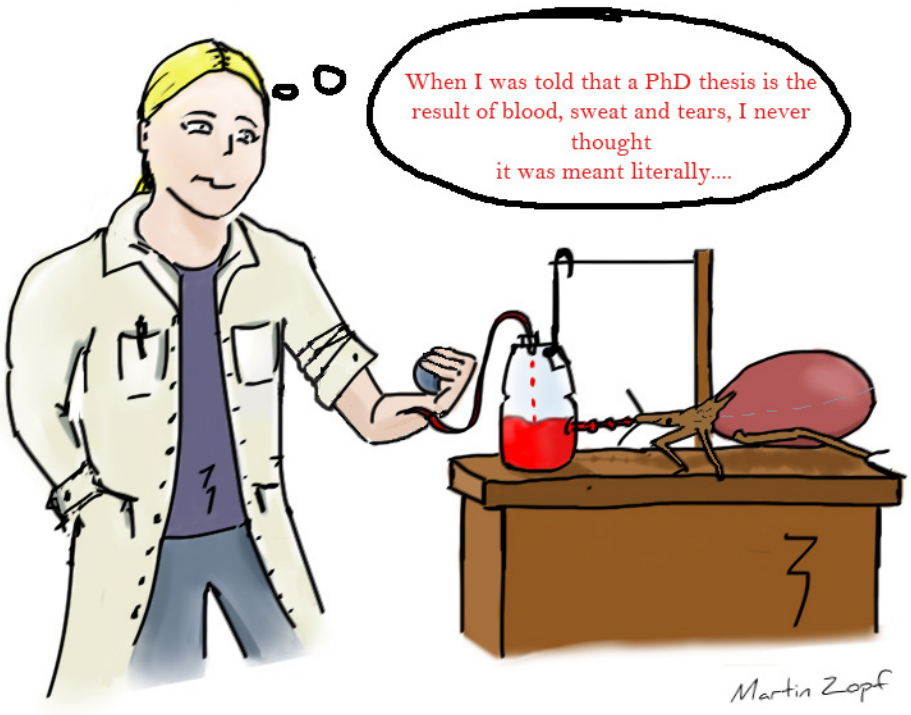
Zopf, L.M., Lazzari C.R., Tichy H. (2014) Differential effects of ambient temperature on warm cell responses to infrared radiation in the bloodsucking *bug Rhodnius prolixus*. *J Neurophysiol*, **111**:1341-1349

Zopf, L.M., Schmid, A., Fredman, D., Eriksson, J. (2013) Spectral sensitivity of the ctenid spider *Cupiennius salei* Keys. *J Exp Biol,* **216**: 4103-4108

Conference Contributions

16.-18. September 2009	11. Konferenz der österreichischen Gesellschaft für Neurowissenschaften (ANA). Salzburg, Austria. *Poster*: Zopf, L.* & Schmid, A (2009) Spektrale Empfindlichkeit der Jagdspinne *Cupiennius salei*.
7.-10. September 2011	European Chemoreception Research Organisation (ECRO) 21. Kongress. Manchester, Great Britain. *Poster*: Zopf, L.M.*, Burgstaller, M., Anton, S. & Tichy, H. (2011) Adaptive bias in the response of the ON and OFF olfactory receptor neurons to creeping changes in food odour concentration.
21.-24. September 2012	105. Konferenz der deutschen zoologischen Gesellschaft (DZG). Konstanz, Germany. *Poster*: Zopf, L.M.*, Lazzari, C.R. & Tichy, H. (2012) Temperature and infrared detection in the bloodsucking bug *Rhodnius Prolixus*.
10. April 2013	IST Austria Neuroscience Network Meeting, Klosterneuburg, Austria. *Poster:* Zopf, L.M.*, Lazzari, C.R. & Tichy, H. (2013) Thermoreceptors in bloodsucking bugs differentiate between heated air and the thermal effect of infrared radiation.
26. Juni 2014	IST Austria Neuroscience Network Meeting, Vienna, Austria. *Talk:* Zopf, L.M.*., Lazzari, C.R. & Tichy, H. (2013) Infrared detection without specialized infrared receptors in the bloodsucking bug *Rhodnius prolixus*

*presenting author


When I was told that a PhD thesis is the result of blood, sweat and tears, I never thought
it was meant literally....
Martin Zopf