Humidity-Dependent Cold Cells on the Antenna of the Stick Insect

Harald Tichy
Faculty of Life Sciences, Department of Neurobiology and Cognition Research, University of Vienna, Vienna, Austria

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Tichy H. Humidity-dependent cold cells on the antenna of the stick insect. J Neurophysiol 97: 3851–3858, 2007. First published March 28, 2007; doi:10.1152/jn.00097.2007. We present the first systematic study of the response of insect “cold cells” to a variation in the partial pressure of water vapor in ambient air. The cold cells on the antenna of the stick insect respond with an increase in activity when either the temperature or the partial pressure of water vapor is suddenly reduced. This double dependency does not in itself constitute bimodality because it could disappear with the proper choice of parameters involving temperature and humidity. In this study, we demonstrate that the evaporation of a small amount of water from the sensillum surface resulting from a drop in the water vapor pressure—leading to a transient drop in temperature and thus to a brief rise in impulse frequency—is the most plausible explanation for this bimodal response. We also show with an order-of-magnitude calculation that this mechanism is plausible and consistent with the amounts of water vapor potentially present on the sensillum. We hypothesize that a film of moisture collects on the hygroscopic sensillum surface at higher humidity and then tends to evaporate when humidity is lowered. The water might even be bound loosely within the cuticular wall, a situation conceivable in a sensillum that contains two hygroreceptive cells in addition to the cold cell.

INTRODUCTION

Many insect species rely heavily on the temperature sense. Their low body weight makes heat exchange with the environment very rapid. To maintain body temperature, insects must actively track a suitable thermal environment. The sensory cells responsible for detection of ambient temperature are located on the surface of the antennae within cuticular extensions termed sensilla (Altner and Prillinger 1980; Loftus 1978; Tichy and Gingl 2001). Two physiologically different types of thermoreceptive cells are distinguished: cold and warm cells. Both types respond to the same temperature changes, but in the opposite direction. The same cooling raises impulse frequency of the cold cell and lowers it in the warm cell. Correspondingly, opposite effects are produced by warming.

Cold cells and warm cells were rarely reported occurring alone in a sensillum. The rule in insects is combinations of sensory cells of different modalities, most often a cold cell with two hygroreceptive cells (Loftus 1978; Tichy and Gingl 2001; Tichy and Loftus 1996). Within this category, several examples of highly sensitive cold cells have been observed. In the stick insect Carausius morosus, the responses to vapor pressure changes are implied in the way it responds to vapor pressure changes. The reaction to changes in water vapor pressure is greater than those to falling temperature, but the impulse frequencies will correspond to drops of 4°C (Tichy 1987).

The present study quantitatively describes the dependency of this cold cell on the sudden decrease in the partial pressure of water vapor. This allows a better assessment of the way in which cold cells supply the brain with information about ambient temperature and also helps examine whether its responses to vapor pressure changes are implied in the way it responds to temperature changes. Our results here rule out the
possibility that the relative humidity and enthalpy are adequate stimuli. Evaporation of water from the sensillum surface, however, might explain the cold cell's reactions to falling humidity. This interpretation clearly assumes that moisture is available for evaporation, although what water is evaporating and where does it come from? We hypothesize that a film of moisture collects on the sensillum surface at higher humidity and then tends to evaporate when humidity is lowered. Direct demonstration of evaporation was not possible. Instead, we estimated how much water would evaporate to produce the observed cooling effect. From the degree of cooling and from the water content of the air stream, we calculated the amount of water potentially available for evaporation from the sensillum surface.

**Methods**

**Animals**

The large, egg-laying stick insects (*Carausius morosus*) used in this study were raised on ivy leaves at temperatures between 21 and 25°C. They were used only when the length of their antennae exceeded 25 mm. Thus the flagellum extended 15 mm beyond the 12th segment, the one from which the recordings were made (Fig. 2A). The insect was fixed on a holder with adhesive tape and one antenna was attached to a narrow support with dental cement (Harvard Cement) for unobstructed stimulation.

**Location and sensillum structure**

The 12th antennal segment bears a field of about 50 sensilla of different types (Fig. 2A). One sensillum consists of a short peg inserted in a pit and surrounded by a broad collar (Figs. 1A and 2, B and C). In serial sections of the sensillum the peg is about 6.4 μm long and 1.4 μm broad at the base; the cuticular wall is 0.6 μm thick and contains no pores (Fig. 2D, from Altner et al. 1978). The outer segments of two sensory cells are unbranched and extend into the lumen of the peg, whereas the branched dendrite of the third sensory cell terminates below the peg’s base (Fig. 1A).

**Recordings**

Action potentials were recorded extracellularly with electrolytically sharpened tungsten wires. One electrode was inserted lengthwise into the tip of the antenna for about 2 mm and the other from an angle of about 45° into the margin of the mound-shaped sensillum. The 45° angle appeared to enhance the probability of reliable recordings.

**Stimulation**

The stimuli were presented by way of three air streams, each emerging at 2.5 m/s from a nozzle 7 mm in diameter. Two of the streams (Fig. 3, A and B) had the same partial pressure of water vapor but different temperatures and two (Fig. 3, B and C) had the same temperature but different partial pressures of water vapor. Each air stream could be directed to a single area about 20 mm from the nozzle.
through two separate units (Fig. 3, mineral wool. The air was then divided into two streams and driven to remove acid vapors, and finally cleaned by flowing over charcoal and through concentrated sulfuric acid and then over KOH pellets to produce rapid changes in water vapor at the same temperature, the air stream moistened in the second unit passed through a heat exchanger that was at the same temperature as that of one of the other two and was then expelled from a third nozzle.

Temperature of the air stream was measured with a precision of 0.03°C by a small (0.25 × 0.4 mm) bead thermistor (BC32L1, Fenwall Electronics) positioned about 3 mm downstream from the sensillum. During fast temperature changes, when one air stream was shifted to another, the time course of the voltage did not reflect the time course of temperature of the sensillum. This is because the sensillum is much less massive than the thermistor and therefore develops much higher transients when the streams are shifted. For this reason the difference between the temperatures of the streams (ΔT = T2 - T1) was used as a stimulus parameter, although the rate of change would probably be a better measure if precise values for it were obtained from measurements within the sensillum. It is extremely difficult to precisely measure the moisture content of an air stream without changing or disrupting its flow. The partial pressure of water vapor was therefore not monitored during the experiments but rather adjusted to psychrometrically precalibrated values. A useful concept in psychrometry is the wet-bulb temperature, which is defined by the dry-bulb temperature and the dew point temperature. The wet-bulb temperature is the temperature reached by a water surface, such as that registered by a thermometer bulb surrounded by a wet wick, when exposed to air passing over it. The wick and thus the thermometer bulb decrease in temperature below the dry-bulb temperature until the rate of heat transfer from the warmer air to the wick is equal to the rate of heat transfer needed to provide for evaporation from the wick into the air stream. The temperature of the wet thermistor remains constant, the wet and dry thermometers can be read. For the psychrometric measurement, a new wet-, now dry-bulb mercury thermometer accurate to 0.1°C was inserted into the tube attached to the outlet of a heat exchanger and the air stream sent through it at the normal flow rate. The bore of the tube was so chosen that it produced an air speed of about 3 m/s around the bulb when it was protected by a covering of wet wick. An air speed of 2 m/s is recommended for psychrometric measurements. Only one partial pressure of water vapor is possible at a given pair of stabilized wet- and dry-bulb temperatures under such conditions. Temperature constancy indicated that the water vapor could be set with a precision of about 3%. Constancy of relative humidity requires constancy of temperature as well as partial pressure of water vapor, because relative humidity is defined as the ratio of partial pressure of water vapor to saturation vapor pressure and saturation vapor pressure increases with temperature. However, because the temperature of the air stream fluctuated only slightly and slowly (no >0.1°C in 5 min), the error was considered tolerable. At 7 torr and 20°C, a difference of 0.1°C produces only a 0.3% change in relative humidity. This is within the limits of accuracy of adjustment of the water vapor, which determines relative humidity. Furthermore, this difference—extended as a mean rate of change over the 5-min period—could well be 10 times smaller than that produced by switching from one air stream to another.

The thermodynamic properties of air, such as dry- and wet-bulb temperatures, moisture content, and heat energy, are taken from...
handbooks of chemistry and physics (Bredicka 1958; Frauenfelder and Huber 1966), drawn from psychrometric charts, a psychrometric slide rule, or a computerized equivalent (www.uigi.com/WebPsycH.html). The mass of water evaporating from the sensillum surface was calculated from the drop in sensillum temperature by using the method described in the explanations of the thermal properties of water (Koehler 2003; also see www.engineeringtoolbox.com/water-thermal-properties; www.engineering toolbox.com/enthalpy-moist-air; and www.world-wide-water.com).

Stimulus protocol

Stimulus changes were provided by switching from one air stream at steady initial conditions to another at different steady conditions for 0.65 s and then back to the first for a 3-min recovery period before the next shift in air streams. During this period, the conditions of the second air stream were altered. After a series of such steplike stimuli, the conditions of the first air stream were then set at another value and allowed to stabilize for 3 min before commencing a new series.

Response magnitude

Impulse frequency was determined by the number of impulses, for a time span of 100 ms in the case of step changes and for 1 s under constant stimulus conditions.

RESULTS

Identification

The cold cell described herein is associated with a moist cell and a dry cell in the mound-shaped sensillum on the 12th segment of the antenna (Fig. 2, B and C). Most recordings (78%, \( n = 35 \)) revealed the activity of all three sensory cells, but in some recordings (22%, \( n = 10 \)) only two were found. The cold cell was identified by its responses to sudden changes in temperature produced by shifting between two air streams at 0 torr partial pressure of water vapor, the first at higher and the second at lower temperature. In the example shown in Fig. 4A, when temperature suddenly decreases from 20.5 to 17.5°C, the firing rate of the cell with the large impulses rose from 7 to 45 impulses/s, whereas the cell with the small impulses displayed no significant change in the discharge rate.

A previous study showed that impulse frequency of the cold cell rose monotonically with the size of the cooling step; it rose more rapidly, however, the lower the initial temperature from which these steps were initiated (Fig. 4 in Tichy and Loftus 1987). Here, we evaluated the relationship for initial temperatures between 18.5 and 22°C. Sixty-six series of cooling steps were tested on 16 cold cells, with each series at a different initial temperature.

The impulse frequency of a single cold cell in response to temperature drops (from three initial temperatures) is illustrated in Fig. 5A. The functions are nearly linear and the slope of the regression lines determined by each set of points steepened by a factor of 1.8. The slopes indicate the differential sensitivity to cooling steps from a given initial temperature. Differential sensitivity shows the mean change in impulse frequency for each degree the step in temperature is increased. At 19.5°C, the step must be increased by 0.15°C to increase impulse frequency by 1 impulse/s. At 19.5°C an average increase of only 0.08°C in step size suffices to elevate impulse frequency by the same amount. (The preceding two elevations in step size are reciprocals of differential sensitivity, -6.4 and -11.3 impulses·s\(^{-1}·°C^{-1}\), respectively.) The negative values for differential sensitivity reflect the downward direction of temperature change yielding a rise in impulse frequency.

The cold cell in Fig. 5A is one of 16 examined similarly. Most series of responses to cooling steps at a given initial temperature displayed a high correlation coefficient. The mean for the 66 series was \( r = -0.9802 \), the range between -0.9982 and -0.9623. The differential sensitivity (the slope of the regression lines) was then plotted against initial temperature (Fig. 5B). All 66 series showed the same relationship as displayed in Fig. 5A: the lower the initial temperature, the steeper the regression. A linear regression indicates that, as the initial temperature decreases from 22 to 18°C, the differential

![Figure 4](http://jn.physiology.org/)

**Fig. 4.** A and B: examples of the activity of the cold cell and the dry cell in a single sensillum, during the last 0.5 s at the end of a 3-min exposure to an air stream with constant temperature and partial pressure of water vapor and after a sudden change to a second air stream different from the first in either the temperature or the water vapor. Large-amplitude impulses (III) are produced by the cold cell, the small impulses (●) by the dry cell.
sensitivity to rapid cooling increased by 2.2 impulses/s \( \cdot \) °C \(^{-2} \) from 2.9 to 10.6 impulses/s \( \cdot \) °C \(^{-1} \). At 22.5°C the step size must be increased by 0.3°C to raise impulse frequency by 1 impulse/s and at 18.5°C by 0.09°C. Thus impulse frequency can be influenced more by increasing the cooling step by 1°C than by decreasing the initial temperature by 1°C.

**Downward steps in partial pressure of water vapor**

Not only rapid cooling but also rapid drying increases the cold cell’s discharge rate (Fig. 4). To determine whether such responses were related to the amplitude of drying (and thus show that the discharge conveys information on both cooling and drying), 14 cold cells were investigated systematically. They were exposed to 62 series of downward steps in partial pressure of water vapor, with each series at a different constant temperature in the 18.5 to 22°C range.

Figure 6A is an example of three drying-step–response curves from the same cold cell shown in Fig. 5A. The curves are nearly linear as in Fig. 5A and, also, the slopes are not the same at all temperatures. Independently of sign, they become steeper as the temperature drops. At 21.5°C the curve indicates an increase of 0.18 impulse/s for a 1% increment in the relative humidity; at 18.5°C the value is almost double, 0.34 impulse/s. Thus at 21.5°C the size of the drying step must increase by 5.5% relative humidity to raise impulse frequency by 1 impulse/s; at 18.5°C the value is only 2.9%.

This relationship was reflected in the same manner when the slopes of the whole sample of response curves were plotted to manifest the temperature dependency (Fig. 6B). The mean correlation coefficient for the 62 curves was \( r = -0.9676 \), the range between \( r = -0.9902 \) and \( r = -0.9234 \). A linear regression indicates that, as the temperature decreases from 22 to 18.5°C, the sensitivity to rapid drying increases by \( -0.04 \) impulse/s \( \cdot \) °C \(^{-2} \) from 0.15 to 0.29 impulse/s \( \cdot \) °C \(^{-1} \). Thus at 22°C the step size must increase by 6.6% relative humidity to raise impulse frequency by 1 impulse/s and at 18.5°C by 3.4% relative humidity. (The negative values for differential sensitivity reflect the downward direction of humidity change yielding a rise in impulse frequency.)

**DISCUSSION**

The term “cold cell” has traditionally been restricted to sensory cells that are excited by a small decrease in temperature and that are insensitive to mechanical or chemical stimul-
lotion (Hensel 1952, 1974; Loftus 1968; Tichy 1979). This definition characterizes insect cold cells only partially because their responses were found to be determined by more than one modality. Impulse frequency of the cold cells of the stick insect Carausius morosus (Tichy 1978) and the cave beetle Speophyes lucidulus (Loftus and Corbière-Tichané 1987) rises to transitory high values when an air stream directed onto the sensillum is replaced by another a few degrees cooler but at the same partial pressure of water. It can also rise to high values when the second stream has the same temperature as the first but a lower partial pressure of water vapor. Here, we present the first quantitative study on the excitation of a cold cell to decreasing water vapor pressure. The results show that a portion of the cold cell’s frequency range is covered by the responses to both cold and dry stimulation. Within this range, each frequency value can be obtained by a set of combinations of initial temperature and temperature drop and by properly adjusting both temperature and humidity drop to a whole variety of values. The response at any moment is ambiguous because different modalities influence it. The high resolving power for temperature drops could lose much of its meaning unless it can be shown that the responses to one of the modalities were ultimately ascribable to the other.

Although the bimodality of the cold cell is clearly defined, the small size of these cells precludes visualizing or directly analysis of the processes that govern their response to falling humidity. In view of the technical obstacles, we attempt to explain the responses in terms of accepted physical principles. We therefore check whether the double dependency would disappear with the proper choice of parameters involving temperature and humidity. Such parameters would fall into two groups: one on the side of water vapor and the other on the side of heat.

Water-vapor–related parameter: relative humidity

A single parameter that might explain the response to changes in both temperature and the partial pressure of water vapor would be the relative humidity—i.e., the ratio of water vapor pressure at a given temperature to saturation pressure at the same temperature. The ratio increases when temperature decreases and water vapor pressure remains constant because the saturation pressure decreases with temperature. This relationship, however, rules out relative humidity as an adequate stimulus. The cold cell responds with an increase in impulse frequency when temperature is lowered and water pressure held constant (Fig. 1B, column e, row 2); impulse frequency rises when water vapor is lowered and temperature remains constant (Fig. 1B, column e, row 4). In the first case the cold cell’s increase in impulse frequency is associated with an increase in relative humidity (Fig. 1B, column d, row 2) and, in the second case, with a decrease in relative humidity (Fig. 1B, column e, row 4).

Heat-related parameters

ENTHALPY. Another single parameter that might explain the response to changes in both temperature and the partial pressure of water vapor would be enthalpy. Enthalpy can be decreased by decreasing the temperature or the moisture content (Bredicka 1958; Frauenfelder and Huber 1966). Assuming that impulse frequency is determined by decreasing enthalpy, then frequency must increase under whatever temperature and humidity conditions enthalpy decreases, although this is not the case. Impulse frequency is the same when enthalpy decreases by 2 kJ/kg, brought about by reducing temperature from 20 to 18°C with dry air (0 torr partial vapor pressure of water; Fig. 4A), or when enthalpy decreases by 50 kJ/kg, brought about by reducing the relative humidity from 80 to 0% at a constant of 20°C (Fig. 4B). These reactions are inconsistent with the idea that enthalpy alone accounts for cold cell response.

EVPAPORATIVE COOLING. Another possibility is evaporative cooling. Evaporation would have to be transient to explain the short duration of the cold cell’s responses to sudden drying. One scenario would be a brief discharge of hemolymph from the electrode insertion wound (a droplet covering the sensillum area). In moist air the droplet’s mass would influence the temperature course in the sensillum and in dry air the moisture would produce cooling. Although no droplet was observed where the electrode was inserted and no moisture film was visible, lesion-induced cooling cannot be dismissed entirely: there might have been enough bleeding to permit evaporative cooling without visible moisture film. This scenario is unlikely because in dry air the film would dry out and remain dry unless fed. In moist air the film would have little effect as long as it was only a film. Slow, continued hemolymph discharge should manifest itself either by a crust over the sensillum or by a growing droplet. We conclude that the responses were not lesion induced.

An alternative might be that part of the sensillum continuously takes up moisture from the hemolymph. The moisture content of the sensillum may achieve equilibrium with the ambient air and will be decreased by evaporation when exposed to dry air. In this case the cold cells were basically “wet-bulb” thermometers. The drier the air, the greater the evaporation and the lower the reading on the wet-bulb thermometer compared with the ordinary temperature (indicated by the dry-bulb thermometer). The temperature difference between the two thermometers—the temperature depression—the dry-bulb temperature, and relative air humidity are interrelated, so that any two define the third one. Therefore the relative humidity and the dry-bulb temperature will reveal whether the temperature indicated by the cold cell’s activity represents the wet-bulb temperature.

Although the temperature of the cold cell within the sensillum cannot be measured directly, it is probably the same as that of the air stream because the receptive site is superficial and the air flow rate moves across the sensillum at a velocity of >106 sensillum diameters or roughly 106 antenna diameters per second. Relative to the sensillum, the mass of air contacting it per second is very great. The air temperature is therefore assumed to be equal to the receptive site and can be used to calibrate the cold cell’s impulse frequency for temperature. Accordingly, the change in the calibrated impulse frequency during the exposure to dry air will indicate the magnitude of the sensillum’s temperature drop.

Using this method, the sensillum temperature drops by about 1°C when, at constant temperatures between 18.5 and 21.5°C, the relative humidity suddenly decreases from 60 to 0% (Fig. 7). Under the same conditions, the wet-bulb temperature would fall by about 7°C. Thus the sensillum does not reach the same
low temperature as does a wet-bulb thermometer. The difference may arise from too low an air speed, from water impurities, and from a water supply insufficient for adequate evaporation. A minimum air speed of 2 m/s is required for accurate readings. Much lower speeds yield a too high wet-bulb value.

In the present study, the air stream moved at 2.5 m/s. An inadequate water supply or accumulating solutes, on the other hand, would retard evaporation. A wet surface must stay wet to produce proper evaporative cooling. When the water supply begins to fail, evaporation falls off. Increasing solute concentrations decrease evaporation even further. Then, “wet-surface” temperatures approach that of ambient air and the wet-bulb temperature depression becomes low. In the case of the cold cell, however, insufficient fluid flow and solute concentrations on the “wet surface” could make the temperature depression smaller than that of a wet bulb. Responses of the cold cell, however, are consistent with the notion that water is evaporating from the sensillum surface.

**HOW MUCH WATER CAN EVAPORATE WHEN THE SENSILLUM IS EXPOSED TO DRY AIR?**

The amount of water that can leave a substrate by evaporation depends on the amount of water the substrate contained before exposure to the air, the temperature, and the concentration of water in the air. The relation between the amount of water in the substrate and its concentration outside is expressed, at any temperature, by sorption isotherms, which are, of course, not available for the sensillum. The general principles underlying the sorption process, however, are the same for different materials; this justifies evaluating the mass of water that can be evaporated from the sensillum. This requires determination of the mass of the sensillum, its heat capacity, and the temperature depression arising from evaporation. The sensillum dimensions are measured from serial sections (Fig. 2D), although the density and heat capacity are not known. In the absence of more appropriate data, the values of water are used. Then the mass of water (m\(_w\)) that can be evaporated from the sensillum surface is

\[
m_w = (m_s \times c_s \times dT)/H_w
\]

where m\(_s\) is the mass of the sensillum, which is \(1.3 \times 10^{-8}\) kg; c\(_s\) is the heat capacity of water, which is 4.18 kJ·kg\(^{-1}\)·K\(^{-1}\); dT is the drop in the sensillum temperature, which is 1 K; and H\(_w\) is the enthalpy of evaporation of water, which is 2,500 kJ/kg.

Therefore a 1°C drop in the sensillum temperature, which is elicited by a 60% drop in the relative humidity at constant temperatures between 18.5 and 22°C (Fig. 7), could evaporate \(2.2 \times 10^{-1}\) kg water from the sensillum surface. This is 0.2% of the mass of the dry sensillum. The sorption isotherms of textile fibers indicate a similarly low evaporation. At 60% relative humidity, these values are 13% for wool, 9% for silk, 7% for cotton, 5% for cellulose, and 3% for nylon (from Fig. 7 in Urquhart 1960).

The comparatively low amount of water that could evaporate from the sensillum surface indicates its possible origin. We can rule out a continuously flowing hemolymph that moistens the surface. Instead, the slightly hygroscopic cuticular wall may come to equilibrium with the humid air. When exposed to air of different humidity, the moisture content of the cuticular wall will change by binding water or evaporating the water bound to the surface.

**The meaning of increasing impulse frequency**

The preceding results are important in interpreting transient elevations in the cold cell’s impulse frequency. The cells are highly sensitive to minute temperature changes (an average increase of 0.06°C in the cooling steps suffices to increase frequency by one impulse/s; Tichy and Loftus 1987) and are therefore effective thermoreceptors. However, because two modalities influence the responses, unambiguous information regarding temperature is not automatically available. Some of the total frequency spectrum is filled by changes in ambient humidity. As demonstrated, there is a range of values in the frequency scale that the cold cell’s response never assumes unless ambient temperature is low and has just changed to still lower values. These values are above the range elicited by humidity changes. As soon as frequency exceeds the maximum for humidity changes, a change in temperature is signaled. At discharge rates below the maximum in response to humidity changes, a change in temperature is signaled. At discharge rates below the maximum in response to humidity changes, the dry cell’s response to the same step stimulus may exceed or change the maximum in response to humidity changes, a change in temperature is signaled. At discharge rates below the maximum in response to humidity changes, the dry cell’s response to the same step stimulus may exceed or change the maximum in response to humidity changes, a change in temperature is signaled.


