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TEMPERATURE SENSITIVITY IN THE PROTHORACIC GANGLION OF THE COCKROACH, *PERIPLANETA AMERICANA*, AND ITS RELATIONSHIP TO THERMOREGULATION

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SUMMARY

1. Activity of neurones in the prothoracic ganglion of the cockroach, *Periplaneta americana*, recorded extracellularly, showed a wide range of temperature sensitivity. These responses were categorized by linear regression.

2. The regression lines with the greatest slopes are proposed to characterize central temperature receptors; warm units with lower slopes may be the result of nonspecific Q_{10} responses of ordinary neurones.

3. An overlap of regression lines from cells with high slopes occurs near the acclimation temperature of the animals; the regression lines of most of the warm-sensitive units reach zero firing rate near the mean chill-coma temperature (10·5 °C) for this species.

4. The temperature selection by the whole animal in a temperature gradient shuttlebox was found to require central temperature receptors as well as the peripheral temperature receptors on either the antennae or tarsi.

5. Both neural and behavioural data indicate a greater sensitivity to heat than cold in cockroach thermoregulatory behaviour.

INTRODUCTION

Obligate ectotherms, such as cockroaches, avoid extremes of temperature. A cockroach acclimated to room temperature prefers an ambient temperature of 27–28 °C, but will avoid temperatures below 10–15 °C and above 33–35 °C (Gunn, 1934; personal observation). This avoidance may be achieved by an on-off coupled mechanism in which the animal does not respond actively to the temperature of its surroundings if the temperature remains between two set points, a high and a low. This zone can be referred to as the thermal refractory zone (Heath, 1970). The animal's preferred temperature will be normally distributed between the two set points, whose values may be affected by acclimation. The assumptions of the on-off coupled mechanism can be tested by experiments designed to elucidate how ectotherms sense temperature

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and how temperature information is received and processed by the central nervous system to direct thermoregulatory behaviour.

Peripheral temperature receptors have been found in cockroach antenna (Loftus, 1966, 1968; Alther, Sass & Alther, 1977) and tarsus (Kerkut & Taylor, 1957). The peripheral cold receptor characterized by Loftus (1968) fires a high frequency burst (peak frequency) as the temperature is lowered and adapts after several seconds to a frequency determined by the new steady temperature. The peak frequency depends primarily upon the initial temperature when the antenna is within the range of 25–32 °C, but above or below this range the extent of temperature change becomes important. So a particular peak frequency can be achieved by several different combinations of initial temperature and magnitude of temperature change. This ambiguity, plus the presence of significant fluctuations in frequency at steady temperature make it unlikely that the receptor is a useful thermometer (Loftus, 1968). A different input pathway may be required for adequate thermoregulatory behaviour. Temperature receptors in the central nervous system acting in conjunction with peripheral receptors could provide the needed specificity and precision. The existence of such central receptors is supported by the finding that the transition between warm-up behaviour and flight in Sphinx moths is dependent upon the temperature of the thoracic ganglia (Hanegan & Heath, 1970).

Temperature-sensitive neurones have been described in a few insect species and from various locations in the central nervous system, for example, in locust metathoracic ganglion (Heitler, Goodman & Frazer-Rowell, 1977) and in cockroach thoracic and abdominal ganglia (Kerkut & Taylor, 1956, 1958). Such neurones are not necessarily functional as temperature receptors. Their temperature sensitivity may be the result of a nonspecific Q_{10} effect (Barker & Carpenter, 1970). Indeed we have little knowledge of the neural control of temperature regulation in insects (Kammer, 1981).

The purpose of this study is to search the prothoracic ganglion of the American cockroach, *Periplaneta americana*, for spontaneously active, temperature-sensitive cells and to investigate the relationship between central and peripheral temperature reception by behavioural experiments in a temperature gradient shuttlebox. The prothoracic ganglion was chosen for these experiments because, of the three thoracic ganglia, its physiology has been the most neglected. *P. americana* has relatively simple thermoregulatory behaviour since it does not exhibit basking responses or postural changes. Changes in cockroach body temperature follow the Newtonian model closely (Buatois & Croze, 1977).

MATERIALS AND METHODS

Cockroaches (both sexes) were obtained from our own colony and were fed Ralston Purina rodent chow and water *ad libitum*. All animals were kept at room temperature (RT; 22–24 °C) until used. The light–dark cycle was usually 8 h light and 16 h dark. Experiments were usually conducted during the daylight hours.

Neural recordings

A cockroach was prepared for neural recording by removing the legs just below the

femur and the wings at their base. The insect was then pinned through the abdomen and edges of the pronotum dorsal side up onto a wax block. A strip of exoskeleton was removed from the pronotum to reveal the underlying tissue. The longitudinal thoracic muscles were cut, a few large trachea were cut and removed, and several masses of salivary gland tissue were removed. The gut was then pinned aside to reveal the prothoracic ganglion. A thermode was carefully placed underneath the ganglion, which was raised slightly to help isolate it from movement of the thorax. The thermode was constructed of thin stainless steel tubes (4 and 2 mm diameter) placed inside one another so that water could flow in through the smaller tube and out through the larger. One end of the thermode was tipped with a small platform of solder coated with wax. Water of different temperatures was circulated through the thermode by vacuum pump to change the temperature of the ganglion. A small copper-constantan thermocouple was waxed onto the opposite face of the thermode from the ganglion and was connected to a Bailey Bat-4 electronic thermometer. The maximum difference in temperature between the thermode and the top of the ganglion was found to be 3 °C at maximum deviation of the thermode from ambient temperature. Surrounding tissues, in particular the mesothoracic ganglion, were affected by the thermode. The temperature at the mesothoracic ganglion was at most 6 °C different from ambient when the thermode was near maximum deviation from ambient temperature.

Extracellular recordings were made using a glass capillary microelectrode. Electrodes for some initial recordings were filled with 3 M-KCl and for later recordings with 3 M-NaCl with no apparent change in the nature of the recordings obtained. Electrical contact with the recording electrode was made with a thin tungsten wire. A silver-chloride coated wire, placed in the thorax several millimetres away from the ganglion, served as the reference electrode. The recording electrode was brought into contact with the ganglion by use of a Narishige micromanipulator and was gently tapped in order to penetrate the ganglionic sheath.

The right-hand, caudal quadrant of the prothoracic ganglion was searched for spontaneously active cells. Recordings were made from the first cell that could be isolated. The recording electrode was then held stationary throughout the recording period. Thermode temperature was held constant for the first 30–45 s to obtain base line activity, and the temperature of the thermode was then rapidly changed. A recording was not considered useful unless the cell had been subjected to at least one complete cycle of temperature change.

The electrical activity of the neurone was amplified by a differential amplifier (Frederick Haer) and recorded together with the output of the electronic thermometer on magnetic tape (Hewlett Packard 3960). Neural recordings (Fig. 1) were replayed through an amplitude analyser and rate interval analyser, and displayed with the temperature record on a two-channel chart record (Brush 220). Suitable recordings over the complete temperature range were obtained from 34 cells; 12 were warm sensitive, six were cold sensitive and nine were insensitive. The remaining seven did not fit the simple linear model ($P < 0{\cdot}05$).

Behavioural experiments

The shuttlebox consisted of a steel box 52 cm long, 26·5 cm wide, 2 cm high and covered by a glass plate. The shuttlebox was divided along its length into five equal

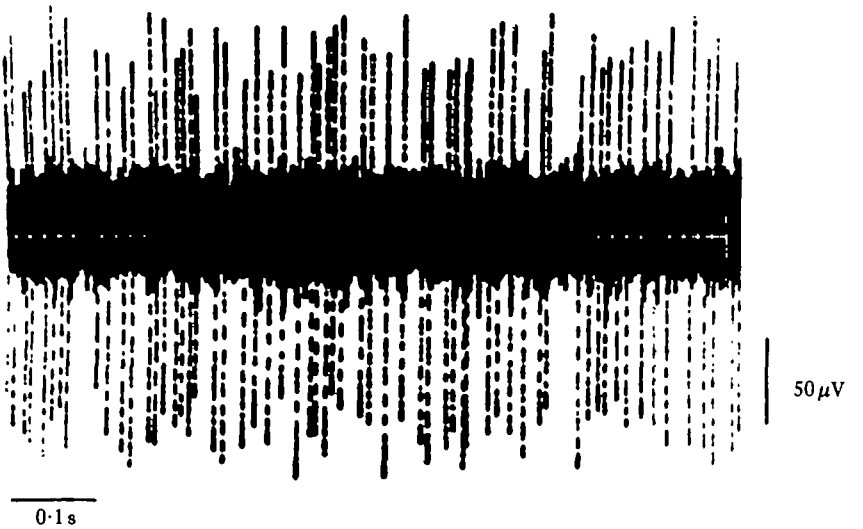

Fig. 1. Oscillograph of the electrical activity from an unidentified cell in the cockroach prothoracic ganglion.

regions, each representing a 10 °C range within the overall range from 0–50 °C. A copper-constantan thermocouple was waxed into place in the base of the box in the middle of each region; every thermocouple was attached to a thermocouple switchbox that was in turn connected to a Bailey Bat-4 electronic thermometer. The output of the electronic thermometer was attached to a one-channel chart recorder (Houston Instruments). The temperature gradient was maintained across the length of the shuttlebox by ice under one end and hot water under the other. The floor of the shuttlebox was covered with wet paper towels to minimize the humidity gradient.

A shuttlebox trial was begun by placing a cockroach in the box in the 20–30 °C region and lowering the glass cover. The time spent by a cockroach in any region over a 20-min period was marked by hand. A total of 20 trials were obtained from 7 to 11 different animals for each group of cockroaches tested. Cockroaches used in one group were not used in subsequent groups. The groups of cockroaches were: no temperature gradient in the box (NTG); room-temperature (RT) acclimated; cold-acclimated (15–17 °C); warm-acclimated (33–35 °C); antennaeless; tarsiless; antennaeless and tarsiless. All groups except the warm- and cold-acclimated groups were acclimated to room temperature. The minimum acclimation time for all animals was 3 weeks, which was considered more than sufficient (Anderson & Mutchmor, 1968). Cockroaches in the coldest region of the box were slowed down and thus may have increased the length of time spent in this region. Those that were unable to leave (5–7 min) were declared cold torpid and the trial aborted. All shuttlebox trials were conducted in lighted conditions.

Data analysis

Chart records of firing rate and temperature were sampled at intervals of 5 s. This permitted an accurate description of each cell's thermal characteristics yet allowed very few high firing rate transients to influence the data analysis. Relatively longer

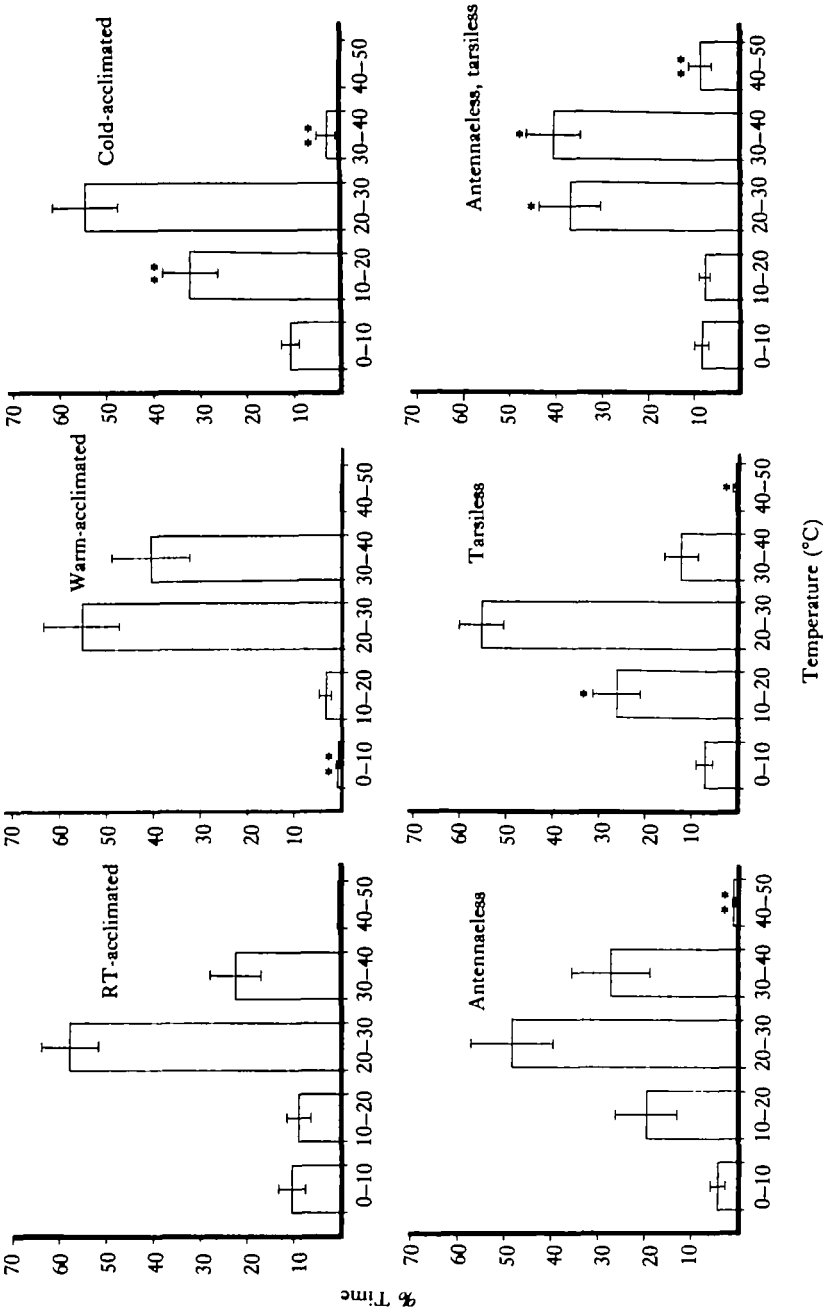

Fig. 2. Percent time histograms for cockroaches in a temperature gradient shuttlebox. All groups were acclimated to room temperature except where stated. The height of each bar represents the mean percent time, the error bars represent the standard error of the mean. Asterisks indicate significance between the mean for that region of that group compared to the mean for the same region for the RT-acclimated group: *—0·05; **—0·01; ***—0·001.

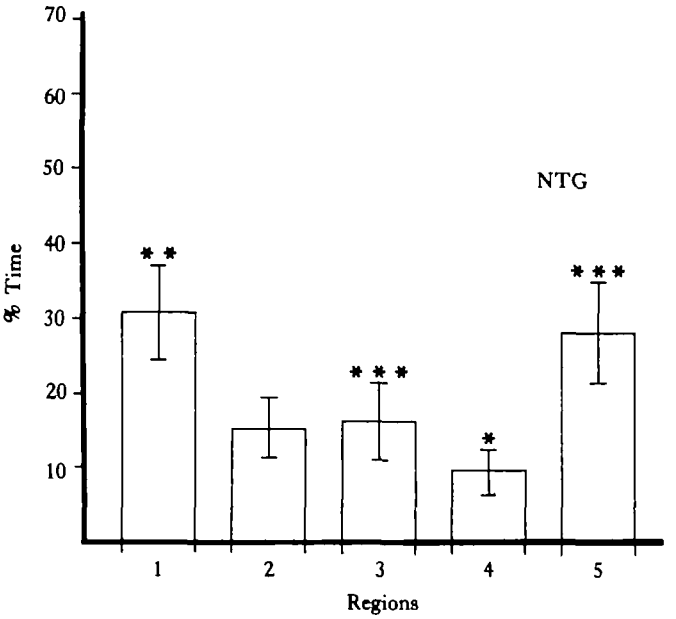

Fig. 3. Percent time histogram for control cockroaches. Normal, room temperature-acclimated cockroaches were released into the shuttlebox in the absence of a temperature gradient: NTG-No temperature gradient. Characteristics of this histogram are the same as those in Fig. 2: *—0·05; **—0·01; ***—0·001. Regions: 1, 0–10 °C; 2, 10–20 °C; 3, 20–30 °C; 4, 30–40 °C; 5, 40–50 °C.

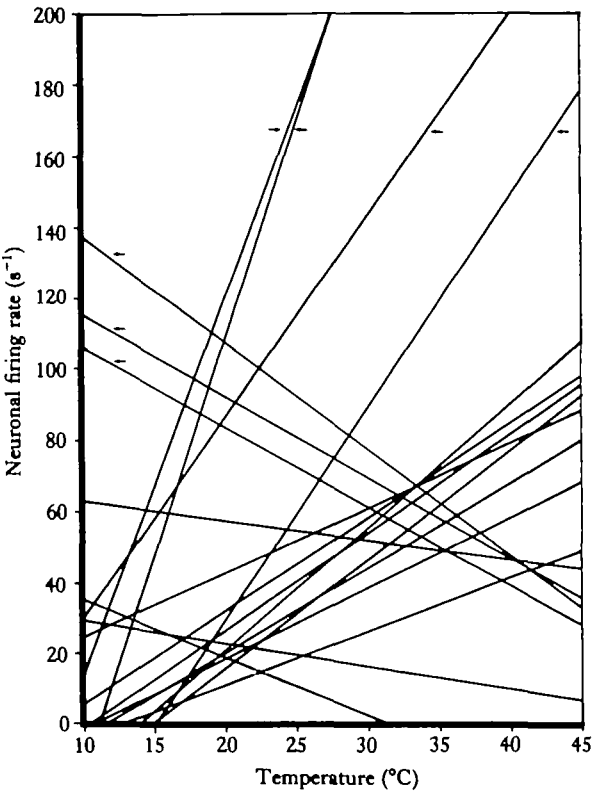

Fig. 4. Composite of the linear regression lines of 18 temperature-sensitive cells. The arrows refer to lines from high sensitivity cells. The y-axis is neuronal firing rate and the x-axis is thermode temperature.

bursts of activity that were obviously from neighbouring cells were omitted from the data analysis (Fig. 6). Shuttlebox data were analysed by Student's *t*-test. Differences were considered significant at a probability level of 0·05.

RESULTS

Behavioural experiments

Cockroaches acclimated to room temperature, but otherwise untreated, sought the temperature range of 20–30 °C when placed in the thermal gradient (Fig. 2). Cockroaches in no thermal gradient preferred the ends of the shuttlebox (Fig. 3). Cold-acclimated animals spent more time below 20 °C and less time above 30 °C than the group acclimated to RT; warm-acclimated animals spent less time below 20 °C

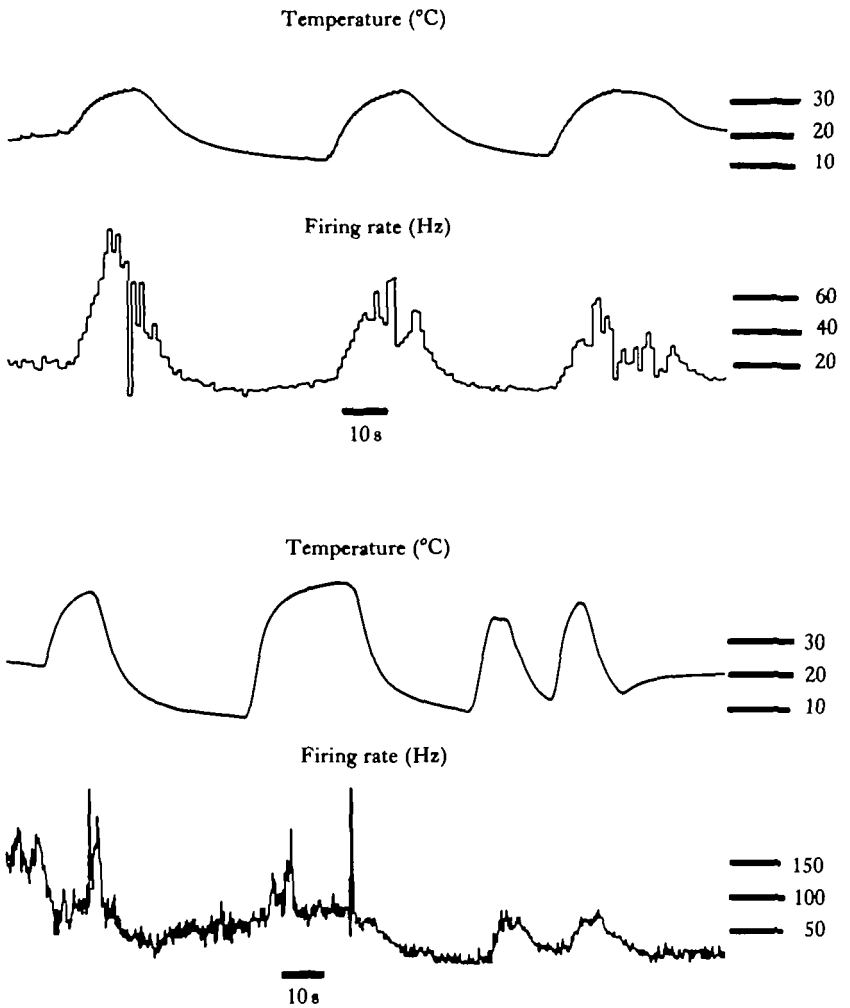

Fig. 5. Chart records of low sensitivity warm units; top unit 7-1-2, bottom unit 5-2-2. In both records the top trace is thermode temperature and the bottom trace is neuronal firing rate. Integration time for the top firing rate is 1 s; for the bottom firing rate it is 0·5 s.

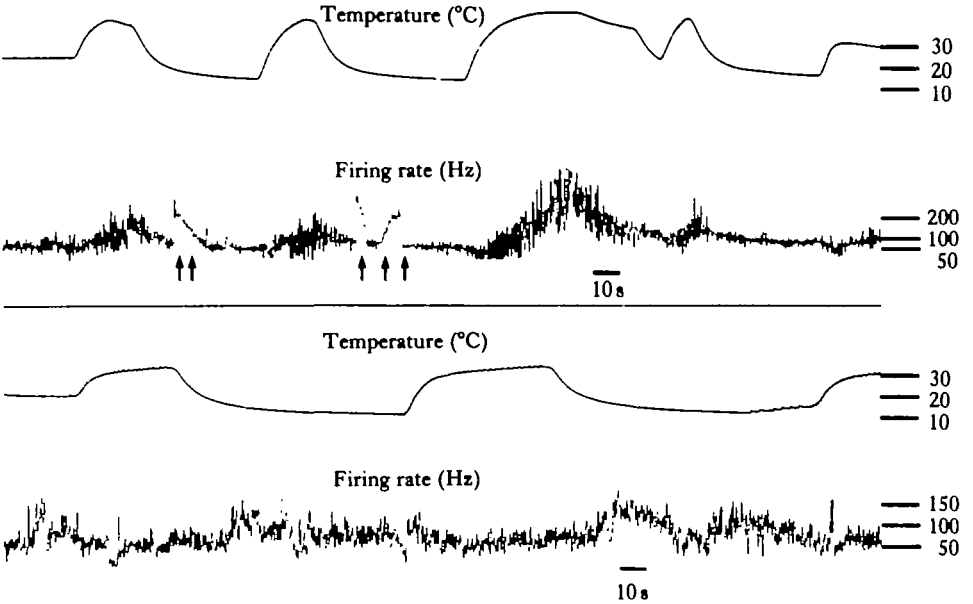

Fig. 6. Chart records of a high sensitivity warm unit (top) 4-1-6 and a high sensitivity cold unit 7-1-9. In both records the top trace is thermode temperature and the bottom trace is neuronal firing rate. The integration time for both firing rate traces is 0·5 s. The arrows refer to points omitted from the data analysis.

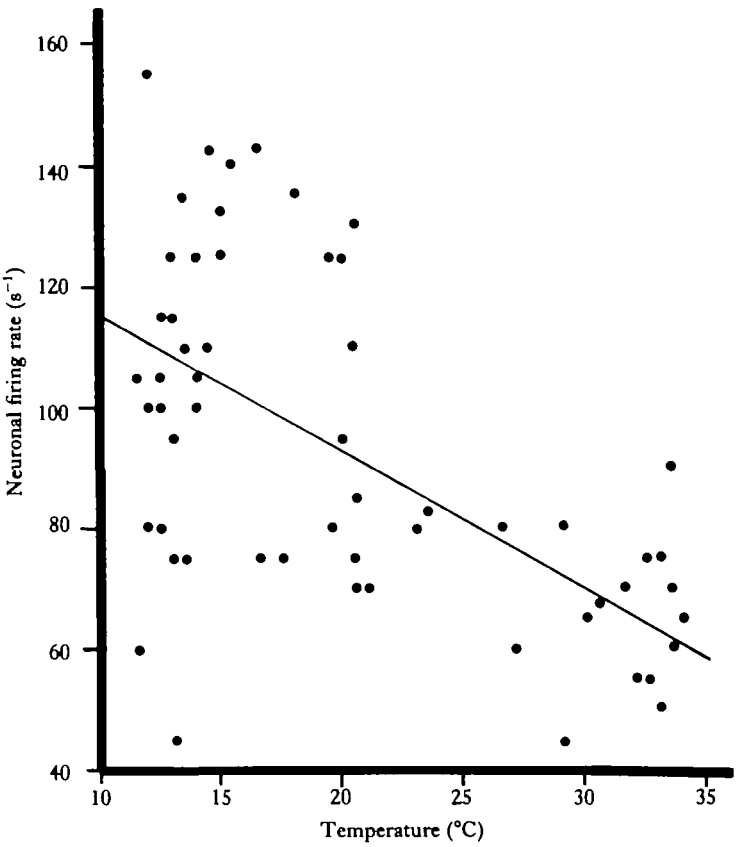

Fig. 7. Scattergram of the high sensitivity cold unit, 7-1-9. The line through the points is a least-squares fitted linear regression line. The y-axis is neuronal firing rate and the x-axis is thermode temperature.

Fig. 2). Animals with only one set of peripheral receptors, those with either antennae or tarsi removed, showed a similar thermal preference to the untreated RT-acclimated animals (Fig. 2). Removal of both antennae and tarsi caused a dramatic shift to the warm end of the shuttlebox (Fig. 2).

Neural recordings

A broad range of temperature sensitivity was found among cells of the prothoracic ganglion. Some units exhibited a much higher sensitivity than others (Fig. 4). For the less sensitive cells, the slopes of the regression lines varied from 1·52 to 3·45 for warm-sensitive units and from −0·52 to −1·66 for cold-sensitive units. Examples of the activity of warm-sensitive units in this category are given in Fig. 5. Cells that displayed the greatest temperature sensitivity are considered to be the most likely candidates for central temperature receptors. Both warm- and cold-sensitive cells were observed (Fig. 6). The slopes of the regression lines of high sensitivity cells varied from 5·70 to 12·16 for warm units and from −2·24 to −3·00 for cold units. A cell was designated as highly sensitive if the slope of its regression line was above 5·00 for a warm unit and below −2·00 for a cold unit. Gaps in slope value, below and above these values respectively (Fig. 4), set these cells apart from the less sensitive cells. The very high firing rates at behaviourally significant temperatures also distinguishes the highly sensitive cells. The scattergrams for two high sensitivity cells (Figs 7, 8) show the extent of variation in firing rate during the exposure of the cells to changing temperature.

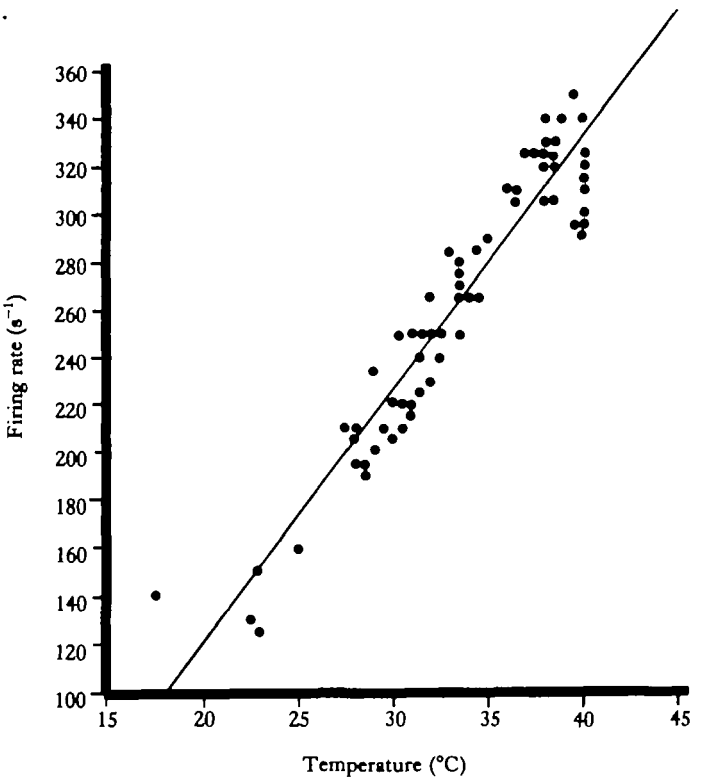

Fig. 8. Scattergram of the high sensitivity warm unit, 4. The line through the points is a least-squares fitted linear regression line. The y-axis is neuronal firing rate and the x-axis is thermode temperature.

Among the high sensitivity cells the firing rates of all but one of the warm units were similar to those of the cold units at temperatures close to acclimation temperature. It should also be noted that three of the four regression lines of the high-sensitivity warm units reach zero firing rate at close to 10 °C.

DISCUSSION

Central temperature receptors should exhibit the characteristics of peripheral temperature receptors: (1) a static discharge at constant temperature; (2) a dynamic response to temperature change; (3) insensitivity to other stimuli; (4) sensitivity to temperature in a behaviourally significant range (Hensel, 1981). In the present study, most temperature-sensitive cells fulfilled three of these criteria; their sensitivity to other stimuli was not observed. Cells whose temperature sensitivity can be attributed to nonspecific Q_{10} effects (Barker & Carpenter, 1970), with a slope lower than 5·00 and higher than −2·00 we assume are not temperature receptors. The remaining highly temperature sensitive cells might be central temperature receptors. They can achieve firing frequencies that are comparable to the peak frequencies of peripheral temperature receptors. However, peripheral receptors tend to be very phasic and nonlinear (Loftus, 1968). The cold units, both high and low sensitivity, may be cells that are inhibited by nearby warm-sensitive cells (Boulant & Demieville, 1977). All cold-sensitive responses may be synaptically driven (Kelso & Boulant, 1982).

If it can be assumed that the high sensitivity cells are central temperature receptors then the similarity of the firing rates of the warm and cold units within a 10 °C range near acclimation temperature may be significant: a difference between the rates may be necessary for a thermal response. Most of the regression lines of the warm-sensitive units reach zero firing rate at about 10 °C (Fig. 4). This is in interesting agreement with Bradfisch, Drewes & Mutchmor (1982), who found that the mean temperature for onset of chill-coma in *Periplaneta americana* was 10·5 °C.

Among the high sensitivity cells, the regression lines for warm units were generally steeper than those for cold units (Fig. 4). A similar finding has been made for temperature-sensitive cells in the brain of the lizard *Tiliqua scincoides* (Cabanac, Hammel & Hardy, 1967), this may reflect the tendency of animals to operate at high body temperature, often within a few degrees of their lethal limits (Kluger, 1981). A response to a small rise in temperature may, therefore, be more important for survival than a response to a small fall in temperature. This difference also manifests itself in the shuttlebox data in that cockroaches spend approximately 40 % of their time either above or below their preferred temperature range (Fig. 2), but the 20 % time spent in the cold side is spread out over a 20 °C range while the 20 % time spent in the warm side is restricted to a 10 °C range. This low temperature skewness is a characteristic common to many ectotherms (DeWitt & Friedman, 1979). Additional evidence for a greater degree of thermal control on the warm side is given in Fig. 2, where the loss of both sets of peripheral temperature receptors leads to significantly more time spent in the hotter regions of the shuttlebox. This implies that peripheral receptors are especially important for high temperature control. Cockroaches quite often become cold torpid in a temperature gradient shuttlebox, but almost never become hot

rpid. This also supports the contention that cockroaches have a greater sensitivity to heat than to cold.

Thermoregulatory behaviour was shown, albeit with less precision, in the absence of either set of peripheral temperature receptors (Fig. 2). We therefore propose that there is a central thermoregulatory system in the cockroach, and that the high sensitivity cells of the prothoracic ganglion may form part of this system. The system may include temperature-sensitive cells in other ganglia, such as those observed by Kerkut & Taylor (1958). Further experiments into the nature of the proposed thermoregulatory centre are underway in our laboratory.

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REFERENCES

ALTHER, H., SASS, H. & ALTHER, I. (1977). Relationship between structure and function of antennal chemo-, hygro- and thermoreceptive sensilla in *Periplaneta americana*. *Cell Tiss. Res.* **176**, 389–405.

ANDERSON, R. L. & MUTCHMOR, J. A. (1968). Temperature acclimation and its influence on the electrical activity of the nervous system in three species of cockroaches. *J. Insect Physiol.* **14**, 243–251.

BARKER, J. L. & CARPENTER, D. O. (1970). Thermosensitivity of neurons in the sensorimotor cortex of the cat. *Science, N.Y.* **169**, 597–598.

BOULANT, J. A. & DEMIEVILLE, H. N. (1977). Responses of thermosensitive preoptic and septal neurons to hippocampal and brain stem stimulation. *J. Neurophysiol.* **40**(6), 1356–1368.

BRADFISCH, G. A., DREWES, C. D. & MUTCHMOR, J. A. (1982). The effects of cooling on an identified reflex pathway in the cockroach (*Periplaneta americana*), in relation to chill-coma. *J. exp. Biol.* **96**, 131–141.

BUATOIS, A. & CROZE, H. O. (1977). Thermal responses of an insect subjected to temperature variations. *J. therm. Biol.* **3**, 51–56.

CABANAC, M., HAMMEL, H. T. & HARDY, J. D. (1967). *Tiliqua scincoides*: Temperature sensitive units in the lizard brain. *Science, N.Y.* **158**, 1050–1051.

DEWITT, C. B. & FRIEDMAN, R. M. (1979). Significance of skewness in ectotherm thermoregulation. *Am. Zool.* **19**, 195–209.

GUNN, D. L. (1934). The temperature and humidity relations of the cockroach (*Blatta orientalis*). II. Temperature preference. *Z. vergl. Physiol.* **20**, 617–625.

HANEGAN, J. L. & HEATH, J. E. (1970). Temperature dependence of the neural control of the moth flight system. *J. exp. Biol.* **53**, 629–639.

HEATH, J. E. (1970). Behavioral regulation of body temperature in poikilotherms. *The Physiologist* **13**(4), 399–410.

HEITLER, W. J., GOODMAN, C. S. & FRAZER-ROWELL, C. H. (1977). The effects of temperature on the threshold of identified neurons in the locust. *J. comp. Physiol.* **117**(2), 163–182.

HENSEL, H. (1981). *Thermoreception and Temperature Regulation*. Monographs of the Physiological Society No. 38. London: Academic Press. 321 pp.

KAMMER, A. E. (1981). Physiological mechanisms of thermoregulation. In *Insect Thermoregulation*, (ed. B. Heinrich), pp. 115–158. New York: John Wiley & Sons.

KELSO, S. R. & BOULANT, J. A. (1982). Effect of synaptic blockade on thermosensitive neurons in hypothalamic tissue slices. *Am. J. Physiol.* **243**(5), R480–R490.

KERKUT, G. A. & TAYLOR, B. J. R. (1956). Effect of temperature on the spontaneous activity from the isolated ganglia of the slug, cockroach and crayfish. *Nature, Lond.* **178**, 426.

KERKUT, G. A. & TAYLOR, B. J. R. (1957). A temperature receptor in the tarsus of the cockroach, *Periplaneta americana*. *J. exp. Biol.* **34**, 486–493.

KERKUT, G. A. & TAYLOR, B. J. R. (1958). The effect of temperature changes on the activity of poikilotherms. *Behaviour.* **13**, 259–279.

KLUGER, M. J. (1981). *Fever, its Biology, Evolution and Function*. Princeton, New Jersey: Princeton University Press. 195 pp.

LOFTUS, R. (1966). Cold receptors in the antenna of *Periplaneta americana*. *Z. vergl. Physiol.* **52**, 380–385.

LOFTUS, R. (1968). The response of the antennal cold receptors of *Periplaneta americana* to rapid temperature changes and to steady temperature. *Z. vergl. Physiol.* **59**, 413–455.