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THE EFFECTS OF TEMPERATURE ON FLIGHT MUSCLE POTENTIALS IN HONEYBEES AND CUCULIINID WINTER MOTHS

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SUMMARY

Amplitudes of extracellular action potentials in indirect flight muscles of honeybees and cuculiinid winter moths decline with decreasing muscle temperatures and fall suddenly to zero. Action potential durations increase with amplitude decline. Amplitudes at 11 °C are only 20 % of values near 30 °C in workers of *Apis mellifera mellifera*. They fall to zero at approx. 10 °C. In the cuculiinid winter moth *Eupsilia devia*, amplitudes at 1 °C are approx. 12 % of values at 27 °C. They fall to zero between 0 and 1 °C. The duration of action potentials in bees and cuculiinid winter moths is about 7 ms at 27 °C and increases to 52 ms at 11 °C in bees and to 66 ms at 1 °C in moths. The ratios of action potential rise time to fall time are about 1 at 27 °C for bees and moths. They decrease to 0·45 at 11 °C in bees and to 0·56 at 1 °C in moths. Results suggest that bees can heat flight muscles only if muscle temperatures are above 10 °C, whereas cuculiinid winter moths can shiver with muscle temperatures near 0 °C.

INTRODUCTION

Insects fly at a wide range of ambient temperatures. Flight muscles need particular temperatures for adequate power output (Esch, 1976; Heinrich, 1981*a*,*b*). Muscle temperatures may have to be raised above or held near environmental temperatures for proper flight conditions. Muscle temperatures can be raised by shivering before flight and may be kept above environmental temperatures through heat produced as a by-product of flight. They can be lowered by convective heat loss or evaporative cooling. Ambient temperatures ultimately determine the range in which flight muscles can be used. Insufficient heat loss sets an upper limit above which muscles overheat. Too much heat loss makes flying uneconomic at lower temperatures since muscles cool down quickly and flight has to be interrupted for reheating (Esch, 1976). Flight muscles cannot be used at all when environmental temperatures are too low for shivering.

Numerous behavioural observations in insects suggested that ambient temperatures must be above 10 °C to allow shivering (Heinrich, 1981*a*,*b*). Preliminary

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measurements of flight muscle action potentials in honeybees showed that action potential duration increases exponentially when muscle temperatures fall below 18°C (Esch & Bastian, 1968). This indicates that the temperature sensitivity of action potential mechanisms limits the ability to shiver at temperatures well above 0°C. However, Heinrich (1987) reported that adult cuculiinid winter moths in northern temperate regions of the United States can begin to shiver when body temperatures are −2°C. Their flight muscles must be distinctly different from those of honeybees.

The work reported here was undertaken to document differences in temperature dependence of action potentials in honeybees and cuculiinid winter moths. Action potentials were recorded in both species at environmental temperatures between 0 and +30°C under similar conditions. One can predict the lowest environmental temperature at which shivering is possible from the results. Since flight muscles are the only internal heat source in these two species, one can understand how muscle physiology and ecology interact.

MATERIALS AND METHODS

Cuculiinid winter moths (*Eupsilia devia*) were caught near Burlington, Vermont, and shipped to Notre Dame, Indiana. Worker bees (*Apis mellifera mellifera*) came from the apiary in the Department of Biological Sciences at the University of Notre Dame.

A small hole was punched through the dorsal cuticle with an insect pin. Copper or platinum wire electrodes (diameter 50 μm) were pushed into dorsoventral or dorsolongitudinal flight muscles. A reference electrode was placed in the head. Electrodes were waxed into place. The animal was attached dorsally to a small wooden rod with tacky wax. A copper–constantan thermocouple (diameter 0·001 mm) was waxed to the dorsal surface to measure muscle temperature (Esch, 1960). A second thermocouple 1 cm above the animal determined the environmental temperature.

The animal held a small piece of paper with its feet and was lowered into a round glass container (diameter 4·5 cm, height 2 cm) resting on a 12×12 cm copper plate, 0·4 cm thick. The glass container was insulated with a 1 cm thick styrofoam layer on the outside, except on the part of the bottom which was in contact with the top of the copper plate. Four water-cooled frigistors attached to the copper plate allowed manipulation of the temperature in the glass container from −5°C to +30°C by varying frigistor current. Electrodes were directly connected to a UFI Model 2122 Bioamplifier (input impedance >10 MΩ). The d.c. output of this amplifier was connected to a Vetter Model B Instrumentation Recorder (FM mode). One-minute samples were taken at various temperatures. Recordings were played back to a Tandy Model 1000 microcomputer through an AD-interface board. Amplitudes, slopes and durations of action potentials were determined from digitized recordings. Results were stored in a database for subsequent evaluation. Time between the first deflection from resting potential and the peak was taken as the rising phase. Duration of the falling phase was determined by the time between the peak and the intersection

with the resting potential of an extrapolated line laid through the peak and the first half of the falling phase. The latter arrangement was necessary since action potentials often showed a considerable junctional component (Esch & Bastian, 1968).

RESULTS

Bees

It was possible to follow single units close to an electrode through the whole temperature range. Units with similar amplitudes (2–4 mV at 20°C) were evaluated (Fig. 1). Results are based on 2803 action potentials from nine individuals. Variability was mostly within-individual variation. Spontaneous action potentials were always seen when body temperatures dropped below 16°C. The amplitude decreased slowly with temperature and fell suddenly to zero between 10 and 11°C (Fig. 1). Action potential frequencies were between 10 and 20 Hz and decreased with temperature in a given preparation (Fig. 2). The last seconds before disappearance at 10°C were marked by a sudden increase in frequency. Then *all* detectable electrical activity ceased. The cut-off point was very distinct. This burst led to no detectable increase in flight muscle temperature. High frequency could not be sustained if body temperature was kept at the temperature of the cut-off point. Activity regularly began near 10°C if bees were warmed again. Bees under experimental conditions often needed to be mechanically stimulated to produce action potentials if their body temperature was above 16°C.

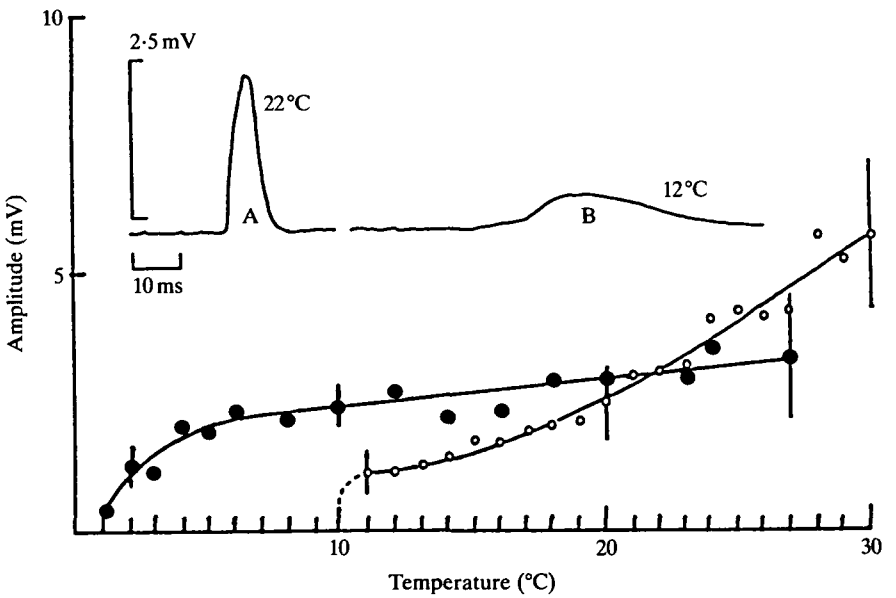

Fig. 1. The amplitude of flight muscle potentials in bees (open circles) and cuculiinid winter moths (filled circles) and muscle temperature. Characteristic standard deviations are given at the beginning, centre and end of curves. Inset: bee action potential (A) at 22°C and (B) at 12°C.

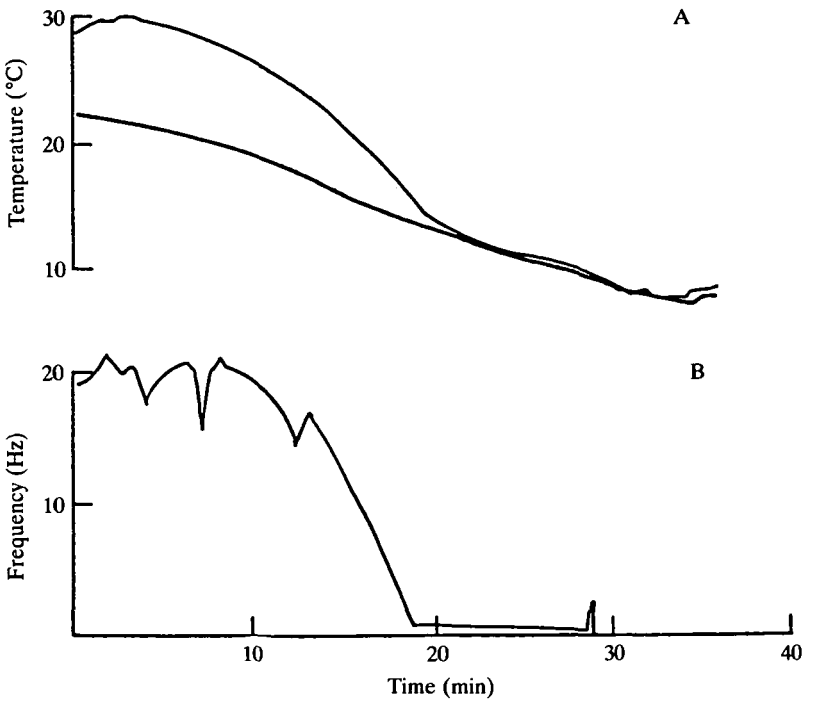

Fig. 2. Body temperature (A, upper trace), environmental temperature (A, lower trace) and action potential frequency (B) of a honeybee worker in a characteristic experiment. All values were computer averaged over 6 s and then plotted.

Muscle potentials lasted about 7 ms at 27 °C. Duration increased with falling temperatures (Fig. 3). The longest value reliably measured was about 52 ms near 11 °C. The curve depicting action potential duration *versus* temperature had an asymptote near 10 °C. At this point action potentials would have infinite durations. Separation of duration into 'rise time' (from resting potential to peak) and 'fall time' (from peak to resting value) showed that fall time contributed the biggest share to long durations near 10 °C (Fig. 4). Rise and fall times were nearly equal at temperatures above 20 °C. Rise time increased somewhat as temperature fell below 20 °C, but fall time increased much more.

Cuculiinid winter moths (Eupsilia devia)

Single units near the recording electrode could be followed through the whole temperature range. Units with amplitudes between 2 and 4 mV at 20 °C were selected for evaluation. Results are based on 957 action potentials from three individuals. Amplitudes decreased with falling temperatures, but not as drastically as in bees (Fig. 1). The decrease was nearly linear down to 4 °C and the amplitude fell quickly to zero at 0 °C. Action potential frequencies were in the range of a few Hz. Animals were cooled further to −5 °C and then heated again. Action potentials reappeared when muscle temperatures reached 1 °C and legs were stimulated by pulling the

paper held by the animals. Individuals did not show spontaneous activity at temperatures near 1 °C. Activity could last for 10–20 min once it was stimulated.

The duration of muscle potentials was not significantly different from the values for honeybees near 27 °C (approx. 7 ms). The increase with falling temperatures was smaller than in bees. The asymptote lies near 0 °C (Fig. 3). The longest reliably measured duration was approx. 66 ms at 1 °C. The curve thus extends much further

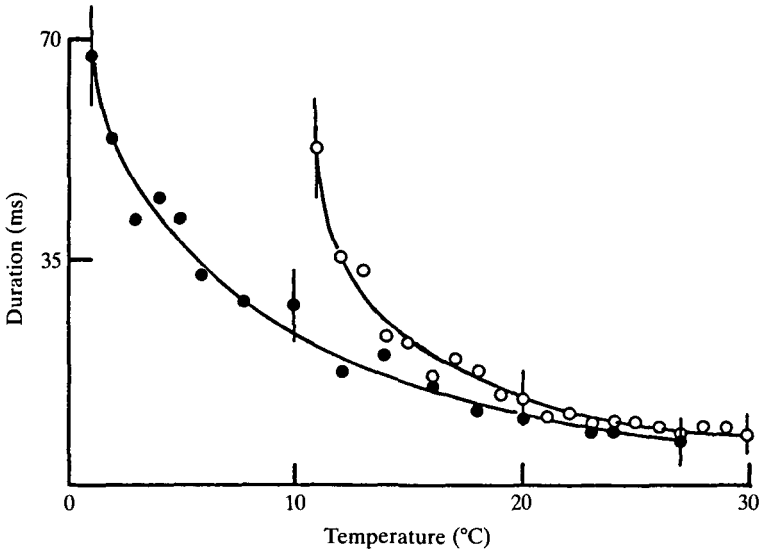

Fig. 3. The duration of flight muscle potentials of bees (open circles) and cuculiinid winter moths (filled circles). Standard deviations as in Fig. 1.

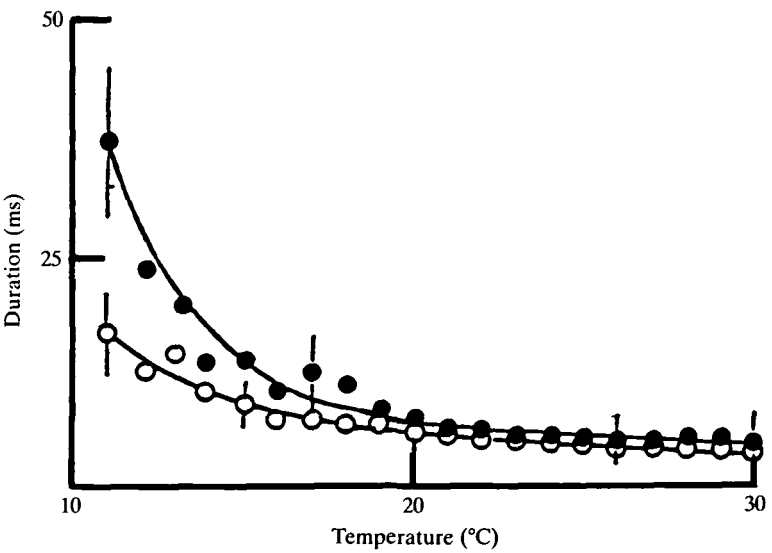

Fig. 4. The duration of rising phase (open circles) and falling phase (filled circles) of muscle potentials in bees with muscle temperature. Standard deviations as in Fig. 1.

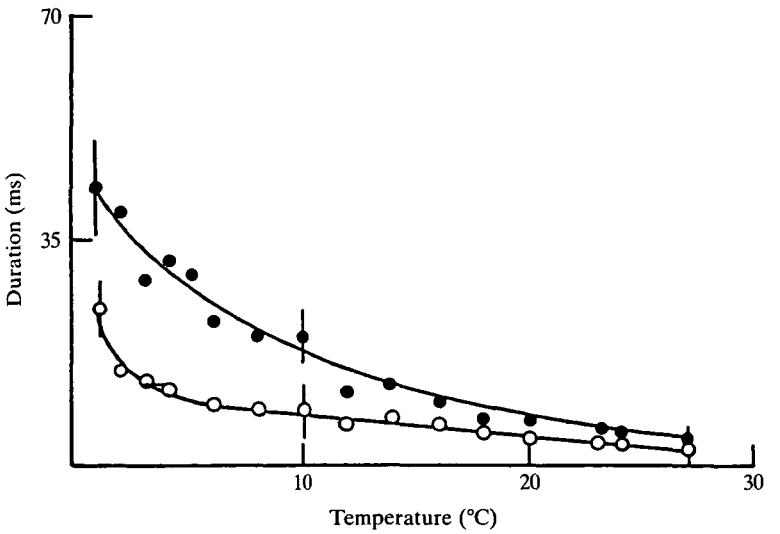

Fig. 5. The duration of the rising (open circles) and falling (filled circles) phases of muscle potentials in cuculiinid winter moths with muscle temperature. Standard deviations as in Fig. 1.

into lower temperatures than in bees. Rise time and fall time were nearly equal at temperatures above 20°C, but fall time increased much faster with falling temperature (Fig. 5).

DISCUSSION

Physiological observations

The electrical processes that control resting and action potentials in squid axon are reasonably well understood. This is not true for insect muscles (Piek & Djie Njio, 1979; Pichon & Ashcroft, 1985). The resting potential of squid axon is practically constant over the range 3–20°C. It decreases at higher temperatures and falls by 10–15 mV near 35°C (Hodgkin & Katz, 1949). The rising phase of squid axon action potentials is caused by the opening of sodium channels and the influx of sodium ions. Resting potential is restored during the declining phase when sodium channels close and potassium channels open. Sodium and potassium movements determine duration and amplitude of the rising and declining phases. Movement of potassium is affected more by falling temperatures than that of sodium. This leads to a proportionally larger increase in the duration of the declining phase as compared with the rising phase (Hodgkin & Katz, 1949). Moreover, action potential amplitudes *increase* with falling temperatures since the restoring effects of potassium movement show later and later.

The opposite can be observed with rising temperatures. An increasingly faster rate of potassium movement leads to a progressively earlier inactivation of sodium influx.

This *reduces* the action potential amplitude and eventually leads to a 'heat block' of propagation near 38°C (Hodgkin & Katz, 1949; Noble, 1966).

These processes cannot be simply applied to insect flight muscles. Sodium and potassium are responsible for the resting potential in a similar way as in squid axon. However, the rising phase of action potentials is caused by the opening of *calcium* channels (Wood, 1957; Hoyle & Smyth, 1963; Hagiwara & Naka, 1964). The declining phase is controlled by potassium movement. Increasing durations of rising and declining phases of action potentials with decreasing temperatures point to a decrease in calcium and potassium movement with falling temperatures. Movement involves ion flux and gating mechanisms. Both processes are temperature-dependent, but the gating mechanisms probably have a much higher temperature coefficient. Potassium movement decreases faster than calcium movement. This is reflected in the proportionally longer declining phase at lower temperatures. The Nernst equation for calcium ions predicts a decrease of a few percent in action potential amplitude over the experimental range. The observed potential changes are much bigger. The Nernst equation cannot explain the *sudden* drop to zero at a given temperature. Reasons for decline in action potential amplitude are not yet understood. Planned intracellular recordings might give a clue.

Action potential amplitudes in insect flight muscles *increase* with rising temperatures. Durations for rise and fall time become equal between 20 and 30°C. There is no indication that fall time might become shorter than rise time with further increase in temperature or that action potential amplitudes might be reduced by earlier onset of inactivation of calcium influx. Action potential amplitudes in bees at 38°C are higher than at 30°C. Wings beat with a very high frequency and maximum lift is observed (Esch, 1976). Heat block does not occur near 38°C.

Behavioural observations

The curves representing amplitudes and durations at various temperatures clearly mark a range in which muscles can operate. Amplitudes fall to zero at distinct temperatures and durations increase towards infinite values. This breakpoint is not the same for cuculiinid winter moths and honeybees. Bees cannot use flight muscles at temperatures below 10°C, even for shivering. For cuculiinid winter moths this critical temperature lies near 0°C. The difference between −2°C (Heinrich, 1987) and 0°C in the above experiments might be explained by species and/or individual differences. B. Heinrich (personal communication) looked at a dozen species and hundreds of individuals. More than 98% of the individuals did *not* warm up from −2°C.

Observations of small groups of worker bees at environmental temperatures between 0 and 40°C (Free & Spencer-Booth, 1958, 1960) and of swarms under similar conditions (Heinrich, 1981*a*,*b*) relate the results to behaviour. The number of dead or immobile animals in small groups (10–200 individuals) during a 24-h period declines sharply if the environmental temperature is raised from 9°C to 11°C (Free & Spencer-Booth, 1958). Only 6·2% of workers taken directly from a hive and kept alone at 9°C moved after 1 h. The number jumped to 95·4% when the

environmental temperature was raised to 10°C. Both observations show that individuals lose control over flight muscles near 10°C and have no means for *individual* heating. They fall into chill-coma or die.

Bees in the mantle of captive swarms hold their body temperature at 15–16°C independent of ambient temperatures from 4 to 13°C (Heinrich, 1981*a*,*b*). They try to avoid cold torpor to be ready to warm up quickly should the swarm decide to move on. Animals fall from small swarms kept at room temperatures of 5°C for several days, but they move again when warmed up. Not only wing, but also leg and body, muscles fail at lower temperatures. In most experiments a loudspeaker was connected to the recording amplifier, and electrical activity from nerves and muscles could be heard in the background. This activity disappeared completely near 10°C and returned with action potentials in flight muscles during warm-up.

Cuculiinids can actively warm their bodies from temperatures near 0°C. They have excellent heat insulation. Heat flow within their bodies is controlled to avoid heat loss to head and abdomen (Heinrich, 1987). They can fly during any winter month in the northeastern United States when environmental temperatures are close to 0°C. Specialization of their flight muscles shows mainly at low temperatures enabling them to shiver. Thoracic temperatures of 30–35°C are required for flight. Similar thoracic temperatures are found during flight in comparable moths from tropical areas (Bartholomew & Heinrich, 1973). It would be interesting to know whether moths from tropical areas *can* shiver near 0°C.

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