

## TEMPERATURE DEPENDENCE OF THE NEURAL CONTROL OF THE MOTH FLIGHT SYSTEM

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### INTRODUCTION

The warm-up period of Lepidoptera can be characterized not only by the increasing metabolic rate and rising thoracic temperature (Hanegan & Heath, 1970; Heath & Adams, 1967; Dorsett, 1962; Dotterweich, 1928; Oosthuizen, 1939; and others) but also by the phasing of contraction of the flight muscles (Kammer, 1968, 1967; Moran & Ewer, 1966).

In Lepidoptera the flight system is neurogenic (Roeder, 1951). There are two major groups of muscles involved in flight: the dorsal longitudinal muscles (DLM), wing depressors, and the dorsoventral muscles (DVM), wing elevators (Nuesch, 1953, 1957). During flight these two muscle groups contract alternately, and the wing movements are of large amplitude. During the warm-up period the wing movements may be of either small or medium amplitude and occur at flight frequencies or higher. The phasing of the depressors and elevators during warm-up varies in different species. In the saturnid moth, *Samia cynthia*, the elevator and depressors contract synchronously and the frequency is higher than observed in flight; however, in the sphingid moth, *Celerio lineata*, the depressors contract out of phase during warm-up and in phase during flight (Kammer, 1968).

The elevators and depressors shift gradually in phase from synchrony to antiphase during the transition from warm-up to flight in the hawk moth, *Mimas tilae*. In *S. cynthia* the transition is abrupt and occurs within one wing-beat cycle (Kammer, 1968). The transition from warm-up to flight must involve alterations in the coupling of the pattern-generating system (Wilson, 1968). Three possible mechanisms to account for this transition have been proposed by Kammer (1968). (1) Command neurones activated by external sensory input effect the transition. (2) Internal temperature receptors which monitor the thoracic temperature exist, and at a critical temperature initiate the transition. (3) The pattern generator itself may be temperature sensitive, and at higher temperatures the flight pattern is more stable than the warm-up pattern. The transition would then occur automatically. Another possible mechanism is that the sensory input from the wing base may initiate the transition. McCrea (1969) found that the frequency of wing beats increased with increasing thoracic temperature during the warm-up period.

In the present study the transition of the warm-up motor pattern to the flight motor pattern in the saturnid moth, *Hyalophora cecropia*, was investigated. The effects of altering the temperature of the thoracic ganglia temperature independently

body of temperature on the transition of warm-up to flight was studied in detail. A model to account for the interactions of the two different motor patterns is proposed.

#### MATERIALS AND METHODS

A culture of *H. cecropia* was raised from eggs on wild cherry trees covered with large Nylon nets.

Potentials from the major indirect flight muscles (dorsal longitudinal, wing depressors, and dorsoventral, wing elevators) were recorded simultaneously during warm-up and flight. The recording electrodes consisted of two 0.004 in diameter copper wire 'fish-hooks' fed through a 25 gauge hypodermic needle. The copper wire was insulated with an enamel coating to the tip. The hypodermic needle was used to insert the paired electrodes into each muscle group. The potentials generated in the muscles were recorded differentially between the paired electrodes using Tektronics 122 pre-amplifiers. The data were stored on magnetic tape (Ampex 1100 tape deck) for future analysis. During the course of the experiments the muscle activity was monitored on a Tektronics 502 dual beam oscilloscope. For permanent records the tape play-back was photographed from the oscilloscope with a Cossor Instrument camera. Text-fig. 1 is a schematic diagram of the experimental arrangement.

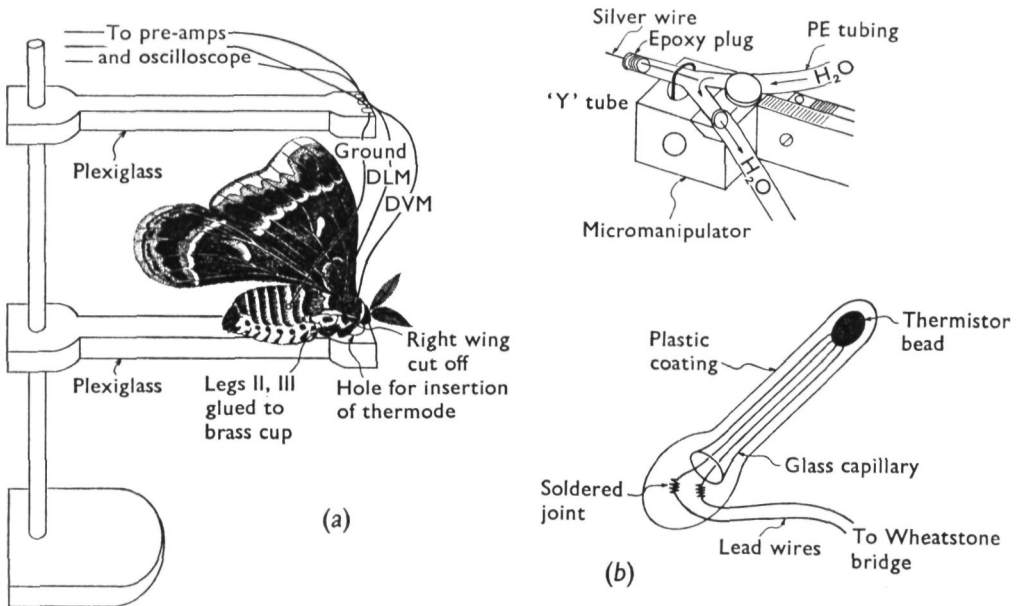
The animal was not free to fly, but the movement of the wings was not hampered and they were able to describe an arc of 180°. The animal was held stationary by glueing a portion of the ventral surface and the severed stumps of the mesothoracic and metathoracic legs to a small brass cup supported by a Plexiglass rod. Weldwood contact cement worked most satisfactorily in holding the animal in position. A second Plexiglass rod supported 4–6 in above the mounted animal was used to prevent the electrode and ground wires from interfering with the movement of the wings (shown in Text-fig. 1).

Two types of thermodes were used to heat the thoracic ganglia independently of body temperature. The first was a Veco 2 K ohm thermistor bead (0.02 in in diameter) mounted in a glass capillary tube and encased in plastic. The leads of the thermistor were drawn through the capillary tube which was then filled with Lucite plastic dissolved in chloroform. At this stage in the construction of the thermode the capillary tube was checked for formation of air bubbles or shorting of the thermistor leads. The capillary tube was then coated with dissolved Lucite plastic to prevent current leaks from the thermode. A modified Wheatstone bridge, in which current could be varied, was used to heat the thermistor bead and also to record thoracic temperature.

The second thermode was constructed from a plastic Y-shaped tube 5 mm in diameter and a short length of silver wire. The silver wire was glued into the base of the Y-tube with epoxy cement. The wire extended inward to the branch of the 'Y' and projected 5 mm from the base outward. Water from a constant-temperature bath was pumped into one branch of the 'Y' and left by the other. The silver wire rapidly reached the temperature of the flowing water and conducted the heat out to its tip. When this type of thermode was used thoracic temperature was measured with a thermocouple implanted into the DLM muscle group from the dorsal side and glued into position with a small piece of cork. Temperature was intermittently recorded on a potentiometer.

meter (Honeywell Elektronik-16). Both the water and thermistor thermodes are shown in detail in Text-fig. 1.

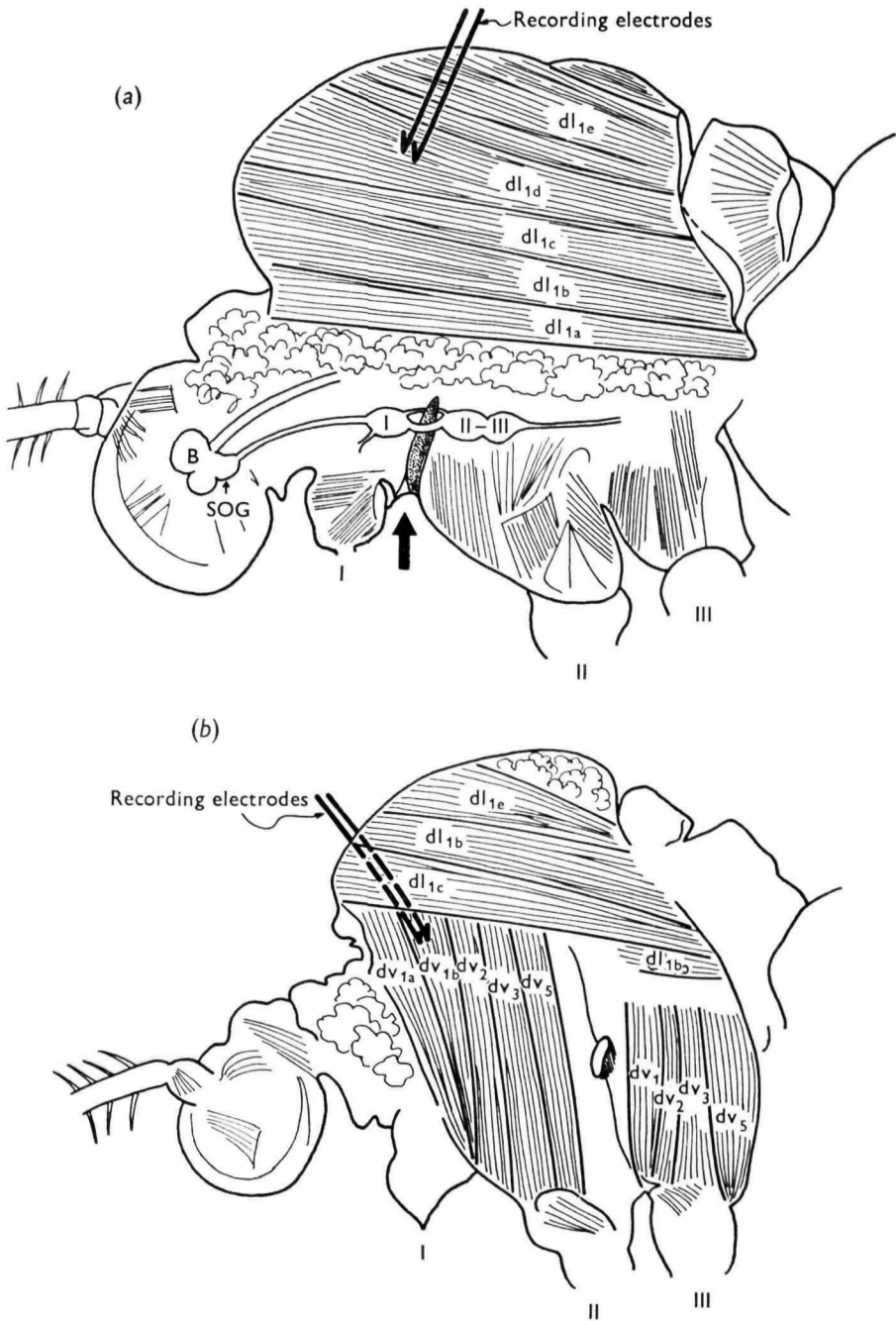
Placement of the electrodes and thermode was determined from histological sections. Several moths were embedded in Celloidin and sectioned at  $125\ \mu\text{m}$ . The sections were cut in a variety of orientations and were stained with haematoxylin eosin to reveal the fine structure of the nerves and muscles of the thorax. From these sections it was noted that an apodeme projected inward between the connective nerves of the prothoracic and pterothoracic ganglia. The apodeme was open to the exterior of the animal and provided access to the thoracic ganglia with minimum damage to the animal. The shape and position of this apodeme was confirmed by boiling the thorax of both male and female moths in 10% KOH to remove all soft tissue. After the skeleton had been cleared the intact apodeme could be seen clearly. Text-fig. 2a is a drawing traced from a photograph of a stained midsagittal section. The tracing indicates both the organization of the DLM muscle group and the relationship of the apodeme to the thoracic ganglia. Text-fig. 2b is a more lateral section from the same animal showing the DVM muscles and the position of the recording electrode in this muscle group.



Text-fig. 1. Experimental arrangement for recording from the indirect flight muscles while heating or cooling the thoracic ganglia. (a) The right forewing and hindwing of the moth have been cut off for clarity. (b) Drawing of the thermodes used for heating and cooling (not drawn to scale).

## RESULTS

*H. cecropia* is incapable of maintaining level flight when the thoracic temperature is below  $34\ ^\circ\text{C}$ , and normally does not initiate flight until the thoracic temperature reaches  $37\ ^\circ\text{C}$  (Hanegan & Heath, 1970). When the thoracic temperature is below  $37\ ^\circ\text{C}$  these moths initiate a warm-up period in which the thoracic temperature rises above ambient at a rate of  $3.5\ ^\circ\text{C}/\text{min}$ .

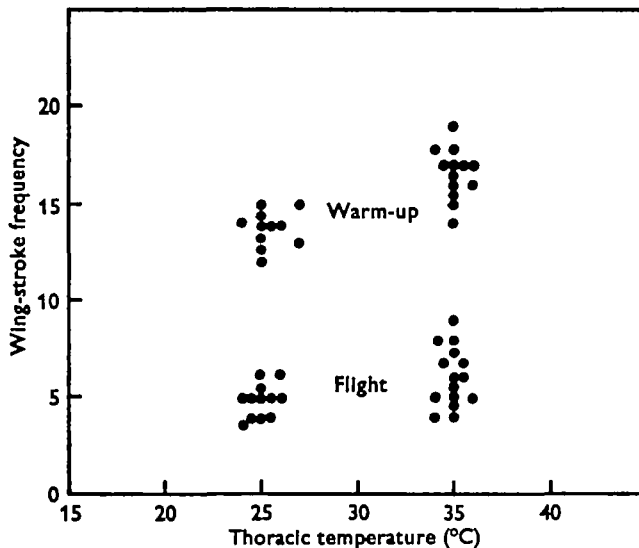


Text-fig. 2 (a). Midsagittal section of a moth (*H. cecropia*) illustrating the organization of the dorsal longitudinal muscles (numbered according to Nuesch, 1953). The arrow points to the apodeme which projects up between the prothoracic and pterothoracic ganglia (II and III in the drawing). I is the prothoracic leg, II is the mesothoracic leg, III is the metathoracic leg, B is the brain, SOG is the suboesophageal ganglia. (b) More lateral section of the same moth showing the placement of the recording electrode in the dorsoventral muscles (dv<sub>1b</sub> or dv<sub>2</sub> from Nuesch, 1953).

During the warm-up period the wings are held horizontally and vibrate at a frequency approximately twice that of the wingstroke frequency in flight. Simultaneous recordings from the wing depressor muscles (DLM) and wing elevators (DVM) indicate that these muscles, normally antagonists during flight, contract synchronously during warm-up (Plate 1). The number of times the muscle is activated per wing stroke (burst length) varies from 1 to 3 during warm-up. The stroke frequency during warm-up is slightly dependent on thoracic temperature, increasing from 15/s at 25 °C to 18/s at 37 °C (Text-fig. 3). The  $Q_{10}$  for this increase in stroke frequency with thoracic temperature is 1.2.

The transition of the warm-up pattern to the flight pattern may occur within one wing-stroke or there may be a period of inactivity between the two patterns (Plate 1). In either event the flight pattern is initiated with a burst of activity in the wing elevator muscles. The frequency of wing stroke varied from 4 to 9/s during flight. The wing-stroke frequency was dependent upon thoracic temperature, and the  $Q_{10}$  was 1.4 (Text-fig. 3). The burst length varied from 5 to 8 during flight.

When the thoracic ganglia were heated independently of body temperature in inactive moths (body temperature equals air temperature when the moth is quiescent) a flight motor pattern was recorded (Plate 2). The time lag between the start of heating and the response ranged from 10 to 60 s. If the response did not occur in the first minute the experiment was categorized as a negative response. In control experiments the thoracic temperature after 1 min of heating did not rise more than 2 °C (25–27 °C).



Text-fig. 3. Frequency of wing-stroke during warm-up and flight plotted against thoracic temperature. The frequency of wing-strokes during flight at low thoracic temperature was taken from thermally stimulated animals.

The wing-stroke frequency in these thermally stimulated animals was used to determine the dependence of stroke frequency on thoracic temperature shown in Text-fig. 3. The motor pattern is essentially the same in these animals and those which

underwent a normal warm-up period before initiating flight. The only apparent difference is the slight decrease in wing-stroke frequency. In two animals the pattern was not a simple flight pattern with continuous heating but rather an alternation of flight and warm-up patterns. Plate 3 shows an example of this response.

The experiments in which the thoracic ganglia were heated in inactive animals show that the flight-pattern generator is temperature sensitive but did not directly indicate that the transition from warm-up to flight was a temperature-dependent phenomenon. In three animals which initiated a warm-up process it was possible to heat the thoracic ganglia and switch the animal from the warm-up to the flight pattern. An example of this change from warm-up to flight as a result of heating the thoracic ganglia is shown in Plate 4. In two of these animals the thoracic temperature was 27 and 29 °C, respectively. The thoracic temperature in the third animal was not recorded, but the animal had not warmed-up for more than 1½ min and presumably the thoracic temperature was well below 34 °C. In these three animals the thermode was turned on for a maximum of 20 s and if the animal did not respond within this time it was considered to have shown a negative response.

Since heating the thoracic ganglia changed the pattern from warm-up to flight, cooling the ganglia would presumably effect the opposite response, i.e. changing the flight pattern in an active moth to the warm-up pattern. Three animals were stimulated to warm-up and flight by pinching the abdomen. Once flight was initiated the thoracic ganglia were cooled with the water thermode (water-bath temperature was 20 °C). In all three animals flight ceased, but the animals did not revert to the warm-up pattern. The flight pattern stopped abruptly and the animals remained quiescent.

In one animal 'passive-cooling' did cause a shift in pattern from flight to warm-up. A quiescent animal was stimulated to generate the flight pattern by heating the thoracic ganglia. After approximately 1 min of flight the thermode was turned off and the animal shifted from the flight pattern to the warm-up pattern. The animal continued to warm-up for about 2 min and then shifted to the flight pattern in a normal transition. Plate 5 shows selected segments of this record.

Table 1. *Summary of thermode experiments*

Transition from warm-up to flight (normal)*	Flight pattern thermode heating (animal quiescent)	Flight pattern thermode heating (animal in warm-up)	Inhibition of flight pattern thermode cooling†
20 (+)	17 (+) 3 (-)	3 (+) 6 (-)	4 (+)

\* In four animals the transition was observed visually and not recorded.

† One animal switched to warm-up; three animals ceased all activity.

Table 1 is a summary of responses of the heating experiments. A normal transition from warm-up to flight was recorded in each animal as a control to insure that the thermode did not damage the thoracic ganglia. In four animals the transition from warm-up to flight was not recorded, but it was observed visually and the animal appeared normal. Seventeen of these 20 animals showed a positive response to heating (generation of flight pattern with no preliminary warm-up) and only three did not

respond. Two of the positive responses were not a continuous flight pattern but an alternation between warm-up and flight motor patterns. Nine of the 20 animals had the thoracic ganglia heated during warm-up and of these only three responded by switching to the flight motor pattern. The time of heating was limited to 20 s, and if the thermode was not precisely positioned the short period of heating may have been insufficient to stimulate the ganglia. Of the animals in which the ganglia were cooled during flight, three ceased all activity (those actually cooled by a thermode) and one switched to warm-up (heating thermode turned off).

The results from four animals were discarded when analysis of the records indicated that the ganglia had been stimulated with d.c. current from a defective thermode. In these animals the DLM units were activated randomly and there was no activity in the DVM units. With increasing heating and current the frequency and magnitude of activity in the DLM increased, but no apparent burst pattern was detected.

#### DISCUSSION

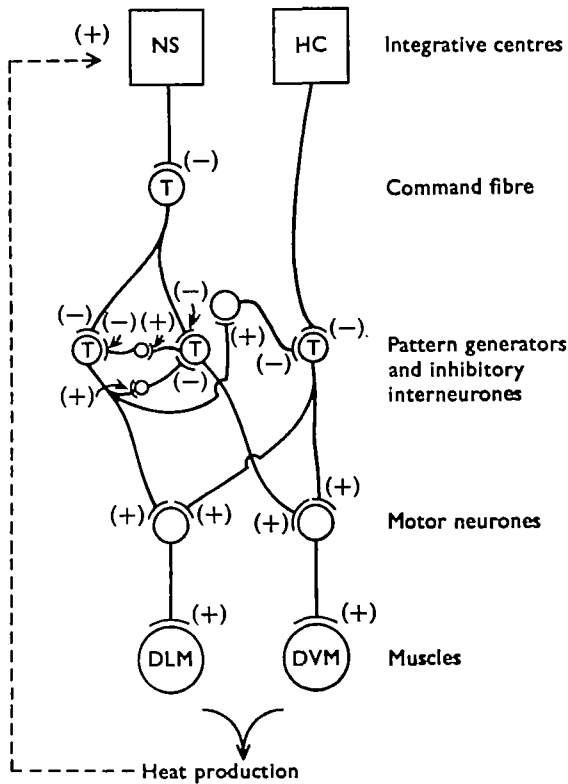
Heating the thoracic ganglia in a quiescent moth stimulates the animal to generate a flight motor pattern in the absence of warm-up activity. The temperature of the ganglia at which this response is generated is unknown, but the temperature of the thermode was 50–55 °C. In a control experiment the thermode was implanted into the dorsolongitudinal muscle, and the temperature of the muscle 1 mm from the tip of the thermode (50 °C) was 33 °C as recorded with a thermocouple. Considering the length of heating of the ganglia (up to 1 min) the temperature of the ganglia must be in the range of 33–40 °C when the flight motor pattern is recorded.

This flight motor pattern stimulated by heating the thoracic ganglia is similar to the shade-seeking response of *H. cecropia* reported by Hanegan & Heath (1970). In shade-seeking the quiescent animal is heated externally at a rate approximating to the warm-up rate. At a mean thoracic temperature of 38.1 °C the animal voluntarily leaves the heated region either by flying or by walking accompanied by wing flapping. In both the shade-seeking and heating of the thoracic ganglia with a thermode it appears that the flight motor pattern is activated at a critical temperature.

In three animals which had initiated a normal warm-up pattern the thoracic ganglia were heated and the motor pattern of flight resulted. These three experiments indicate directly that the transition from warm-up to flight is dependent on the temperature of the thoracic ganglia. The reverse transition from flight to warm-up occurred in one animal as a rebound from thoracic heating (stimulation of the flight pattern). Actual cooling of the ganglia during flight after a normal warm-up period caused the flight motor pattern to stop. Therefore in modelling the responses of this system three basic patterns must be accommodated: (1) normal transition from warm-up to flight; (2) flight with no previous activity through heating; and (3) transition from flight to either no activity or warm-up through cooling of the thoracic ganglia. A model of a simple nervous system which would respond appropriately is shown in Text-fig. 4.

The model is based on pacemaker cells (tonically active) and also on network properties of neurones to generate the oscillatory pattern of flight. Kammer (1968) states that pacemakers are unlikely since phase shifts between elevators and depressors occur in both warm-up and flight. Her recordings indicated that the subalar (direct

flight-control muscle involved in wing supination and depression) shifted phase with respect to the dorsolongitudinal muscle in both warm-up and flight. This phase shift of a direct flight-control muscle with respect to the indirect wing depressors (DLM) could be expected since it would be disadvantageous to have a control muscle tightly coupled to the basic flight-pattern generator.



Text-fig. 4. Model for the generation of both flight and warm-up motor patterns. The details of the model are discussed in the text. HC indicates higher integrative centres; NS is non-specific input to the nervous system (including temperature effects); T indicates a tonically active cell; (-) is inhibition, (+) is excitation.

Kammer's (1968) suggestions of command neurones and temperature-receptors, tonically active neurones which change their rate of firing with temperature, have been incorporated in the model. In the proposed model a command fibre tonically inhibits the flight pattern generator. This command fibre is itself inhibited by increased rate of firing from non-specific input. The input to the command fibre from non-specific stimuli would be a function of temperature, increasing with increasing thoracic temperature. Other non-specific inputs such as noxious stimuli, wind on the head of the animal, stimulation of the tarsal reflex, and others could also inhibit the command fibre and initiate the flight motor pattern.

The decision to initiate activity is made in higher integrative centres (HC in Text-fig. 4). When the thoracic temperature is low this would result in reducing the inhibition on the tonically active warm-up pattern generator. The pattern generator



would drive sub-sets of positively coupled motor neurones which in turn drive the respective antagonistic flight muscles (DLM and DVM) in synchrony at a frequency of 13–18/s depending upon the thoracic temperature. This synchronous contraction of the indirect flight muscles generates heat and causes the thoracic capsule to increase in temperature (3.5 °C/min).

The rise in thoracic temperature would cause an increase in firing of the non-specific stimulus input to the command fibre. This may be a simple reaction having a  $Q_{10}$  of 2. The non-specific input would inhibit the tonic activity (inhibitory) of the command fibre to the flight pattern generator. The pattern generator may be a network of tonically active cells with cross-inhibition between the cells driving the motor neurones for the DLM and DVM units, as postulated by Wilson & Waldron (1968). There would also have to be inhibitory pathways through interneurons to the warm-up pattern generator. Therefore when the thoracic temperature reaches a critical value (through the warm-up mechanism) the flight pattern is automatically turned on and the warm-up pattern is inhibited.

The pacemakers in the model for the generation of the warm-up motor pattern and flight motor pattern are distinct although they need not be separated anatomically as proposed by Moran & Ewer (1966). Both the burst length and the frequency differ in warm-up and flight. In Plate 1*a* the frequency of warm-up is 17/s and the wing-stroke frequency during flight is 4.5/s. In Plate 1*b* the warm-up frequency is 16/s and the wing-stroke frequency during flight is 5.5/s. Therefore the wing-stroke frequency during flight is not a simple submultiple of the warm-up frequency, and it is unlikely that one pacemaker could generate two patterns of different frequencies and different burst lengths.

The experimental results fit this model in that heating the ganglia in a quiescent animal generates a flight motor pattern without going through warm-up. Furthermore, the transition from warm-up to flight can be initiated by heating the ganglia even though the thoracic temperature is low. Cooling the ganglia stops the flight pattern by decreasing the rate of firing of the non-specific input to the command fibre. The tonic activity of the command fibre resumes and inhibits the flight-pattern generator. The alternation of warm-up and flight patterns (Plate 3) may result from movement of the thermode with a resulting change in rate of heating of the ganglia. This assumes that the animal is voluntarily initiating activity (warm-up) at the same time. This would be an improbable event, and indeed is seen in only two animals out of 17 that responded positively to thermode heating.

The rebound from thermode heating in which the moth switched from a flight pattern to a warm-up pattern when the thermode was turned off could be accounted for in a similar manner. That is, the moth initiated a warm-up pattern during the experiment, and this was overridden by the flight pattern stimulated by thermode heating. When the thermode was turned off the flight pattern generator was tonically inhibited by the command fibre and the inhibition through interneurons to the warm-up pattern generator was therefore removed. This would result in a switch in the motor output from the flight pattern to the warm-up pattern.

## SUMMARY

1. The transition from the warm-up motor pattern to the flight motor pattern in the saturniid moth *H. cecropia*, is described.
2. The transition from warm-up to flight was found to be dependent on the temperature of the thoracic ganglia.
3. A model to account for the two different motor output patterns and the transition of the warm-up pattern to the flight pattern is proposed.

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## EXPLANATION OF PLATES

## PLATE 1

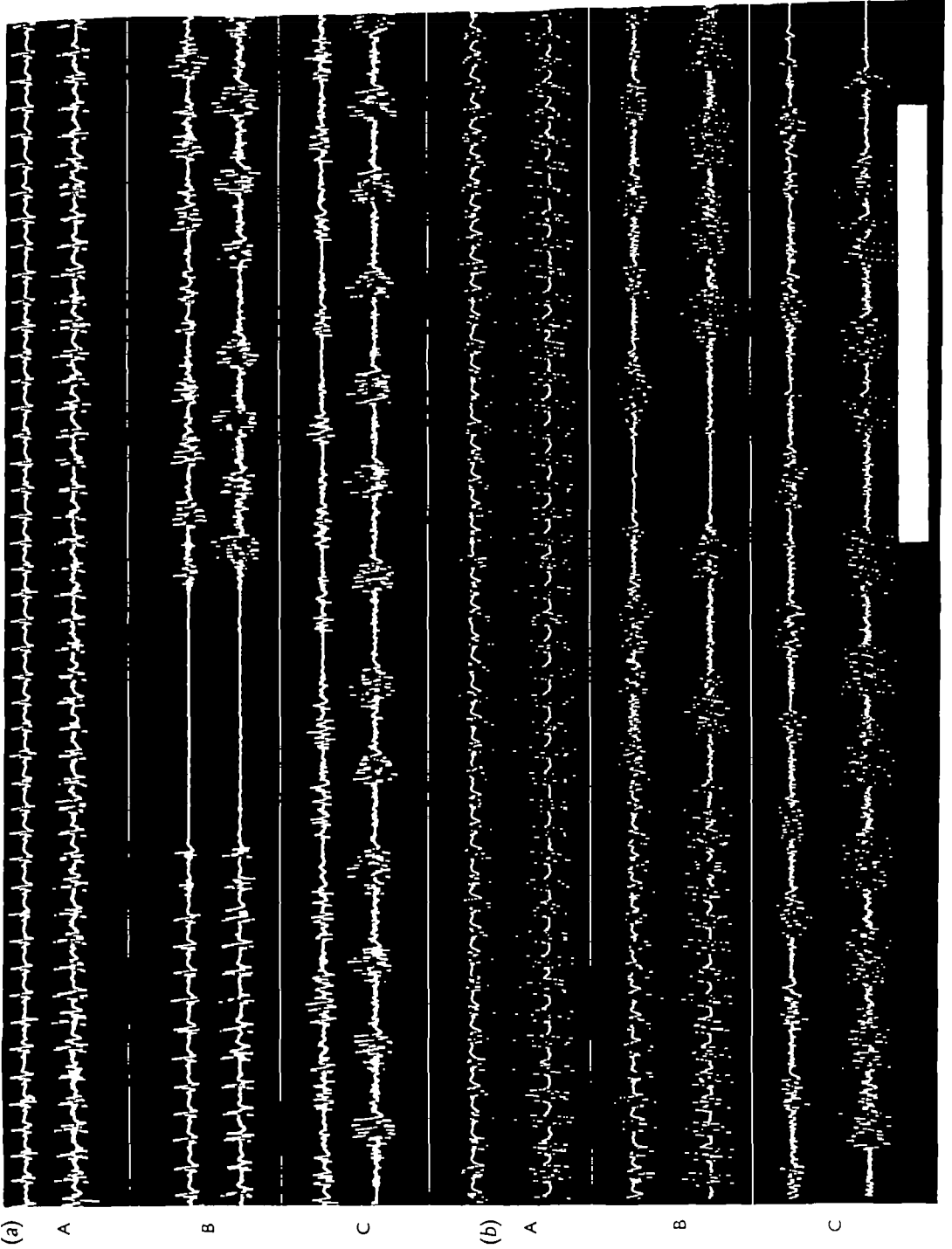
(a) Normal transition from warm-up to flight in which there is a time lag between the two patterns. Note only one muscle action potential per burst during warm-up. (b) Normal transition from warm-up to flight which occurs within one wing-stroke. Note three muscle action potentials per burst during warm-up. The upper trace in both records is the DVM units and the lower trace is the DLM. (The bar represents 1 sec.)

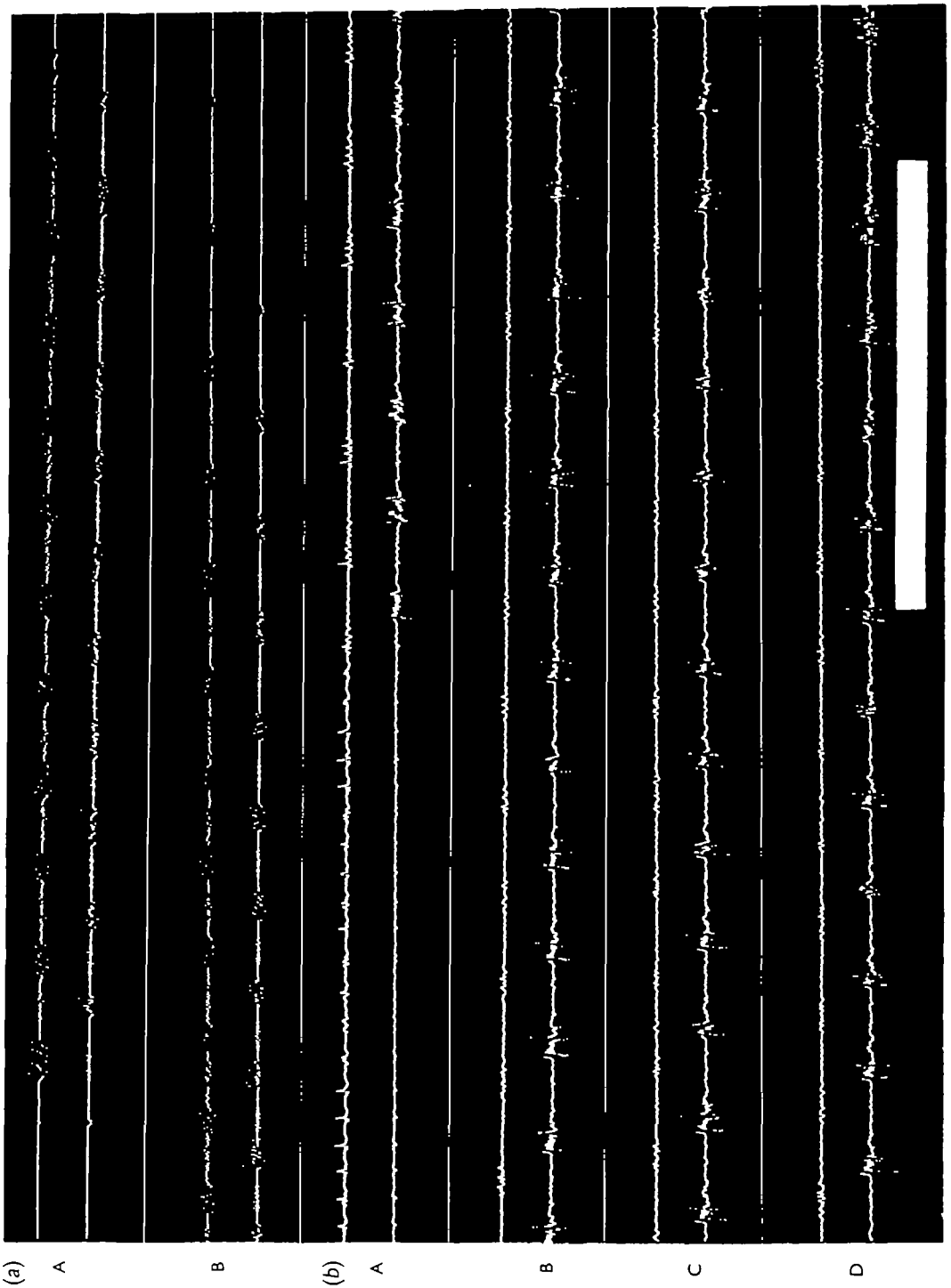
## PLATE 2

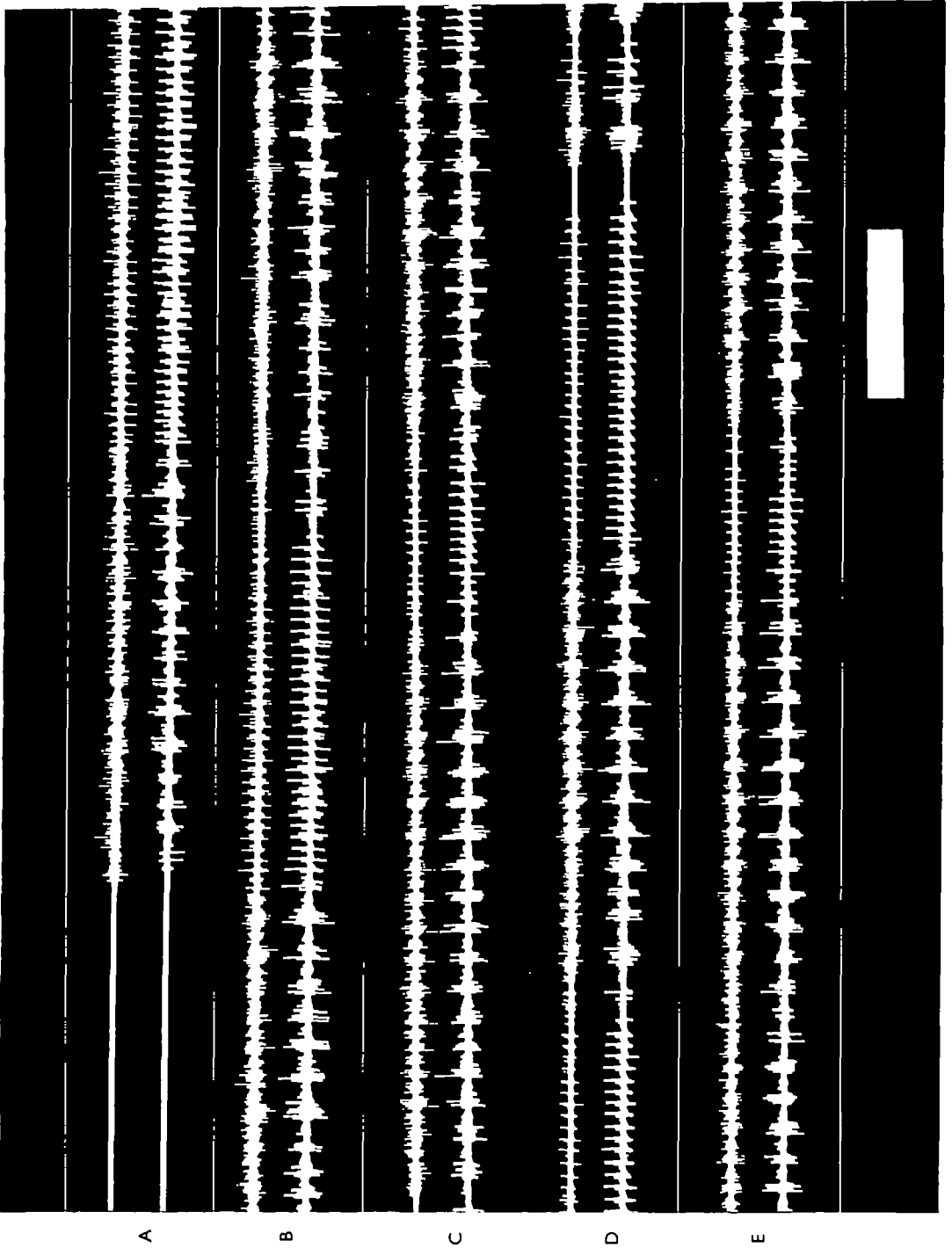
(a) Stimulation of the flight motor pattern by heating the thoracic ganglia in a quiescent animal. (b) Similar record showing generation of the flight motor pattern when the ganglia were heated; however, in this record the flight pattern was preceded by a very short period of warm-up. Upper traces DVM, lower trace DLM. (The bar represents 1 sec.)

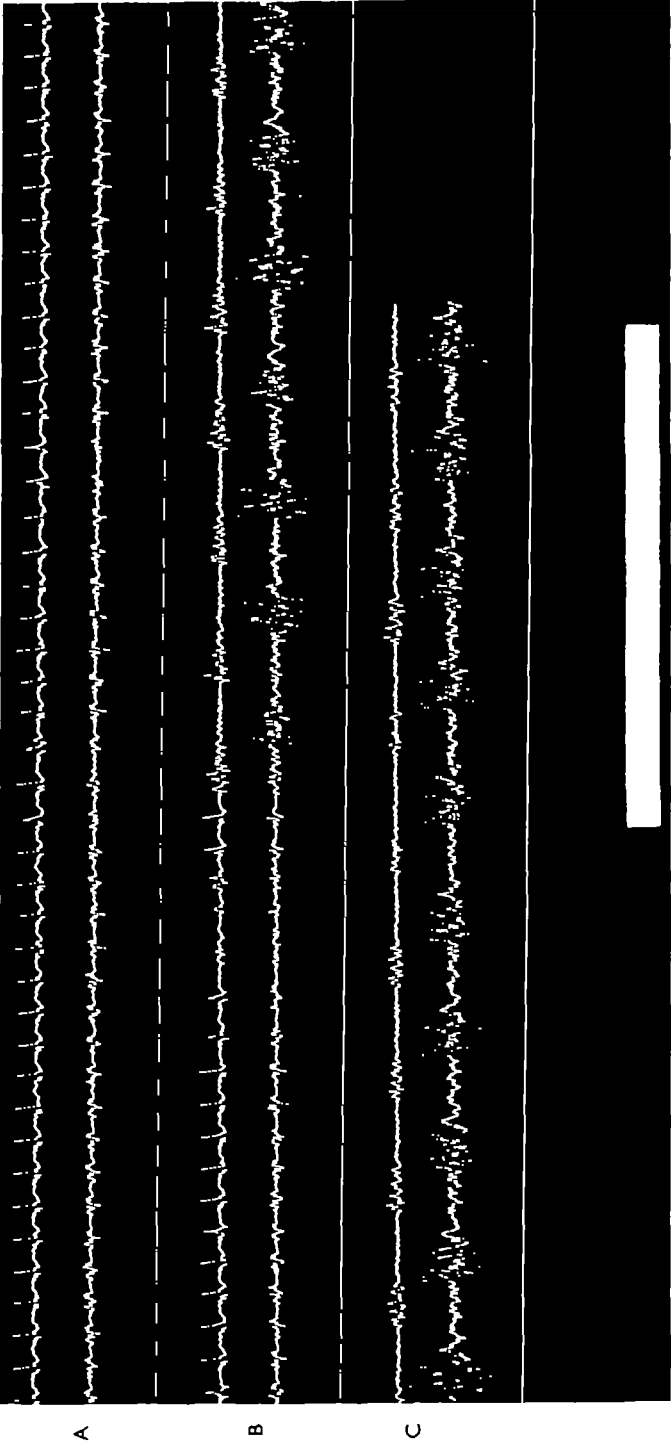
## PLATE 3

Alternating patterns of warm-up and flight during heating of the thoracic ganglia. Upper trace DVM, lower trace DLM. (The bar represents 1 s.)









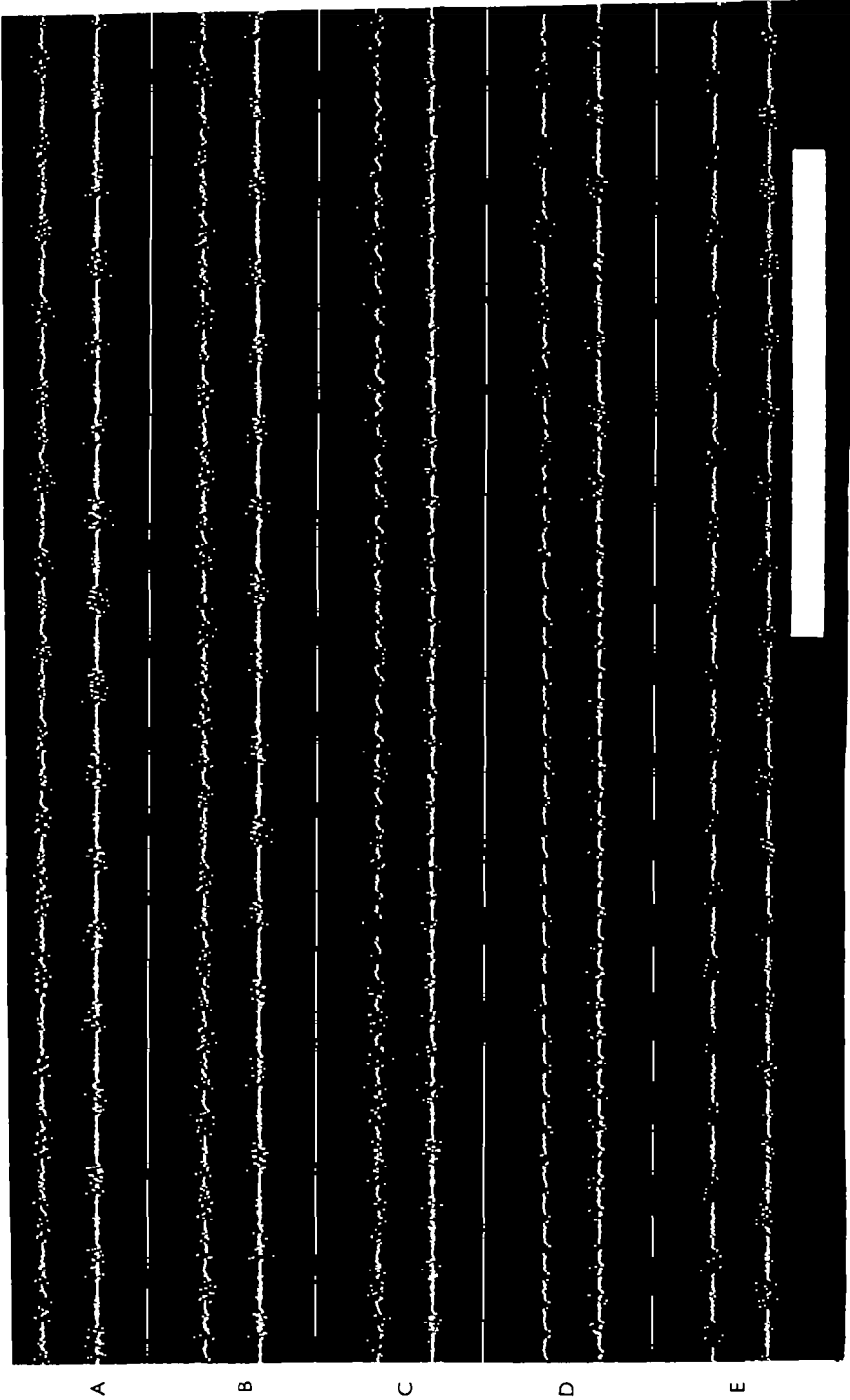


PLATE 4

Heating the thoracic ganglia during warm-up shifted the motor pattern from warm-up to flight (middle record). Upper trace DVM, lower trace DLM. (The bar represents 1 s.)

PLATE 5

Heating the thoracic ganglia produced the flight motor pattern (*A, B*). Thermode turned off in *C* and the motor pattern shifted to warm-up (muscles contract in synchrony). On the right side of record *D* the animal makes the normal transition to the flight pattern. Upper trace DVM, lower trace DLM. (The bar represents 1 s.)