J Comp Physiol B (1991) 161:602–610

Journal of Comparative Physiology B Biochemical, Systemic, and Environmental Physiology

Heartbeat rate modulation mediated by the ventral nerve cord in the honey bee, *Apis mellifera*

Ernest R. Schwab[1]**, Robert A. Chilson[1], and C. Douglas Eddleman[2]*

[1] Department of Biology, La Sierra University, Riverside, CA, 92515 USA, and
[2] Department of Anatomy, Loma Linda University, Loma Linda, CA 92354 USA

Accepted June 14, 1991

Summary. Using impedance conversion as a recording technique, heartbeat patterns were established for intact adult honey bee workers during rest and during nectar feeding and locomotion, activities that both result in accelerating heartbeat. Heartbeat patterns were then described separately for bees in which assumed neural regulation was disrupted (the ventral nerve cord was transected), and for bees in which assumed neurohormonal regulation was disrupted (blood circulation to the abdomen was blocked). Heartbeat patterns from these latter two groups were compared with the baseline data from the former three groups. Results showed that normal modulatory patterns occurring during feeding and locomotion persisted when the neurohormonal pathway was disrupted, but the same modulation was absent when the neural pathway was disrupted. These results offer clear support for the conclusion that neural mechanisms provide a primary form of regulation of heartbeat in the honey bee.

Key words: Insect – Heart regulation – Dorsal vessel – Neural modulation

Introduction

The circulatory system of insects, like that of other arthropods, is classified as an open circulatory system. The blood, or hemolymph, is not contained within a closed system of vessels but circulates freely through the body cavity, or hemocoel, bathing organs and tissues directly. Circulation is maintained and directed by a tubular, contractile dorsal vessel in concert with a variety of accessory pulsatile organs. Although the structure of the dorsal vessel and the extent of its contractility can vary significantly between taxa, the abdominal portion is always contractile and is designated as the heart (Jones 1977).

During the first half of this century, students of insect heart physiology were divided in their opinions regarding the origin of beat generation. The elaborate nervous system of *Periplaneta* (Alexandrowicz 1926) led earlier investigators to believe insect hearts were neurogenic, while more recent interpretations of the same system argue in favor of a myogenic heart (Miller 1968a, b). Attempts to resolve the issue by describing cardiac-associated neural architecture have not been useful, simply because of the neuroanatomical spectrum that has emerged. System structures range from complex types like that of *Periplaneta* to apparently aneural hearts, such as that in *Anopheles* (Jones 1954). Studies using Lepidoptera (Tenney 1953; McCann 1966, 1967; Heinrich 1970; Hanegan 1973) and Diptera (Ballard and Holcomb 1965; Normann 1972; Miller 1979; Cook and Meola 1983), whose cardiac-associated neuroanatomy falls between these two extremes, strongly suggests their hearts to be myogenic. While McCann (1970) believes the distinction between neurogenic and myogenic is artificial, Miller (1985) states that any classification other than myogenic is pedantic. Wigglesworth (1972) attempts to deal with the issue by hypothesizing that in such a diverse taxon as the Insecta, all three schemes (neurogenic, myogenic, and aneural) are possible.

The regulation of contractions is closely associated with the origin of excitation. Hypotheses regarding regulatory mechanisms have been as controversial as those on beat origin, some proponents arguing for a hormonal regulatory mode (Davey 1961, 1962, 1963, 1964; Kater 1968), while others postulate neural (Jones 1977; Miller 1985) and metabolic-ionic regulation (Esch 1982). The most unequivocal evidence in favor of a particular mode was produced by Heinrich (1970), who demonstrated the importance of heart pumping during thermoregulation in sphinx moths. He found that transection of the ventral nerve cord obliterated thermoregulation by disrupting regulation of heartbeat, a clear indication of a neural component to heart regulation.

This study provides convincing evidence for a neural component of heartbeat rate regulation in the honey bee, *Apis mellifera*. This is of particular interest since a cardiac nervous system like that found in Orthoptera is absent in *Apis* (Morison 1928). In fact, neural innervation of *Apis* myocardium is unknown, with the exception of one unsubstantiated report (Rehm 1939) of cardiac ganglion cells on one myocardial segment of a single individual. However, the results of this study suggest that

* *Present address:* Dept. of Nematology, University of California, Riverside, CA 92521 USA

** To whom offprint requests should be sent

a thorough histological analysis of the pericardial sinus should reveal elements innervating the heart.

Materials and methods

Bees were obtained from hives belonging to the Department of Biology, La Sierra University, Riverside, Calif. As a result of the locally mild, Mediterranean climate, active foragers were available during all seasons. To facilitate microscopic viewing of the heart in intact bees, only individuals with light-yellow, semi-transparent abdominal tergites were selected for experiments.

The study was carried out in three steps: (1) to discover factors eliciting an increase in heartbeat rate (such a factor can be used as a switch, allowing activation of heartbeat acceleration in a controlled fashion); (2) to determine whether heartbeat acceleration could be activated following transection of the ventral nerve cord; (3) to determine whether acceleration could be activated following interruption of circulatory input to the abdomen.

Step 1: Factors affecting heartbeat rate. Individual bees were netted at the hive entrance, confined in a stoppered flask, and cooled to a state of narcosis. Narcotization was considered complete when appendicular movement became barely discernible. Individuals removed from the cold flask at that point were generally able to walk haltingly and protrude their sting after 1–2 min. Bees prepared in this fashion rapidly revived and soon resumed normal behavior.

Narcotized bees were fixed to a holder (Fig. 1) using a small drop of resin/beeswax mixture. The holder was attached at the midline of the mesothoracic tergum and immediately lateral to the dorsal midline at the fifth abdominal tergite. The mounted bee was given a small (2 cm diameter) styrofoam ball which was grasped and held. Individuals either remained motionless while holding the ball, or walked and/or ran on it. The ball was not normally dropped except when an animal was either physically disturbed or engaged in wing buzzing (stationary flight).

Following attachment, a small puncture was made through the sclerite of the fifth abdominal segment immediately dorsal to the heart. Two 44-gauge copper recording electrodes, insulated except at the tip, were gently pushed through the puncture and positioned either immediately above or lateral to the heart. The electrodes were connected to a UFI (Morro Bay, CA) Model 2991 Impedance Converter and used to record heartbeats. Signals at the AC coupled output were recorded on a Vetter (Rebersburg, PA) Model G 16-channel FM Instrumentation Recorder. Both filtered (Krohn-Hite Model 3750 Active Electrical Filter, Avon, MA) and unfiltered versions of output signals were simultaneously recorded on separate channels. The additional filtering of the AC-coupled output was occasionally needed to attenuate slower unwanted signals arising from intersegmental and/or alary muscle contraction. Signals were simultaneously monitored using a Tektronix (Beaverton, OR) Model 5111 Storage Oscilloscope for real-time feedback. Slower heartbeat rates were confirmed visually using a Bausch and Lomb (Leica, Buffalo, NY) stereo dissecting microscope.

Two stimuli were tested as potential modulators of heartbeat rate: (1) Feeding; the bee was presented a small droplet of honey on the tip of a dissecting probe. Data were collected only from individuals feeding for 30 s or longer. Such individuals were allowed to eat freely. (2) Locomotory activity: rapid walking, running, or wing buzzing. Activity was induced using tarsal reflex and/or tactile stimulation to the abdomen. Once aroused, bees generally remained active for several minutes. In each testing category, an individual bee was used only once.

Step 2: Effect of ventral nerve cord transection on heart activity. Bees were narcotized as in Step 1, then securely fastened ventral-side-up in a small table-top vise. A minuten pin secured in a Pin Vise (BioQuip, Gardena, CA, 4845) was used to puncture the sternal membranous conjunctiva between the propodeum and the petiolar segment. Both connectives of the ventral nerve cord were simultaneously gently lifted slightly up out of the ventral channel of the

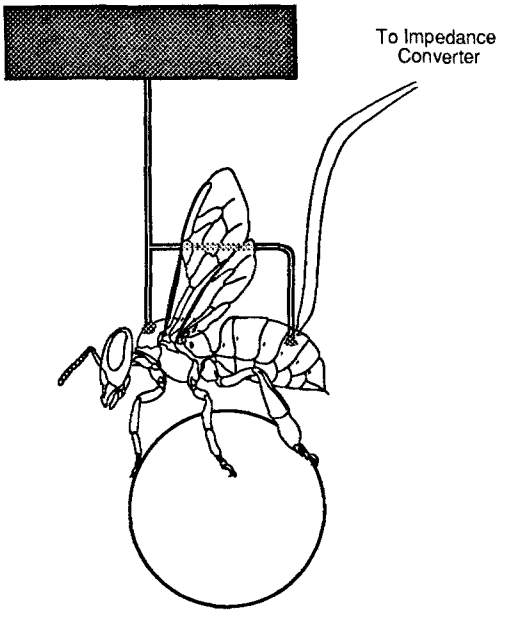

Fig. 1. Diagram depicting a resting, mounted bee with recording electrodes in place. Individual bees were mounted by gluing a probe to the prothoracic and mesothoracic tergites. Abdominal heartbeat was recorded at the dorsal midline of abdominal segment 5

petiole and severed using vannas-type microdissecting scissors. Individuals were then mounted on the recording holder as described under Step 1 and left undisturbed until they became quiet.

The effect of nerve cord transection on the bee's ability to modulate heartbeat rate was tested by either presenting honey or inducing locomotory activity. To rule out procedural effects as causative factors for observed deviations in these experiments, controls were tested under conditions identical to those for the experimental groups, except that ventral nerve cords were only lifted out of the petiolar channel and were not transected.

Step 3: Effect of circulatory block on heart activity. The circulatory block was constructed at the petiole. A tapered glass micropipette was pulled from 50-µl glass capillary tubing using an Industrial Science Associates (Ridgewood, NY) Model M1 Micro-Pipette Puller. The tapered micropipette was clamped in a Jena (Jena-Seiler, St. Louis, Mo) Micromanipulator and attached via a short length of Tygon tubing (1.8 mm OD, 1.0 mm ID) to a tuberculin syringe fitted with a 20-gauge needle.

A narcotized bee was secured ventral-side-up in a vise as described under Step Two. In rapid sequence, the syringe was filled with dental impression material (COE Absolute, Type 1, low viscosity, vinyl polysiloxane, Coe Laboratories, Inc., Chicago), the micropipette was maneuvered into position for ventral injection of impression material into the petiole and advanced through the ventral intersegmental membrane of abdominal segments two and three. Injection was promptly initiated and terminated when the yellow polymer was observed to fill the entire petiole. Complete polymerization required between 90 and 140 s. Following polymerization, a fine strand of nylon was tied around the petiole to seal the membranous conjunctiva around the plug.

Following completion of the above procedures, bees were immediately released from the vise and offered honey. Only those that ate and subsequently flew were retained for experiments. Those bees were later narcotized and attached to the recording holder and instrumentation as described under Step 1. Since locomotory activity produced the most dramatic changes in heartbeat rates, it was used exclusively to test heartbeat rate modulation in this series of experiments.

To test soundness of the seal, 2 µl of hemolymph analog bearing a radioactive marker ($9.62 \cdot 10^{-10}$ g; $1.0 \cdot 10^{-3}$ µCi ^{14}C-labeled

inulin) were injected thoracically into plugged bees. Injection was made through the left sternopleural membrane where it interfaces with the left prothoracic coxa, the furcasternum, and the left anterior edge of the pronotum. The physical disturbance accompanying injection stimulated the necessary motor activity. Heartbeat was recorded for periods varying among individuals; from 3 min (the approximate latency from onset of motor activity to appearance of increased heartbeat rate) to a maximum of 20 min. At the end of this period, the abdomen was severed at the petiole.

The separated abdomen and thorax (with head attached) were individually placed in separate Tenbroeck (Wheaton) tissue grinders containing 1 ml hemolymph analog and ground until sclerotized parts were thoroughly fragmented. A further 2 ml analog was then added to each homogenate and mixed. From each resulting mixture a 2-ml sample was drawn and placed in a scintillation vial with 10 ml scintillation cocktail (Scintanalyzer Scinti Verse II*, Fisher Sci. Co.). Disintegrations were counted in each sample using a Packard (Downers Grove, IL) TriCarb Model 2002 Liquid Scintillation Spectrometer. Samples showing only background counts in the abdomen were known to have had a complete abdominal seal at the petiole.

The hemolymph analog used in this set of experiments was prepared using electrolytes, sugars, and amino acids known (Florkin and Jeuniaux 1963; Brandt and Huber 1979; Sidie 1977) to be present in the hemolymph of *Apis mellifera*. Final osmolality of the analog was adjusted with glycine to 493 mosmol (mean value of hemolymph samples from our colonies) as determined using a Precision Systems (Sudbury, MA) Micro Osmette Osmometer.

Hardcopy of heartbeat data was generated on a Narco (Houston, TX) Physiograph CPM chart recorder. Heartbeat rates were determined by counting the number of beats in 10-s intervals and determining a mean heartbeat rate. Prestimulus and poststimulus mean rates were compared, and differences in sample means tested for significance using a *t*-test for paired comparisons.

Results

The initial objective of Step 1 was to characterize heartbeat in a resting bee. A resting bee was defined behaviorally as one quietly holding a styrofoam ball, virtually unmoving. When external movement was visible, it included only short segments of low-frequency, low-amplitude leg vibration, gentle antennae waving, or slow preening. It did not include walking (even slow walking) or wing buzzing.

Heartbeat in a resting bee was nearly always erratic, consisting of bursts of beating separated by varying periods (interburst periods) of quiescence or lower amplitude, lower frequency beating (Fig. 2). Rates within a burst varied among bees, but were typically around 5 Hz. Interburst beating, when it occurred, exhibited frequencies usually falling between 1 and 3 Hz.

Bursts of beating often began abruptly with high amplitude, high frequency contractions. At other times, the onset of a burst was gradual, beginning with lower frequency and amplitude, followed by an increase in both parameters. Termination of a burst was as likely to be gradual as abrupt. Contraction amplitude and frequency could change rapidly, often between consecutive beats.

Although bursts were immediately preceded by either quiescence or low frequency, low amplitude beating, their onset did not correlate with any externally observable event. Duration of neither bursts nor interburst periods appeared to be predictable.

Heartbeat was clearly visible and individual contractions were easy to detect through the microscope. During periods of quiescence, the heart in abdominal segments 3 and 4 was often observed "whipping" from side to side. This whipping behavior also occurred during beating with amplitudes often obscuring the heartbeat. Those contractions were electronically filtered from the raw data and are not evident in the accompanying records.

In a small number of resting individuals, frequency and amplitude of beating was steady and regular (data

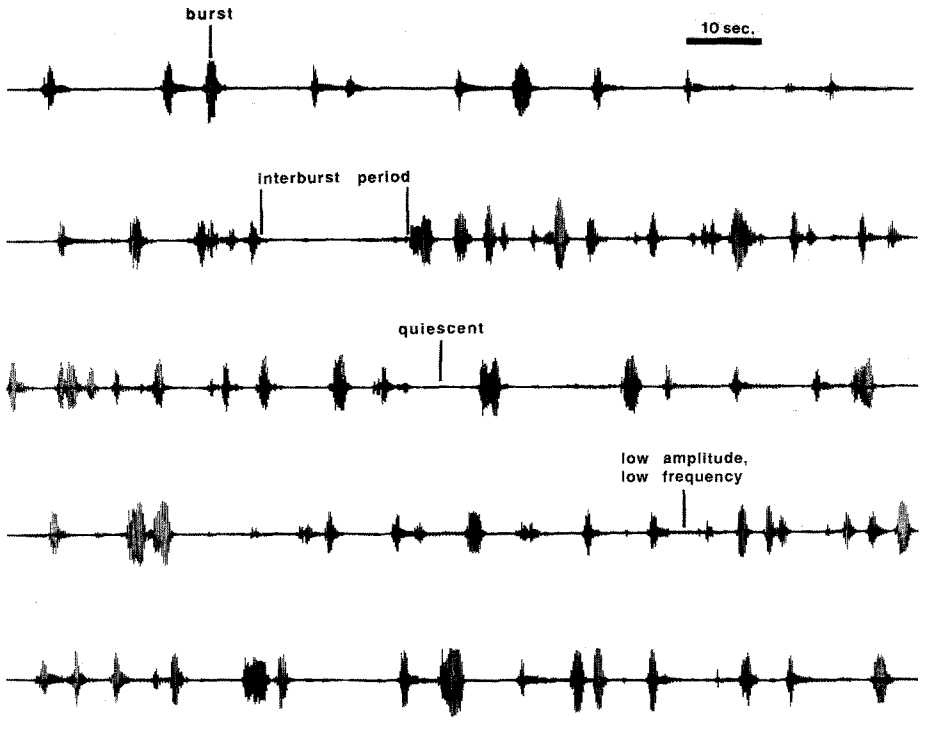

Fig. 2. Impedance conversion recording of heartbeat in a resting honey bee. Pattern of heartbeat consists of short bursts of beating, punctuated by longer periods of quiescence, or in some instances, lower amplitude, lower frequency contractions. Intra-burst heartbeat rate varies from 3 to 6 Hz. Lower amplitude interburst heartbeat rate (when present) varies from 1 to 3 Hz. This record proceeds left to right, top to bottom

not shown). Typical heartbeat rates in these bees were between 1.5 and 3.0 Hz. In the vast majority of cases, however, steady, regular beating occurred only during periods of feeding or locomotory activity.

Heartbeat in resting bees was characterized to provide an index for discriminating modulatory changes in active bees. Patterns recorded during rest were compared with patterns occurring in bees that were feeding and otherwise active, including slow or fast walking, running, bursts of wing buzzing, or continuous wing buzzing, referred to as "flying".

Figure 3 shows the heartbeat of a fully intact bee initially resting, and the transition that occurred as it began eating. Initial beating was the bursting type typical of resting bees, with a mean pre-feeding rate of 3.2 Hz. As feeding began, lower amplitude contractions were replaced by increasingly higher amplitudes, and frequency increased to a mean of 4.2 Hz. All bees that were fed honey exhibited a significant increase in heartbeat rate during feeding (Table 1). Both frequency and amplitude became noticeably regular during feeding. Pattern of beating returned to a resting pattern following cessation of feeding.

Although heartbeat rates increased significantly during feeding, they did not achieve the higher values associated with locomotor activity. Figure 4 illustrates a characteristic record beginning with a resting bee and culminating with locomotor activity. In this example, resting heartbeat immediately preceding activity was slow (2–3 Hz) and regular rather than erratic. However, approximately 30 min earlier this individual had exhibited erratic heartbeat, but a gradual change distin-

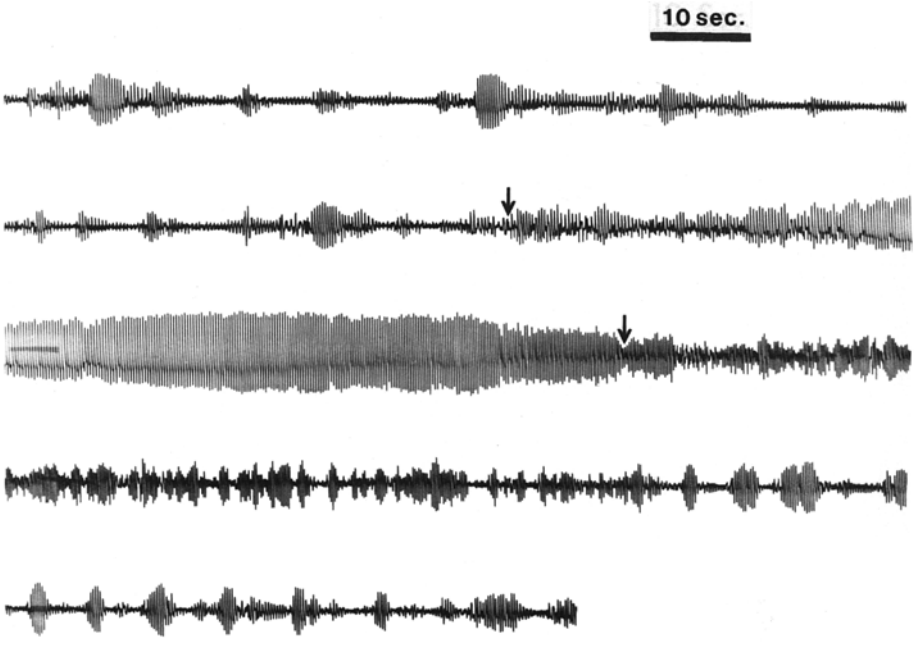

Fig. 3. Impedance conversion recording of heartbeat in a feeding honey bee. Initial portion of record depicts period during which bee is inactive (resting). Initiation of feeding is shown at *arrow on line 2*, and continues through *arrow on line 3*. Following cessation of feeding, heartbeat pattern resumes resting characteristics

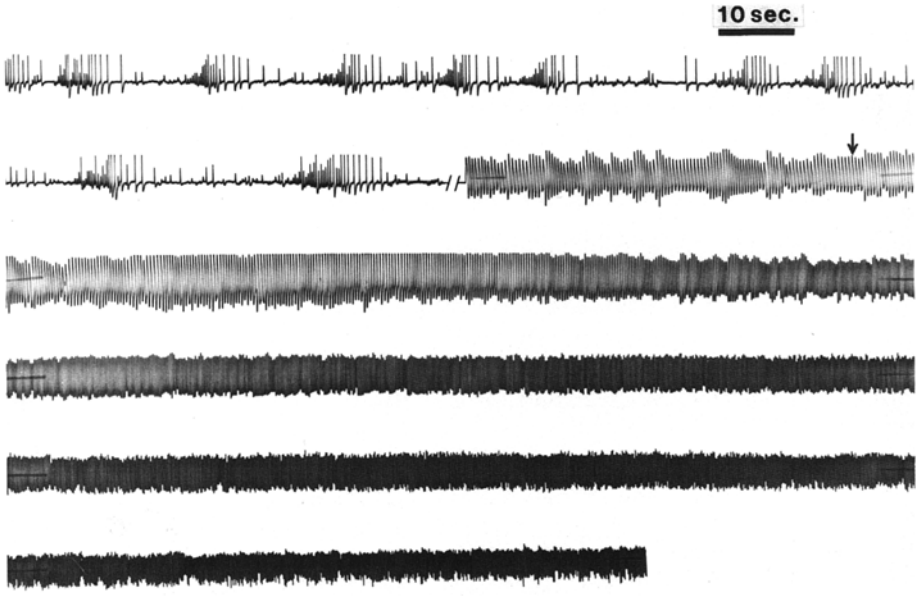

Fig. 4. Impedance conversion recording of heartbeat during locomotor activity by a honey bee. Locomotor activity was initiated at *arrow on line 2* and continued for duration of recording. Notice that frequency more than doubles as a result of the high level of motor activity

guished by increasingly lengthy bursts of beating and fewer and shorter interburst periods eventually led to the continuous resting pattern shown.

Locomotor activity began with a slow walk, increasing to a rapid walk and ultimately to an extended period of running. Heartbeat rate simultaneously increased to 7.5 Hz. Although locomotor activity ended abruptly, heartbeat rate slowed gradually. In all bees tested, heartbeat rate during locomotor activity was significantly higher than pre-locomotor rates (Table 2).

The objective of this study was to determine the type of regulation controlling heart function in the honey bee. Two general hypotheses for regulatory pathways emerge from the literature. A neural pathway is proposed by earlier investigators hypothesizing a neurogenic heart (Krijgsman 1952; Prosser and Brown 1961; Senff 1966) and by more recent advocates of an innervated myogenic heart (McCann 1970). The second general pathway is hemal (hemalcoelomic), used to transport brain hormones and neurosecretions particularly if neurosecretory cells are not located near the abdominal heart. Hemal pathways would also serve in the case of ionic regulation as suggested by Esch (1982). The second and third steps of this study were intended to disrupt these two pathways and determine whether heartbeat rate was still modulated.

Neural pathways to the abdominal heart were disrupted by severing the ventral nerve cord at the petiole. Feeding did not produce changes from prefeeding heartbeat characteristics in bees with a transected nerve cord (Fig. 5). Resting heartbeat in those bees was nearly always as shown in this example, i.e., continuous and regular with no predictable increase in heartbeat rate

Table 1. Effect of feeding on heartbeat rate (Hz) of the honey bee

Bee no.	(R_1)	(R_2)	($R_2 - R_1$)
1	4.2	5.5	1.3
2	3.2	4.2	1.0
3	3.8	6.5	2.7
4	5.2	6.7	1.5
5	3.3	4.9	1.6
6	2.3	3.8	1.5
7	2.8	4.5	1.7

$\bar{D} = 1.61$; $S_D = 0.20$; $t = 8.05$, $v = 6$; $P \ll 0.001$; R_1 = Mean prestimulus heartbeat rate; R_2 = Mean poststimulus heartbeat rate; ($R_2 - R_1$) = difference between mean rates; $\bar{D}$ = mean difference; S_D = standard error of mean difference

Table 2. Effect of locomotor activity on heartbeat rate (Hz) in the honey bee

Bee no.	(R_1)	(R_2)	($R_2 - R_1$)
1	3.2	6.7	3.5
2	4.8	7.8	3.0
3	3.2	6.6	3.4
4	3.3	7.8	4.5
5	3.6	7.6	4.0
6	5.2	6.7	1.5
7	3.7	5.2	1.5
8	3.1	8.1	5.0
9	2.3	6.3	4.0
10	1.7	5.7	4.0
11	4.0	6.6	2.6

$\bar{D} = 3.36$; $S_D = 0.34$; $t = 9.84$, $v = 10$; $P \ll 0.001$; R_1 = Mean prestimulus heartbeat rate; R_2 = Mean poststimulus heartbeat rate; ($R_2 - R_1$) = difference

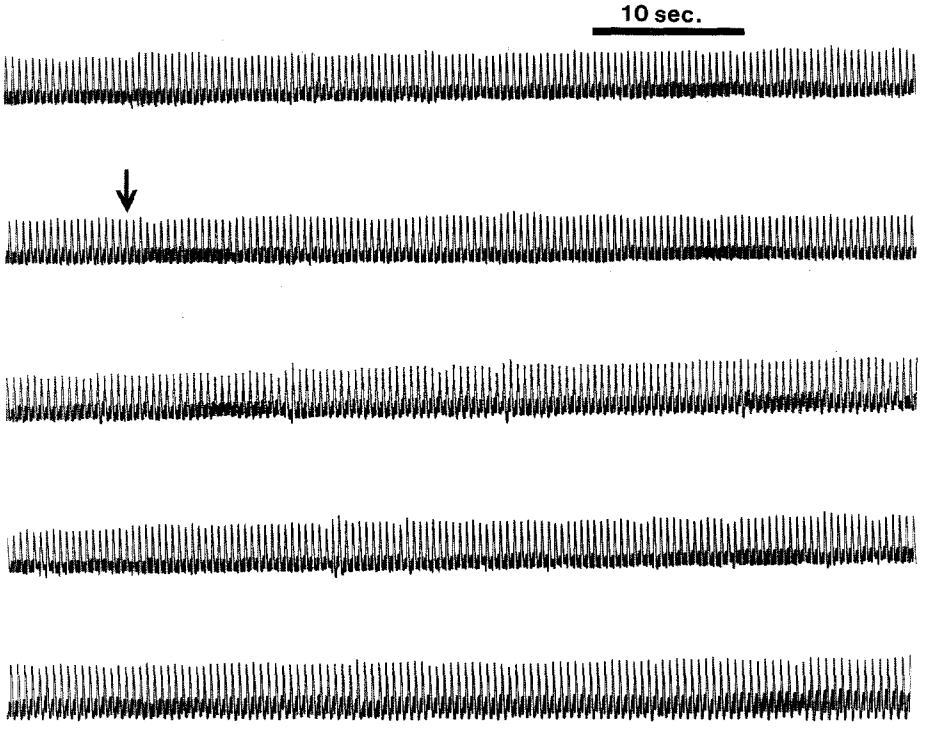

Fig. 5. Impedance conversion recording of heartbeat during feeding by a honey bee with ventral nerve cord transected. Feeding began at *point of arrow on line 2* and continued for duration of the recording. Note absence of any change in frequency or amplitude corresponding with act of feeding

Table 3. Effect of feeding on heartbeat rate (Hz) of the honey bees with transected ventral nerve cord

Bee no.	(R_1)	(R_2)	($R_2 - R_1$)
1	5.6	5.9	0.3
2	3.0	1.6	−1.4
3	1.8	1.7	−0.1
4	2.2	2.2	0.0
5	1.0	1.1	0.1
6	0.7	0.9	0.2
7	1.5	1.5	0.0
8	2.5	2.6	0.1
9	5.8	6.2	0.4

$\bar{D} = -0.04$; $S_D = 0.18$; $t = -0.25$, $v = 8$; $P < 0.9$; R_1 = Mean prestimulus heartbeat rate; R_2 = Mean poststimulus heartbeate rate; ($R_2 - R_1$) = difference

Table 4. Effect of feeding on heartbeat rate (Hz) of the honey bees with sham ventral nerve cord transection

Bee no.	(R_1)	(R_2)	($R_2 - R_1$)
1	4.1	5.9	1.8
2	1.5	3.4	1.9
3	3.6	3.9	0.3
4	3.9	4.5	0.6
5	3.0	5.3	2.3
6	3.9	5.4	1.5
7	4.0	6.1	2.1
8	2.7	5.4	2.7
9	4.9	5.5	0.6

$\bar{D} = 1.53$; $S_D = 0.28$; $t = 5.43$, $v = 8$; $P \ll 0.001$; R_1 = Mean prestimulus heartbeat rate; R_2 = Mean poststimulus heartbeat rate; ($R_2 - R_1$) = difference

(Table 3). The mean rate in this example was 2.2 Hz. Table 4 shows that controls for a procedural effect responded in the same way as intact bees, rather than bees with a severed nerve cord.

Locomotor activity also failed to increase heartbeat rate in bees with transected ventral cords (Fig. 6). In this example, mean pre-locomotor resting rate was 3 Hz, declining over time to 2.4 Hz. This was a general trend in all bees tested (Table 5), with noticeable decreases occurring after a period of hours. It is thus important to note that the probability level given in Table 5 results from a gradual negative change in heartbeat rate and does not denote a statistically significant increase in rate. The outcome of sham surgery indicated that experimental results were not due to procedural effects (Table 6). Clearly, transection of the ventral nerve cord disrupted the modulatory pattern normally associated with feeding and locomotory activity.

The hemal pathway was disrupted by blocking hemolymph flow through the petiole. With hemolymph flow to the abdomen blocked, locomotor activity still produced a significant increase in heartbeat rate (Table 7). Because heartbeat response was normal, it was necessary to insure that the circulatory block was functional. The effectiveness of the block was tested by injecting ^{14}C-labeled inulin thoracically into each bee and allowing it to circulate for a minimum of 3 min (approximate time required for heartbeat rate to rise to the high level associated with locomotor activity). Its presence was then tested for in abdominal homogenates. Scintillation counts revealed that a complete circulatory block was achieved in four of the seven bees tested (Table 7). A breach occurred in the remaining three; the amount of hemolymph entering the abdomen as a result of the breach was less than 1%, and the time required for this penetration to occur varied between 4.5 and 20 min. In

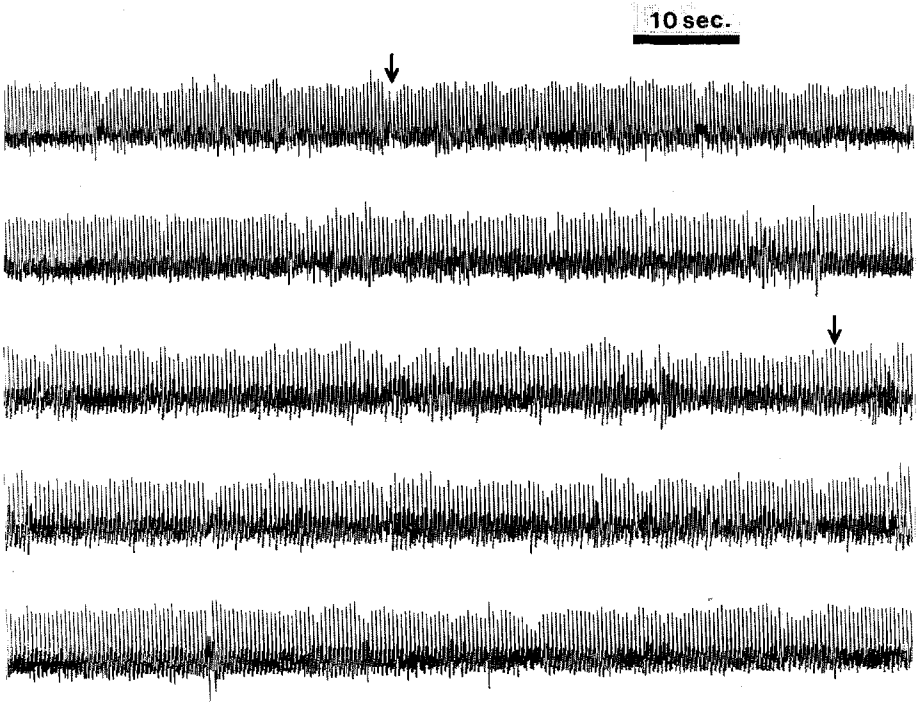

Fig. 6. Impedance conversion recording of heartbeat during locomotor activity by a honey bee with ventral nerve cord transected. Locomotor activity begins at *arrow on line 1* and continues through *arrow on line 3*. Note the conspicuous absence of heartbeat rate increase in response to locomotor activity

Table 5. Effect of locomotor activity on heartbeat rate (Hz) of honeybees with transected ventral nerve cord

Bee no.	(R_1)	(R_2)	(R_2-R_1)
1	6.0	5.6	−0.4
2	1.6	1.5	−0.1
3	3.0	2.4	−0.6
4	1.3	1.4	−0.1
5	2.7	2.3	−0.4
6	1.8	1.8	0.0
7	1.9	2.0	0.1
8	1.7	1.5	−0.2

$\bar{D}=-0.21$; $S_D=-0.08$; $t=-2.55$, $v=7$; $P<0.05$; R_1 = Mean prestimulus heartbeat rate; R_2 = Mean poststimulus heartbeat rate; (R_2-R_1) = difference

Table 6. Effect of locomotor activity on heartbeat rate (Hz) of honeybees with sham-transected ventral nerve cord

Bee no.	(R_1)	(R_2)	(R_2-R_1)
1	4.6	7.1	2.5
2	2.9	6.8	3.9
3	1.8	5.4	3.6
4	3.2	6.4	3.2
5	3.9	5.6	1.7
6	5.4	7.8	2.4
7	4.0	6.0	2.0
8	1.6	7.9	6.3
9	5.5	6.7	1.2
10	2.4	8.2	5.8

$\bar{D}=3.26$; $S_D=0.54$; $t=6.09$, $v=9$; $P<0.001$; R_1 = Mean prestimulus heartbeat rate; R_2 = Mean poststimulus heartbeat rate; (R_2-R_1) = difference

Table 8. Time required for circulation equilibrium of thoracically injected ^{14}C in the honey bee. Mixing ratio is calculated as the proportion of abdominal counts to total body counts. Post-injection time interval is a measure of the time period between injection of radioisotope and excision of abdomen

Bee no.	Post-injection time interval	Thoracic count	Abdominal count	Mixing ratio	Mean Mixing ratio
1	5	26964	10487	0.28	
2	5	33139	8495	0.20	
3	5	32665	4193	0.11	0.18
4	5	31842	5540	0.15	
5	5	32786	5692	0.15	
6	30	21458	18421	0.46	
7	30	25624	12763	0.33	
8	30	14592	16433	0.53	0.45
9	30	17257	17905	0.51	
10	30	129	205	0.61	
11	30	674	204	0.23	
12	120	8434	13871	0.62	
13	120	15181	17339	0.53	
14	120	10428	6375	0.38	0.51
15	120	6898	9197	0.57	
16	120	20899	18210	0.47	
17	240	16948	8807	0.34	
18	240	18487	18457	0.50	
19	240	10363	12010	0.54	0.50
20	240	7755	7161	0.48	
21	240	6571	10657	0.62	
22	480	11221	9900	0.47	
23	480	10876	12161	0.53	
24	480	17341	17819	0.51	0.47
25	480	20890	17517	0.46	
26	480	18383	11986	0.39	

Table 7. Effect of locomotor activity on heartbeat rate of honeybees with circulation blocked by a petiolar plug. Mixing ratio is calculated as the proportion of abdominal counts to total body counts. Post-injection time interval represents the period in seconds between injection of radioisotope and excision of abdomen

Bee no.	1	2	3	4	5	6	7
(R_1)	4.5	2.2	2.5	3.0	3.8	1.8	2.3
(R_2)	5.1	5.1	7.1	5.7	5.7	4.0	5.7
(R_2-R_1)	0.6	2.9	4.6	2.7	1.9	2.2	3.4
Thoracic radiation count	55510.0	73097.0	73301.0	64299.0	68505.0	67196.00	62895.0
Abdominal radiation count	355.0	0.0	0.0	490.0	0.0	0.0	495.0
Mixing ratio	0.007	0.0	0.0	0.008	0.00	0.0	0.008
Post-injection time interval	264.0	624.0	240.00	552.0	372.0	300.0	1176.0

$\bar{D}=2.61$; $S_D=0.47$; $t=5.53$, $v=6$; $P<0.005$; R_1 = prestimulus heartbeat rate; R_2 = poststimulus heartbeat rate; (R_2-R_1) = difference

bees without a circulatory block, circulatory equilibrium (mixing ratio =0.50) was achieved in approximately 0.5 min (Table 8). Measured against this index, the circulatory rates in bees with breached blocks were much too low to have accounted for the normal cardiac response observed if modulation was mediated by a hemolymph-borne substance. Thus, it can be assumed that all seven bees had functional blocks and hemal pathways were not contributing noticeably to heartbeat rate modulation.

Discussion

Despite years of research, an understanding of the mechanism(s) regulating insect heart has yet to emerge. Most authors agree that the heartbeat is probably myogenic, but have yet to agree on whether it is regulated neurally or neurohormonally. With the possible exception of Heinrich's (1970) study of thermoregulation in sphinx moths, no assays producing unequivocal demonstration

of a specific mechanism have been developed (Miller 1979).

The purpose of this study was to determine the heartbeat regulatory mechanism in honey bee. The initial aim was to discover factors that always increased heartbeat rate, thus providing means for activating the heartbeat accelerator. If these factors failed to produce normal acceleration when either the neural or hemal information pathway was blocked, that pathway would stand implicated as a control pathway for heartbeat rate modulation.

Both feeding (eating honey) and locomotor activity consistently produced increases in heartbeat rate, locomotor activity producing the highest rates. If heartbeat rate is correlated with total motor activity, the higher rates accompanying locomotion would be expected, since both more and larger muscles are contracting during locomotor activity than during feeding. Although the data in Tables 1 and 2 suggest that heartbeat rate might be related to total motor activity, this was not pursued, since the immediate objective was to discover factors producing heartbeat acceleration.

The hypothetical neural control pathway, was first disrupted by transecting the ventral nerve cord at the petiole, where it is easily accessible with a minimum amount of damage to the bee. Feeding and locomotor activity did not produce heartbeat acceleration in bees with transected nerve cords. Controls which underwent the entire procedure except for transection itself exhibited the usual increases in heartbeat rate during feeding and locomotion, indicating that lack of heartbeat acceleration in experimental animals resulted from transection of the nerve cord rather than from surgical trauma.

From these results one could propose that the ventral nerve cord serves as an efferent control pathway to the heart. However, the same results could also be expected if efferent control occurred hemally, assuming that abdominal sensory input to a hypothetical neurohormonal regulatory center utilized the ventral nerve cord. In this model, sensory information from the abdomen would be conducted via ventral nerve cord neurons to a cephalad neurohormonal regulatory center for the heart, stimulating secretion of cardioacceleratory neurohormones which would be carried by the hemolymph, posteriorly, to the heart. Transection of the ventral nerve cord would thus block sensory input stimulating release of neurohormones rather than efferent neural control signals. To determine whether transection of the ventral nerve cord had actually disrupted efferent motor pathways to the heart, or simply afferent sensory pathways that turned on cardioacceleratory neurosecretion, the neurohormonal (hemal) pathway to the abdomen was blocked.

Disruption of this hemal pathway was dependent on disruption of the circulation. When using procedures to completely block circulation to the abdomen, the honey bee (or other Hymenoptera) is a most suitable experimental animal. The petiolar constriction at the intersegmental joint between the propodeum and abdominal segment 2 is a conduit through which all circulating hemolymph must flow; by plugging the petiole, a complete circulatory block to the abdomen can be achieved without disrupting neural information flow.

Locomotor activity was promoted in the plugged bees. Although the plug prevented circulation to the abdomen, the ventral nerve cord was intact and all bees exhibited heartbeat rate increases. Since the neurohormonal (hemoceolomic) pathway was blocked, modulation of heart contractions must have been under neural control.

Disruption of the hemoceolomic pathway by blocking hemolymph flow, rather than by attempting to selectively block neurohormone movement, carried the inherent advantage of blocking any putative hemal-borne acceleratory factor. Esch (1982) suggested that heartbeat rate regulation in honey bees might depend on hemolymph sodium ion concentration, which could vary as a result of water production by flight muscles during flight. By blocking circulation at the petiole, factors based on ionic regulation were discounted together with hypothetical hemal-borne or -conducted control factors originating anterior to the petiole, including endogenously produced heat.

Changes in heartbeat rate of honey bees have been suggested by at least one study to correlate with changes in thoracic hemolymph temperature (Esch 1982). In that study, heartbeat rate increased simultaneously with an increase in thoracic temperature during stationary flight. However, in a more extensive study in which both abdominal and thoracic temperatures were monitored, Heinrich (1979, 1980a, b) found that increases in endogenously generated thoracic temperature did not generate noticeable increases in abdominal temperature. More important to this study (and to the assertion that temperature might serve as a medium regulating heartbeat) was the discovery that endogenously generated increases in thoracic temperature produced ambiguous responses of the abdominal heart. In most experiments, thoracic heating had little or no effect on abdominal heart activity and, in some instances, heartbeat frequency declined during thoracic heating. Predictable increases in heartbeat frequency occurred only in response to direct exogenous heating of the abdomen. Heinrich (1980b) ultimately concluded that the abdomen was, on the whole, thermally insulated from the thorax, in spite of blood flow that occurs between those tagmata. This innate thermal insulation, coupled with a complete circulatory block at the petiole, should effectively counter suggestions that heartbeat rate in this study was directly modulated by temperature changes at the abdominal myocardium.

Other characteristics of the heartbeat seem to support neural control of frequency modulation. Resting heartbeat is nearly always erratic. Bursts of pulses occur, punctuating periods of quiescence. Cook and Meola (1983), using *Stomoxys*, and Lhotsky et al. (1975), using *Locusta*, made similar observations. The virtually instantaneous onset and cessation of many of these bursts strongly support an argument for neural regulation. Neurohormonal regulation would be expected to produce more gradual changes. In addition, severing the abdomen from an intact bee nearly always resulted in stabilization of heartbeat at a low frequency and am-

plitude, regardless of the preceding beat characteristics. Similar results were obtained by Normann (1972) using *Calliphora* in which this stability was attributed to loss of inhibitory control when the abdomen was separated from the thoracic ganglionic mass. Heinrich (1970, 1971a, b) found thoracic temperature regulation in *Manduca* to be linked to heartbeat frequency and amplitude and blood flow through the dorsal vessel. Transection of the ventral nerve cord (posterior to the second abdominal segment) eliminated those responses, inferring that the abdominal heart responded to overheating of the thorax through neural influence.

Virtually instantaneous changes in frequency and amplitude of contractions were often observed. Not uncommonly, halving or doubling of contraction rate occurred between consecutive beats. Amplitude changes, sometimes of an order of magnitude, also occurred between consecutive beats. Lhotsky et al. (1975) observed changes in frequency and amplitude between consecutive beats in grasshopper heart, attributing this to control exerted by an external timing mechanism and mediated by means of the nervous system. Changes occurring rapidly enough to be manifest between consecutive beats (at rates of 4–5 Hz) would appear to require neural control.

Although data from this study do not entirely rule out participation of neurohormones in cardioregulation in honey bees, they do reveal a predominant role for the central nervous system in that process.

References

Alexandrowicz JS (1926) The innervation of the heart of the cockroach (*Periplaneta orientalis*). J Comp Neurol 41:291–309

Ballard RC, Holcomb B (1965) An investigation of the house fly heartbeat through transection experiments. Ann Soc Am 58:608–611

Brandt NR, Huber RE (1979) Carbohydrate utilization in the thoraces of honey bees (*Apis mellifera*) during early times of flight. J Insect Physiol 25:483–486

Cook BJ, Meola S (1983) Heart structure and beat in the stable fly, *Stomoxys calcitrans*. Physiol 8:139–149

Davey KG (1961) The mode of action of the heart accelerating factor from the corpus cardiacum of insects. Gen Comp Endocrinol 1:24–29

Davey KG (1962) The nervous pathway involved in the release by feeding of a pharmacologically-active factor from the corpus cardiacum of *Periplaneta*. J Insect Physiol 8:579–583

Davey KG (1963) The release by enforced activity of the cardiac accelerator from the corpus cardiacum of *Periplaneta americana*. J Insect Physiol 9:375–381

Davey KG (1964) The control of visceral muscles in insects. Adv Insect Physiol 2:219–245

Esch H(1982) Neuronal (?) control of heart rate in honey bees. In: Breed M, Michener C, Evans H (eds) The biology of social insects. Westview Press, Boulder, Colo, pp 347–351

Florkin M, Jeuniaux C (1963) Cationic pattern of hemolymph in adult Hymenoptera. (Life science, no. 12) Pergamon Press, Oxford New York, pp 982–985

Hanegan JL (1973) Control of heart rate in *Cecropia* moths: response to thermal stimulation. J Exp Biol 59:67–76

Heinrich B (1970) Nervous control of the heart during thoracic temperature regulation in a sphinx moth. Science 168:606–607

Heinrich B (1971a) Temperature regulation of the sphinx moth, *Manduca sexta:* II. Regulation of heat loss by control of blood circulation. J Exp Biol 54:153–166

Heinrich B (1971b) An analysis of pre-flight warm-up in the sphinx moth, *Manduca sexta*. J Exp Biol 55:223–239

Heinrich B (1979) Keeping a cool head: honeybee thermoregulation. Science 205:1269–1271

Heinrich B (1980a) Mechanisms of body-temperature regulation in honeybees, *Apis mellifera:* I. Regulation of head temperature. J Exp Biol 85:61–72

Heinrich B (1980b) Mechanisms of body-temperature regulation in honeybees, *Apis mellifera:* II. Regulation of thoracic temperature at high air temperatures. J Exp Biol 85:73–87

Jones JC (1954) The heart and associated tissues of *Anopheles quadrimaculatus* Say (Diptera: Culicidae). J Morphol 94:71–123

Jones JC (1974) Factors affecting heart rates in insects. In: Rockstein M (ed) The physiology of Insecta, 2nd ed. Academic Press, New York, pp 119–167

Jones JC (1977) The circulatory system of insects. Charles C. Thomas, Springfield

Kater SB (1968) Cardioaccelerator release in *Periplaneta americana* (L.). Science 160:765–767

Krijgsman BJ (1952) Contractile and pacemaker mechanisms of the heart of arthropods. Biol Rev 27:320–346

Lhotsky S, Phaneuf C, Langlois J, Gagné S, Poussart D (1975) Optocardiography: in vivo measurements of the insect cardiac activity with a new optical method. J Insect Physiol 21:237–248

McCann FV (1966) The effect of intracellular current pulses on membrane potentials in the moth heart. Comp Biochem Physiol 17:599–608

McCann FV (1967) The effect of metabolic inhibitors on the moth heart. Comp Biochem Physiol 20:399–409

McCann FV (1970) Physiology of insect hearts. Annu Rev 15:173–200

Michener CD (1974) The social behavior of the bees. Belknap Press, Cambridge

Miller T (1968a) Role of cardiac neurons in the cockroach heartbeat. J Insect Physiol 14:1265–1275

Miller T (1968b) Response of cockroach cardiac neurons to cholinergic compounds. J Insect Physiol 14:1713–1717

Miller T (1979) Nervous versus neurohormonal control of insect heartbeat. Am Zool 19:77–86

Miller T (1985) Structure and physiology of the circulatory system. In: Kerkut G, Gilbert T (eds) Comprehensive insect physiology, biochemistry, and pharmacology, vol. 3 Pergamon Press, New York, pp 289–354

Morison GD (1928) The muscles of the adult honey-bee (*Apis mellifera* L.) Q J Microsc Sci 71:563–651

Normann TC (1972) Heart activity and its control in the adult blowfly, *Calliphora erythrocephala*. J Insect Physiol 18:1793–1810

Prosser CL, Brown FA (1961) Comparative animal physiology. Little, Brown, New York

Rehm E (1939) Die Innervation der inneren Organe von *Apis mellifica*. Zugleich ein Beitrag zur Frage des sog. sympathischen Nervensystems der Insekten. Z Morphol Ökol Tiere 36:89–122

Senff RE (1966) The electrophysiology of the adult heart of *Periplaneta americana:* evidence for a neurogenic heart. PhD thesis, Purdue University, Lafayette, Ind, pp 70

Sidie J (1977) Chemical changes in the hemolymph of foraging honeybees as a result of flight. Am Zool 17:918

Tenney SM (1953) Observations on the physiology of the Lepidopteran heart with special reference to reversal of the beat. Physiol Comp Oecol 3:286–306

Wigglesworth VB (1972) The principles of insect physiology, 7th ed. Chapman and Hall, London