MODULATION OF THE VENTILATORY RHYTHM OF THE HELLGRAMMITE *CORYDALUS CORNUTUS* BY MECHANOSENSORY INPUT

by

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Introduction

The conclusion that cyclic, repetitive movements do not require peripheral feedback for their generation is now supported by a large mass of evidence and is one of the few broad generalities so far discovered by neuroscientists (Kennedy and Davis 1977; Kristan et al. 1977; Stein 1978; Delcomyn 1980). However, sensory feedback can modify centrally generated rhythms, and elucidation of the means by which rhythmic movements are controlled is an important part of the ultimate goal of understanding behavior.

If peripheral input exerts its effect during the same cycle in which it is produced, the effect is termed phasic; longer lasting effects are termed tonic. With some exceptions (Wendler 1974; Wong and Pearson 1976; Duysens 1977; Beltz and Gelperin 1980), recent work has revealed primarily tonic effects of sensory input on rhythmic behaviors (Seigler 1977; Wyman 1977; Gelperin et al. 1978). When phasic effects were seen, they usually were found to aid in adjusting timing or contractile strength in response to environmental variables (Pearson and Duysens 1976; Zarnack and Mohl 1977). Such adjustments often affect all appendages associated with the behavior rather than only the stimulated appendage. This report focuses on the role of mechanosensory input in the ventilatory rhythm of the dobsonfly larva, Corydalus cornutus (L.).

Previous work (Kinnamon 1981; Kinnamon and Kammer, in preparation) showed that ventilation of the dobsonfly larva involves movement of a pair of tracheal gills attached to each of the first seven abdominal segments. These gills retract or "beat" in a metachronal wave with an unusual sequence: the gills on segment 3 beat first, followed by those on segments 4, 5, 6, 7, 2, and 1. This sequence is repeated

rhythmically 25 to 120 times each minute, although in any one animal the frequency remains fairly constant over short periods of time. Ganglion 3 is almost certainly the most important one in determining the frequency of the final motor output. Evidence for the dominance of this oscillator is that segment 3 normally leads the rhythm, and cycle-to-cycle variations in the rhythm occur first in its output. Furthermore, the burst frequency of ganglion 3 in isolation is nearer to the frequency of an intact animal than the burst frequency of isolated ganglion 2 (the only other ganglion which will cycle in isolation).

The ventilatory rhythm in Corydalus is simple in that each gill retraction is caused by a burst of impulses in a single motor neuron innervating the gill retractor muscle (Fig. 1; Kinnamon and Kammer, in preparation). The motor neuron is contained in a branch (nerve V1) of the ventral nerve, which displays little other activity during normal ventilation. It thus provides a convenient site for recording motor output (Fig. 1b). Motor output associated with ventilation cannot be detected in the ventral nerve distal to this branch; this distal part of the ventral nerve is suitable for recording sensory input or for electrical stimulation of putative afferents. The preparation is thus amenable to investigation of the influence of peripheral input on the motor output of the oscillator.

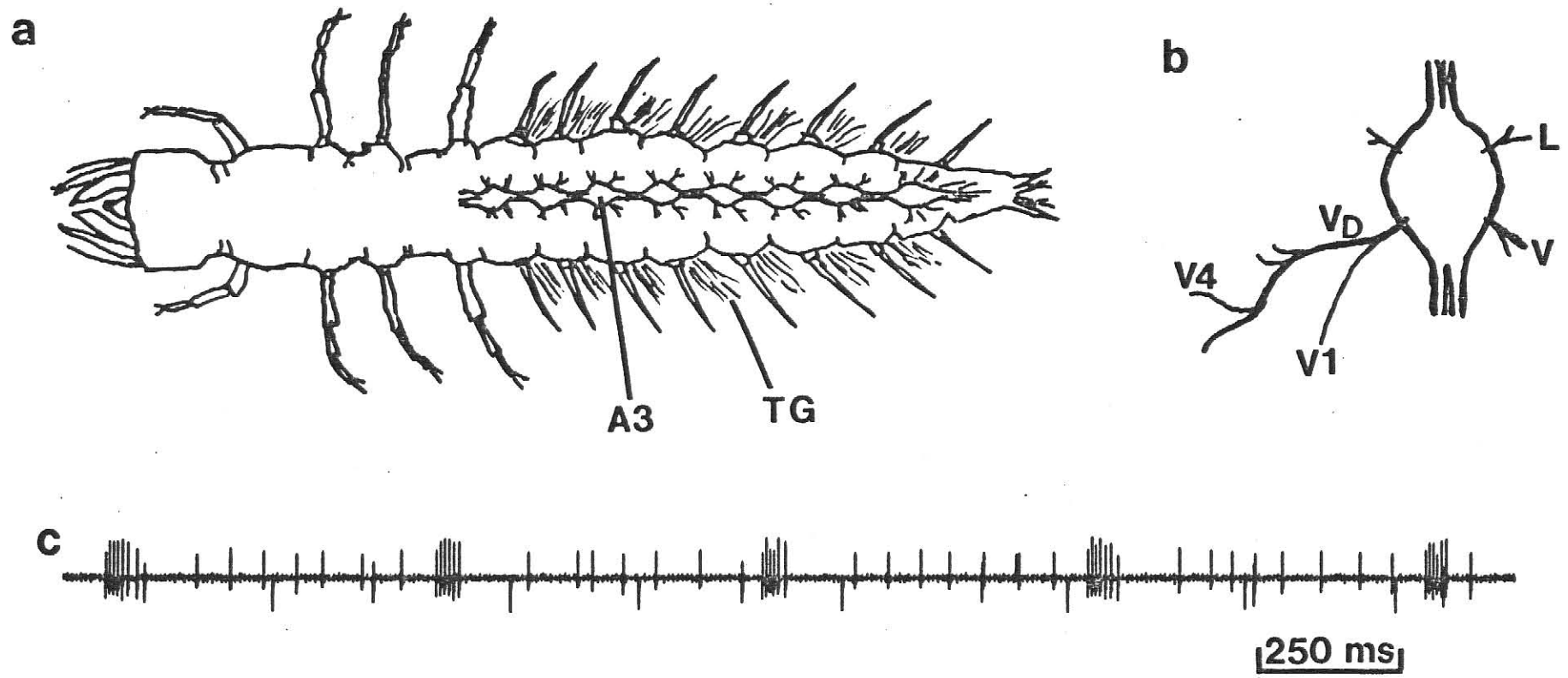
A series of apparently homologous appendages is often involved in a rhythmic behavior produced by a segmentally arranged animal such as the hellgrammite. Normally, more than one body segment contains an oscillator capable of producing the rhythmic behavior. Little work has been done regarding consequences of sensory input as a function of the dominance of the oscillator to which it is applied. Because the

Fig. 1. Corydalus cornutus, abdominal nervous system and ventilatory motor pattern.

(a) Diagrammatic dorsal view of a dissected hellgrammite showing segmental body plan and ventral nerve cord with one ganglion per segment. Each of the first seven abdominal segments supports a pair of tracheal gills (TG). The oscillator normally responsible for ventilatory motor output is located in the third abdominal ganglion (A3). Animals range up to 10 cm in length. (Modified from Hammar, 1908).

(b) Diagrammatic ventral view of one ganglion. Two nerve trunks, ventral (V) and lateral (L), leave the ganglion on both sides. During ventilation, a single motor neuron in the first branch (V1) of the ventral nerve is responsible for gill retraction. No motor activity is present distal to the first branch, which was used to monitor ventilatory motor output. The main trunk of the ventral nerve distal to the first branch, V_D, contains afferents that carry mechanosensory information from the gill tubercle. V_D was used to monitor sensory input and as a site for electrical stimulation, usually in the region between the first two branches and occasionally distal to branch V4.

(c) Representative recording from branch V1 showing ventilatory motor output. Each burst of impulses produces one gill retraction.


a
A3
TG
b
L
V_D
V
V4
V1
c
250 ms

hellgrammite has two oscillators that will produce a ventilatory rhythm when isolated from other segments, one of which is the dominant one for the rhythm, the preparation is ideal for investigation of differential effects of sensory input on dominant and subordinate oscillators.

This study first addresses the following questions involving modulation of the ventilatory rhythm by sensory input: Are the influences of sensory input tonic or phasic? Does response to the input vary according to when during the cycle it falls? Can sensory input modulate frequency of the rhythm? Do inputs to one segment affect other segments as well? The study then deals with comparisons of the effects of sensory inputs to oscillators in different segments. A portion of the data presented in this paper has been published in abstract form (Fitch and Kammer 1981; Kinnamon et al. 1981).

Materials and Methods

Dobsonfly larvae (hellgrammites) used in these experiments were collected locally from creeks and rivers and stored without food in tap water. Large animals (head width 7-11 mm) were usually used. Most experiments were performed after animals had been in the laboratory for about two weeks. Some animals were maintained for longer periods of time at 6°C; they were acclimated to room temperature (22°C-24°C) for about two weeks prior to use. Experiments were performed at room temperature.

After removal of its legs, an animal was pinned on its dorsum in a small chamber so that the gills "faced" the experimenter and gill movement was not restrained. Enough saline to completely cover the animal was added to the chamber. The saline had the following composition (in mM): 53.0 NaCl, 9.3 KCl, 6.1 $CaCl_2$, 7.9 $MgCl_2$ 114.0 Na-methanesulfonate, 55.0 glucose. A longitudinal cut made ventrally along the midline exposed the ventral nerve cord. The tracheal system was left as intact as possible, although it was impossible to avoid cutting some small tracheae. In some experiments, the gut was removed through a dorsal incision before the above dissection was performed. Removal of the gut generally increased ventilatory frequency slightly, but it did not affect the actions of sensory input. No measurements were made before 30 minutes after the dissection because the rhythm was sometimes unstable immediately postoperatively. In experiments involving severance of the ventral nerve cord, a comparable time was allowed to pass before activity was measured. Data for all figures in this paper were taken from animals with intact ventral nerve cords.

For purposes of this paper, any number referring to a ganglion designates the position of the ganglion in the chain of abdominal ganglia numbered from the most anterior abdominal ganglion. Each ganglion associated with gill-bearing segments (1-7) has a left and a right ventral nerve, the first branch of which (nerve V1) contains the motor neuron to the gill retractor muscle (Fig. 1a, 1b; Kinnamon and Kammer, in preparation). Extracellular recordings from an intact nerve V1 or from the peripheral end of severed nerve V1 show that nerve V1 carries no sensory information during normal ventilation; nerve V1 was thus used to monitor ventilatory motor output in all experiments. Most recordings were made from nerve V1 of ganglion 3. Chains of ganglia that excluded ganglion 3 were monitored by recording from nerve V1 of ganglion 2. In some experiments, simultaneous recordings were obtained from more than one segment to determine if a change in the rhythm occurred in all ventilating segments.

Preliminary anatomical studies of preparations stained with methylene blue (Stark et al. 1969) indicated that the lobes bearing the gill filaments are innervated by branches of the ventral nerve distal to nerve V1. This portion of the ventral nerve distal to the first branch, designated V_D, carries no ventilatory motor impulses during normal ventilation. Therefore, nerves V_D of abdominal ganglia 1, 2, 3, and 4 were used to record sensory input during ventilation. Nerves V_D from the first seven abdominal ganglia were used as sites for electrical stimulation. In some experiments, electrical stimulation was delivered to V_D distal to nerve V4 (and therefore presumably distal to any motor neuron) in order to insure that antidromic stimulation of motor neurons was not responsible for any stimulus-induced effects. In some

experiments, the ventral nerve was severed distal to nerve Vl, and electrical stimulation was delivered to the central end of V_D. Similar results were obtained from stimulating the central end and the intact nerve.

A single mechanical stimulus, which caused brief movement of the saline around a gill or gills, consisted of a puff of air from the mouth of the experimenter through a 2mm diameter glass capillary tube. Repeated mechanical stimulation consisted of a continuous stream of air delivered through the capillary tube. Electrical stimulation was delivered via a saline-filled extracellular suction electrode connected to a Grass SD9 stimulator. Sensory activity and motor output were monitored with extracellular suction electrodes on nerves V_D and Vl, respectively (Fig. 1b), and displayed on an oscilloscope. Some recordings were stored on magnetic tape and displayed on a Brush chart recorder for later analysis.

Results

Sensory input during ventilation

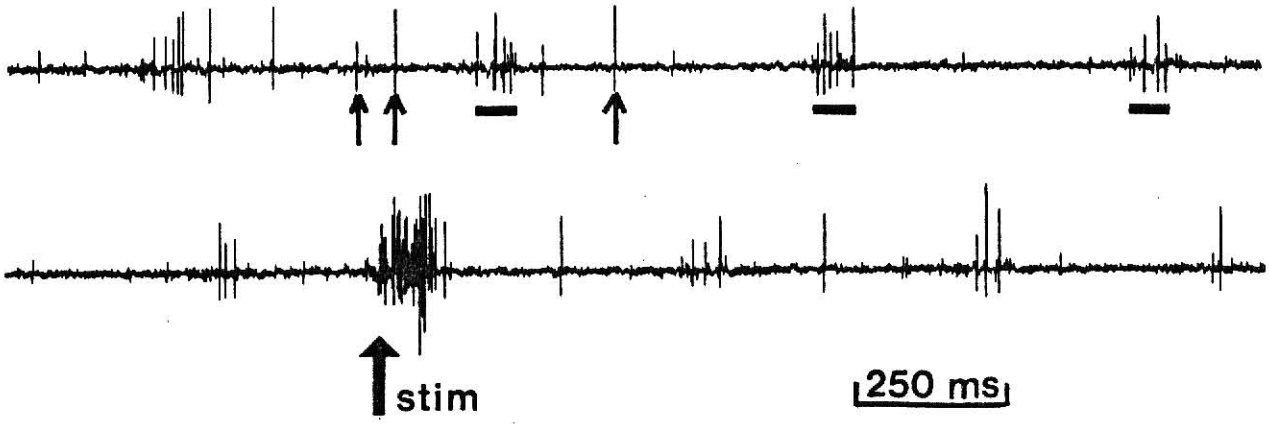
Bursts of sensory impulses were recorded from nerve V_D in unstimulated ventilating preparations (Fig. 2). The bursts, phase-locked with the rhythm, were associated with ventilatory movements -- either movements of the gill on the segment from which the recording was made, as shown by cutting nerves VI to the other thirteen gills to stop their movement, or movements of gills other than the one recorded from, as shown by stopping that gill. Selectively stopping various gills by cutting nerves to the gill retractor muscles showed that the ipsilateral gill immediately anterior to the gill being recorded from was most likely to stimulate receptors near that gill. Stopping all gills abolished the phase-locked input. Presumably both gill self-stimulation and stimulation by an anterior gill can occur within a cycle. Lack of sufficient time lag between the two types of stimulation prevented the demonstration of both in one animal.

Sensory impulses that are not phase-locked with the rhythm are also present in recordings from V_D (Fig. 2). Scattered impulses from several units were usually apparent whether or not the animal was moving its gills. Touching the gill or causing water movement around the gill greatly increased the discharge frequency of these units. The precise location of the mechanoreceptors has not been established, nor have the response properties of individual receptors been defined.

Effects of deafferentation

Isolation of abdominal ganglia 1-7 entailed severing the ventral nerve cord immediately anterior to ganglion 1 and posterior to ganglion

Fig. 2. Extracellular recording from nerve V_D of ganglion 3 in a ventilating preparation (continuous record). V_D was severed and the recording made from its peripheral end to insure that all activity was sensory. In the absence of experimental stimulation, both non-rhythmic activity (indicated by small arrows) and activity that is phase-locked with the ventilatory rhythm (horizontal bars) are present. A brief increase in activity occurs in response to a mechanical stimulus (large arrow) consisting of brief movement of the saline surrounding the gills.


stim
250 ms

7 and cutting all roots associated with ganglia 1-7. Cutting all roots abolished gill movement; the rhythm was monitored by recording motor bursts in nerve V1. When thus isolated, abdominal ganglia 1-7 exhibited a ventilatory frequency in the normal range [25-120 beats/minute (n=12)]. Furthermore, after additional cuts were made between ganglia, isolated ganglion 2, ganglion 3, or any chain of ganglia that included 2 or 3 exhibited a normal ventilatory frequency, confirming the observations of Kinnamon and Kammer (in preparation). Thus, the rhythm is centrally generated and not dependent on sensory cues for its production, and at least two ganglia can individually produce the rhythm.

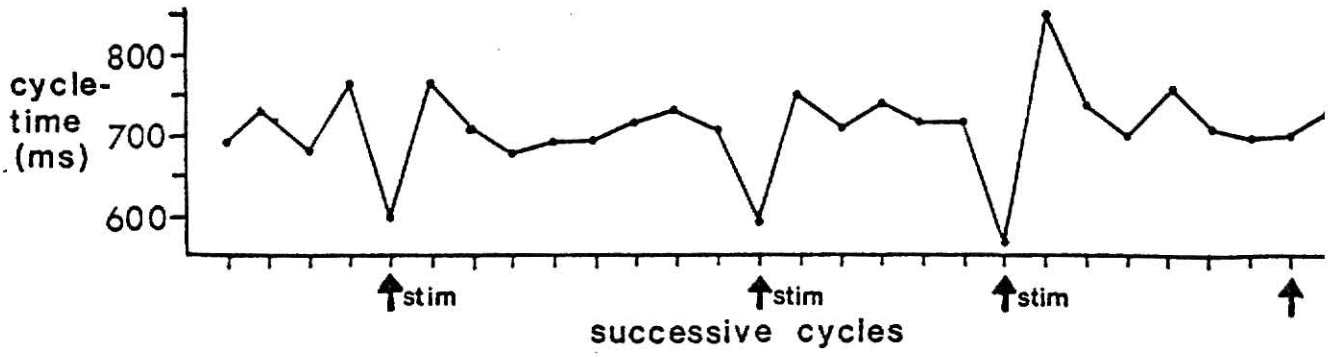
Although sensory input from the periphery is not necessary for production of the ventilatory rhythm, it could nonetheless have an effect on its frequency. This possibility was examined by comparing the frequency of the rhythm before and after deafferentation. The initial preparation consisted of ganglia 1-4. After isolating this chain of ganglia from the rest of the CNS, frequency of the rhythm was measured by recording from nerve V1 of ganglion 3. The nerves (right and left nerves V_D) carrying gill mechanoreceptor activity to all four ganglia were cut, and frequency of the rhythm was again measured. A paired t-test on data from eight animals showed that deafferentation had no significant effect on ventilatory frequency ($p < .05$). Performing the experiment on isolated ganglion 2 or isolated ganglion 3 likewise caused no consistent change in frequency, nor did cutting all other peripheral nerves.

Effects of experimental stimulation on the rhythm

(a) Single mechanical stimulation

The finding that removal of peripheral input did not appreciably change the frequency of the rhythm does not preclude the possibility that the input provides cycle-to-cycle timing cues that are not manifested as frequency changes nor the possibility that additional sensory input can affect the rhythm. It was previously shown that a mechanical stimulus that caused movement of saline around a gill or gills increased the frequency of discharge of afferents in nerve V_D. To determine if added sensory input affects the ventilatory rhythm, motor output was examined during application of the same mechanical stimulus. When a puff of air of short duration was delivered through a capillary tube to the saline covering the gills, the next burst of the gill retractor motor neuron often came sooner than expected. Advance of the burst shortened the time of the cycle in which the input occurred by as much as 40% (Fig. 3). Similar results were obtained with seven other animals. Simultaneous recordings from several segments showed that cycle shortening occurred not only in the motor output of the segment nearest the stimulus but in the motor output of other segments as well; thus the input affected the entire rhythm, and intersegmental coordination was maintained. A shortened cycle was often followed by two or three cycles with variable cycle-times; this phenomenon varied greatly between preparations. Usually, but not always, a shortened cycle was immediately followed by a longer-than-expected cycle. A long cycle usually did not differ as much from the mean unstimulated cycle-time as did the preceding shortened cycle. Some (about 15%) air

Fig. 3. Effect of brief mechanical stimulation. Cycle-times (intervals between onsets of consecutive bursts in the motor neuron to the gill retractor muscle) of twenty-eight consecutive ventilatory cycles in one animal were measured from an extracellular recording from nerve Vl of ganglion 3. Arrows mark cycles during which a stimulus (brief water movement around the gills) was delivered. The first three stimuli caused the cycle in which they fell to be shortened; the fourth stimulus had no effect. The shortened cycles were followed by cycles with cycle-times slightly greater than 710 ms, the mean unstimulated cycle-time.


cycle-
time
(ms)
800
700
600
stim
stim
stim
successive cycles

puffs in all preparations had no effect on the cycle in which they occurred nor on any subsequent cycle. The strength of the stimulus and the phase of the cycle during which the stimulus occurred were difficult to control or measure, but in light of later experiments it is presumed that air puffs having no effect occurred in an improper phase.

(b) Repeated mechanical stimulation

The above results demonstrate that phasic sensory stimulation can affect the timing of the burst in one cycle. No long-lasting or tonic effects were seen, however, although such effects would be necessary for frequency modulation. Nonetheless, all effects were burst advances rather than delays, suggesting the hypothesis that continuous mechanical stimulation could increase the frequency of the rhythm by a series of burst advances. That is, a continuous stimulus might act like a series of individual stimuli, each affecting one cycle, and thus in combination result in frequency modulation. The magnitude of the expected effect will be dependent in part on the percentage of stimuli that are ignored (as mentioned above) and in part on possible complications caused by the tendency of the cycle after a shortened one to be longer than the average cycle in the absence of stimulation. Two other factors made frequency modulation by mechanical stimulation hard to demonstrate. Many restrained, dissected animals ventilated near the presumed maximum ventilatory frequency. In addition, some stimulated animals contracted a host of abdominal muscles in a "struggling" behavior that lasted for one to three seconds, and that greatly affected the ventilatory frequency. In experiments with five animals, however, these factors were absent, and steady streams of air that caused saline movement

around the gills for 60 seconds increased mean ventilatory frequency during the 60-second periods (Fig. 4). Removal of stimulation was followed immediately by a return to the unstimulated frequency. This effect could be repeated in these animals. The most important consideration in showing this effect seemed to be a stable, but relatively low (i.e. 30-45 beats/min) frequency in the absence of stimulation. Thus, under proper conditions, it was possible to modulate the frequency of the rhythm with peripheral input.

(c) Single electrical stimulation

Given the above effects of sensory stimulation on the rhythm, one might expect electrical stimulation of the nerve containing sensory afferents from the gill region to yield similar results. Results of electrical stimulation are more amenable to analysis because strength and timing of the stimulus are more easily controlled.

In animals with intact nervous systems, electrical stimulation of the left or right nerve V_D of ganglion 3 with a single pulse (1 ms, 3.5 volts) often advanced the next burst of the gill retractor motor neuron in all ventilating segments (Fig. 5 ; similar results were obtained with eleven animals). When properly timed, the stimulus decreased cycle-time by as much as 50%, a decrease similar to that caused by a single mechanical stimulus. Stimulating the central end of V_D after severing it also yielded the same result. Stimulating nerve V_D distal to branch V4 and thus presumably distal to any motor neuron also gave the same result. Thus, the stimulus was neither affecting the rhythm via motor neurons in nerve V4 which are not normally involved in ventilation nor by antidromic stimulation of motor neurons in nerve V_D.

Fig. 4. Effect of continuous mechanical stimulation. Each bar represents a 60 second period during which the animal was either not stimulated (gray bars) or mechanically stimulated throughout the 60 second period with continuous saline movement around the gills (black bars). Ventilatory frequency, determined by recording from nerve Vl in ganglion 3, increased in response to stimulation.

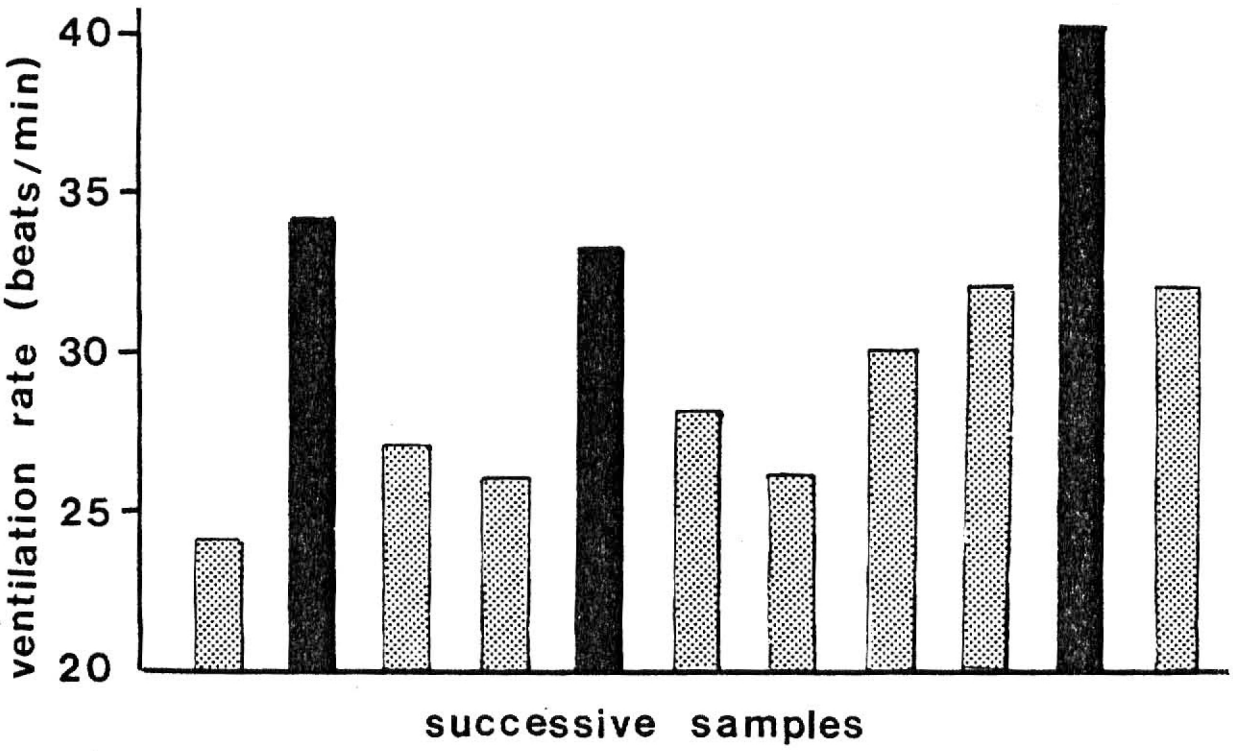
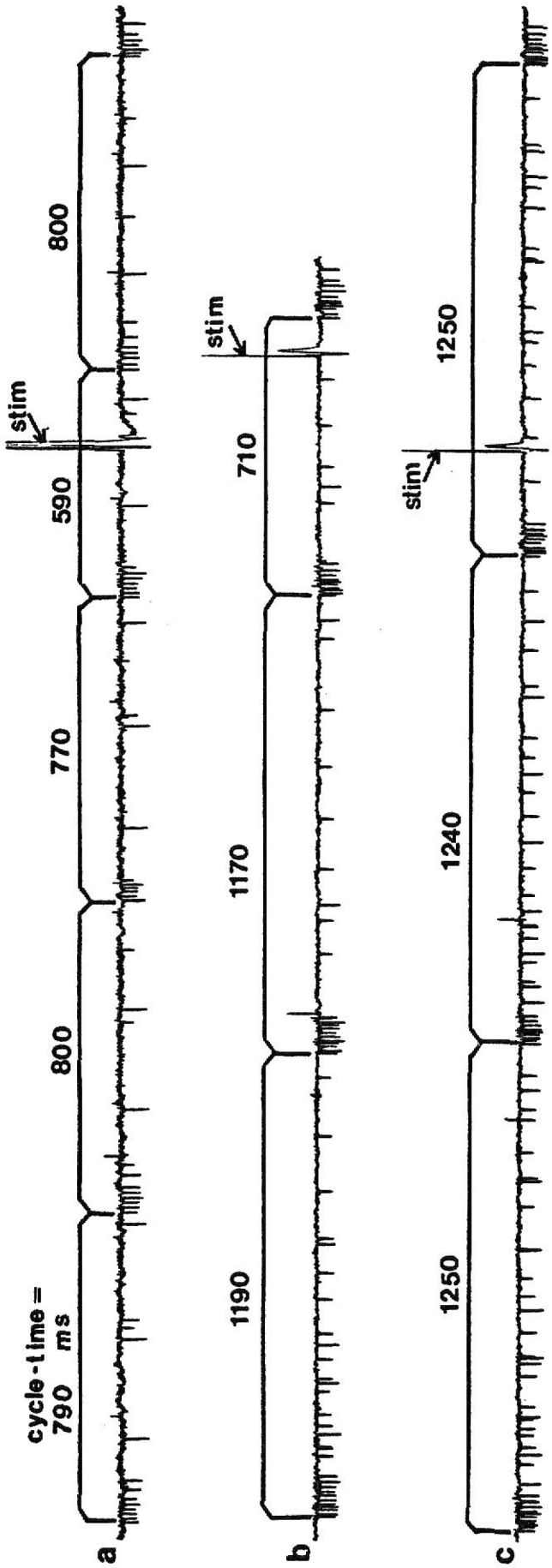

ventilation rate (beats/min)
40
35
30
25
20
successive samples

Fig. 5. Effect of a single electrical stimulus on ventilatory motor output. The left nerve V_D of ganglion 3 was stimulated. (a) recording from the right nerve Vl of ganglion 4 in one animal; (b) and (c) recordings from the right nerve Vl of ganglion 3 in a different animal. The electrical pulses (seen here as stimulus artifacts) in (a) and (b) shortened the cycle in which they were delivered by advancing the next motor burst. The pulse in (c) was delivered too early in the cycle (at a phase of about 0.2) to have an effect on motor output.


cycle-time =
790 ms
800
770
590
800
stim
a
1190
1170
710
stim
b
1250
1240
1250
stim
c

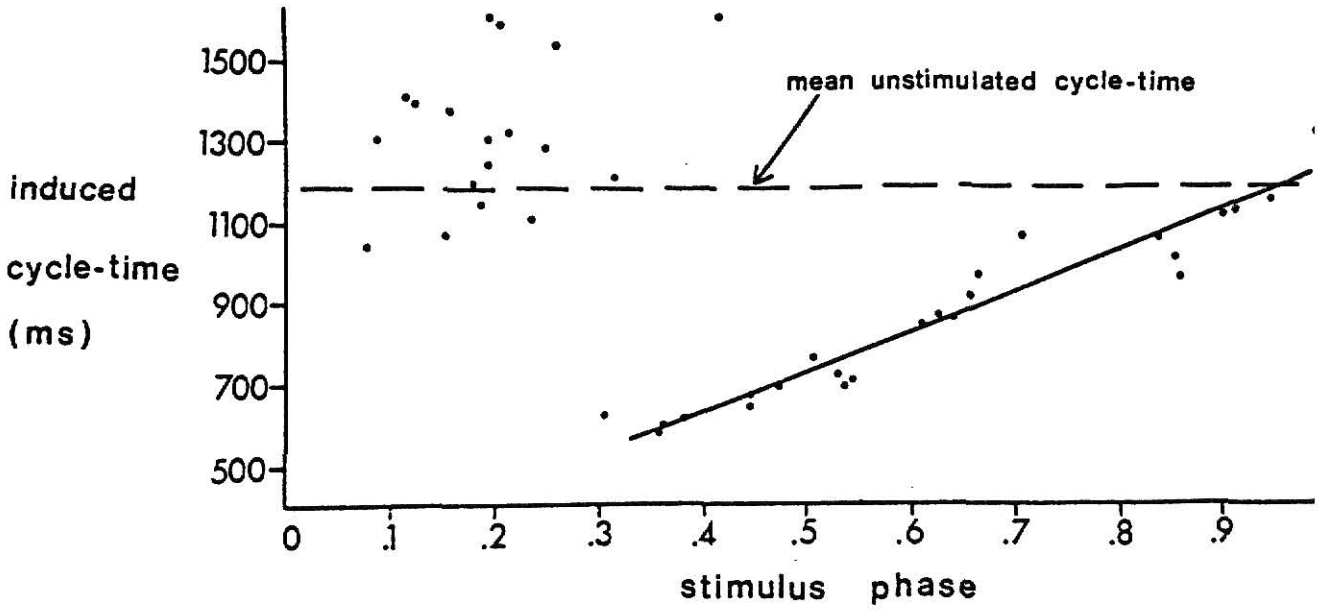
Similar results were obtained with slightly different stimulus voltages. Pulses of 3.5 volts were used for the subsequent studies.

To determine if chains of abdominal ganglia isolated from the rest of the CNS could similarly respond to a single electrical pulse, numerous combinations of abdominal ganglion chains (with or without the head and thoracic nervous system) were produced by severing the ventral nerve cord anywhere anterior and/or posterior to ganglion 3. Electrical pulses to afferents of ganglion 3 caused advances of gill retractor motor neuron bursts in all of these combinations of ganglia, including ganglion 3 in total isolation. As with mechanical stimulation, burst advances occurred in all active gill-bearing segments still connected to ganglion 3. Thus, a pulse to ganglion 3 advanced the next bursts in each of the first seven interconnected abdominal ganglia, and it advanced them in such a way that the proper interganglionic relationships were maintained.

As with air puffs, not every electrical stimulus advanced a burst. A plot of induced cycle-time (duration of a cycle during which a stimulus fell) as a function of stimulus phase (fraction of the expected cycle completed when the stimulus occurred, determined by dividing latency of the stimulus with respect to onset of the previous burst by the mean of the five preceding cycle-times) showed that a stimulus had an effect only if it fell during the last 70% of the expected cycle (Fig. 6). Since stimulus phase is a percentage measure (with unstimulated cycle-time = 100%), preparations exhibiting different unstimulated ventilatory frequencies could be compared using such plots.

With almost no exceptions, a stimulus in the effective phases (that is, in the last 70% of the expected cycle; see Fig. 6) advanced the next

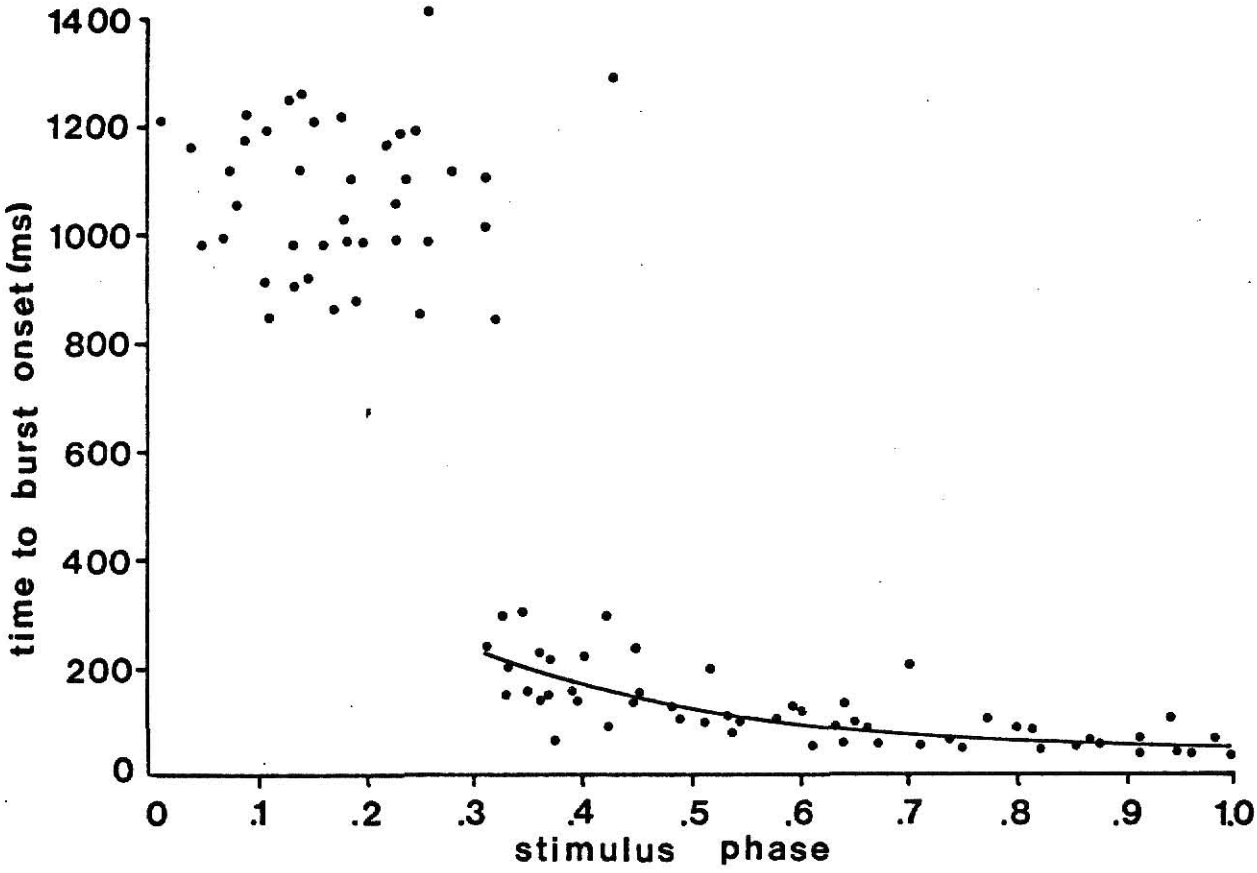
Fig. 6. Effect of stimulus phase. The duration of a cycle during which a stimulus was delivered (induced cycle-time) is plotted as a function of the fraction of the expected cycle which had passed when the stimulus occurred (stimulus phase) in one representative animal. Each stimulus consisted of a single electrical pulse to nerve V_D of ganglion 3 delivered approximately every tenth cycle at a random time within the cycle. Induced cycle-time was obtained by recording motor output from nerve V1 in ganglion 3; stimulus phase was determined by dividing latency of the stimulus with respect to the previous burst onset by the mean of the five preceding cycle-times. A stimulus only advanced the next burst if it fell in the last 70% of the expected cycle. The earlier a stimulus fell in the effective phases, the more it shortened the cycle.


induced
cycle-time
(ms)
1500
1300
1100
900
700
500
mean unstimulated cycle-time
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
stimulus phase

burst (441/454 tests in 11 animals). In no animal did stimuli in the first 30% of the expected cycle advance or delay the next burst. Animals whose unstimulated ventilatory frequencies ranged from 51 to 73 beats/minute were tested; the effective phases did not change with changes in frequency, although experimental variability made it difficult to determine exactly when the effective phases started and could have obscured small changes. The earlier in the effective phases a stimulus occurred, the more it shortened a cycle (Fig. 6). This result should not be interpreted to mean that the oscillator is more sensitive to an early stimulus than to a later one, because in the case of a late stimulus most of the expected cycle had already passed so that only a small amount of cycle shortening is possible. In fact, an examination of how quickly a stimulus is followed by the burst advanced by it shows that a stimulus occurring late in the expected cycle is more effective than one early in the expected cycle (Fig. 7). Stimuli early in the effective phases were followed by burst onsets with time lags of about 200 ms whereas values decreased to about 50 ms for late stimuli.

The finding that an electrical pulse can advance the next ventilatory motor burst suggests that the pulse is resetting the ventilatory rhythm. For the rhythm to be reset, the cycles following the shortened one should have durations similar to the duration of an unstimulated cycle. However, as with cycles shortened by a single mechanical stimulus (Fig. 3), a cycle immediately following one shortened by an electrical pulse sometimes had a cycle-time greater than the mean unstimulated cycle-time. This phenomenon, termed compensation, would advance only a single burst instead the entire rhythm if the shortened cycle and the lengthened cycle differed from an unstimulated

Fig. 7. Relationship between the latency with which a burst followed a single electrical stimulus and the phase of the stimulus. Stimuli were delivered and motor output monitored as in Fig. 6. Data from four animals with mean unstimulated cycle-times ranging from 996-1092 ms. A stimulus that advanced a burst (i.e. one in the last 70% of the expected cycle) was more quickly followed by a burst if it occurred late in the expected cycle. Line was drawn by eye.


1400
1200
1000
800
600
400
200
0
time to burst onset (ms)
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1.0
stimulus phase

cycle by an equal amount. That is, the stimulus may have affected only the motor neuron and not the oscillator in such a way that all subsequent bursts occurred at the times expected had there been no stimulus. Data analysis showed no tendency of any cycle to be lengthened except the first one after a shortened cycle. Consequently, only the cycle immediately following an induced one need be examined. The inequality $2\bar{n} - (C_S + C_{S+1}) > \frac{\bar{n} - C_S}{2}$, in which $\bar{n}$ is the mean of the five cycle-times preceding the one during which the stimulus occurred, C_S is the duration of the cycle during which the stimulus occurred, and C_{S+1} is the duration of the next cycle, was derived to investigate the question of oscillator resetting. The left side of the inequality represents the amount of time that the entire rhythm is shifted by the stimulus. If no compensation occurs this amount will equal the amount of shortening ($\bar{n}$-C_S) in the cycle during which the stimulus is delivered. If the cycle after the shortened one is lengthened such that it compensates less than half for the shortening in the cycle during which the stimulus is delivered, then the entire rhythm (the left hand side of the inequality) must be shifted by at least half the amount of shortening ($\bar{n}$-C_S) of the shortened cycle. Thus, by dividing the amount of shortening by 2 (producing the right side of the inequality), an expression is obtained which contains the arbitrary criterion that compensation of less than 50% is required before one can argue that the oscillator has been reset. Eighty-eight percent of the stimuli delivered to 5 animals (n=138) reset the oscillator when judged by this criterion.

A graphic representation of the durations of cycles immediately following cycles during which a stimulus occurred can also be used to

investigate the question of oscillator resetting (Fig. 8). If the oscillator were not being reset, these points would fall on a line that is opposite in slope to the line generated in Fig. 6 and that intersects the mean unstimulated cycle-time at phase 1.0. That is, each cycle following an induced one should be as much longer relative to an unstimulated cycle as the corresponding induced cycle was shorter. This was obviously not the case, which indicates that the oscillator was indeed being reset.

(d) Repeated electrical stimulation

Mimicry of a single air puff by a single electrical pulse led to suspicion that a rhythmic series of pulses could duplicate the increase in frequency caused by continuous mechanical stimulation. In fourteen animals, when 3.5-volt pulses were delivered to nerve V_D of ganglion 3 at a rate slightly higher than the unstimulated ventilation frequency, rhythm frequency was increased. Furthermore, the increase involved an absolute 1:1 entrainment of the rhythm to the stimulus pulses (Fig. 9). As with single pulses, the effect could be demonstrated on any combination of ganglia that included ganglion 3 as the site of stimulation. As with single pulses, intersegmental coordination was not altered by stimulation.

The first few pulses of rhythmic electrical stimulation often caused a massive contraction of abdominal muscles immediately after which entrainment occurred. Cessation of stimulation was followed by an immediate return to the unstimulated frequency. A driven rhythm was usually entrained such that each stimulus occurred at a phase of 0.5 to 0.7 with respect to the interval between the burst preceding it and the

Fig. 8. The duration of a cycle immediately following an induced cycle as a function of the phase of the stimulus in the preceding cycle. These data are from the same set of stimuli as in Fig. 6. A shortened cycle is often followed by a longer than average cycle, shown by the points falling above the mean unstimulated cycle-time at phases later than 0.3. However, the long cycles do not completely compensate for the shortened cycles, since they do not fall along the line that is a mirror immage of the line generated in Fig. 6. The latter result is evidence that the stimuli are at least partially resetting the rhythm.

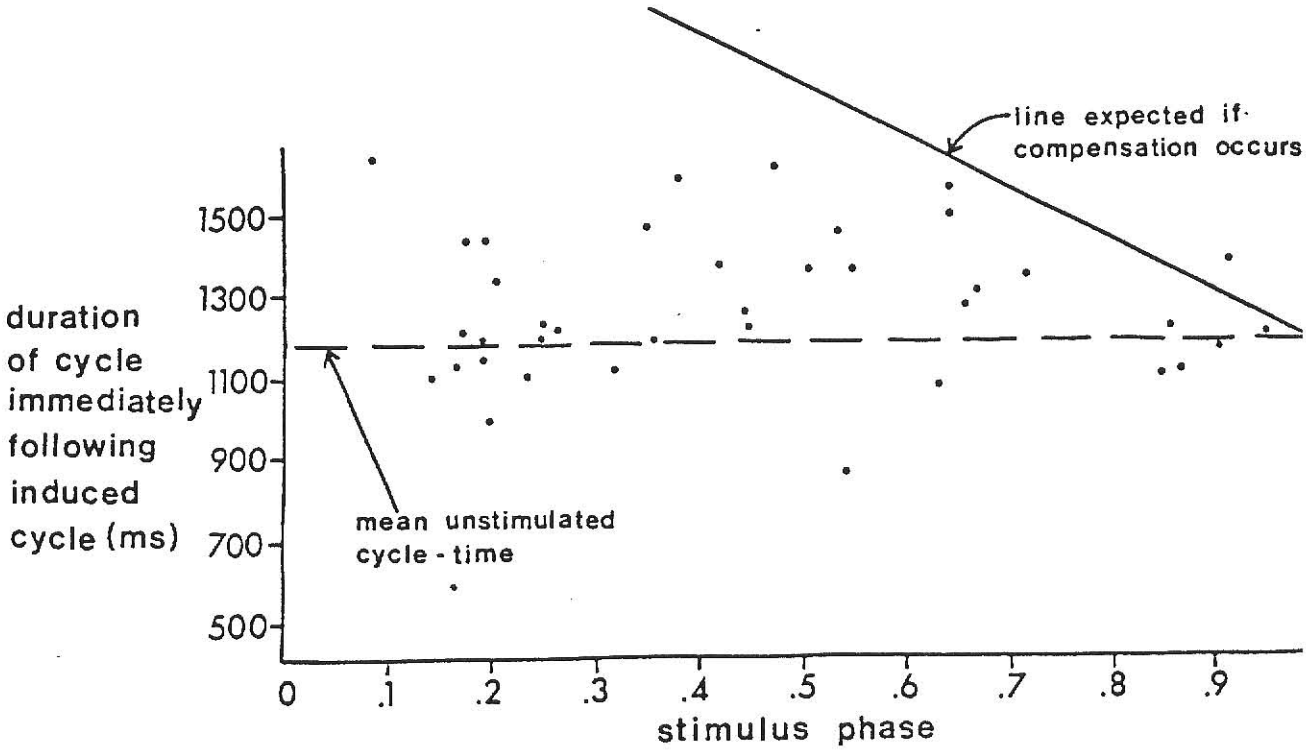
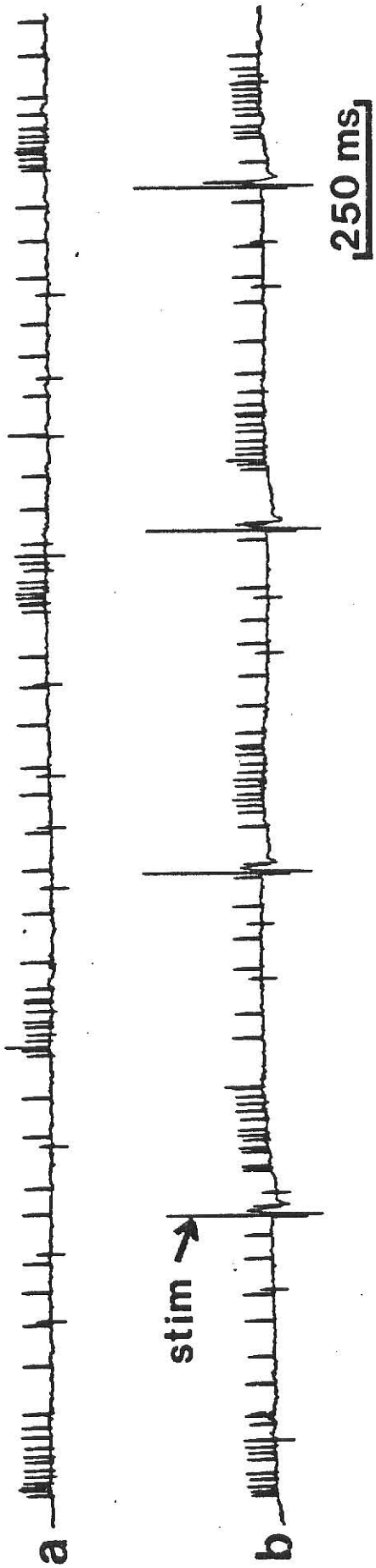

line expected if compensation occurs
duration of cycle immediately following induced cycle (ms)
1500
1300
1100
900
700
500
mean unstimulated cycle - time
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
stimulus phase

Fig. 9. Entrainment of the ventilatory rhythm to rhythmic electrical stimulation. Recording from nerve Vl of ganglion 3; stimuli delivered to nerve V_D of ganglion 3.

(a) The unstimulated rhythm with a frequency of 84 beats/min.

(b) 1:1 entrainment of the ventilatory rhythm to electrical stimulation at a frequency of 108 beats/min.


a
b
stim
250 ms

expected time of the next burst (as calculated from the mean unstimulated ventilation frequency). This was an expected result, as a stimulus occurring at a phase earlier than 0.3 is not effective (Fig. 6).

The range of frequencies over which the rhythm could be driven was 100% to 135% of the frequency before stimulation. Stimulating at a frequency lower than the unstimulated ventilatory frequency did not slow the rhythm. This observation is consistent with the fact that a single pulse cannot delay a burst.

(e) Stimulation of afferents in other ganglia

The effects of electrical stimulation described above were produced by stimulation of afferents in the ventral nerve of segment 3. The effects of stimulating afferents in the ventral nerves of other gill-bearing segments were less dramatic than were the frequency modulation and burst advances produced by stimulation of segment 3. Because of the difficulty of causing water movements that impinged on just one gill, only electrical stimulation of ganglia will be considered here.

In animals with intact central nervous systems, a single pulse of relatively high voltage (15-20 volts) delivered to nerve V_D of ganglion 2 or ganglion 4 sometimes caused a quick retraction of that gill, as determined by visual observation. This result was different from previously described results in that the stimulus often affected no other gills (not even the contralateral gill on the same segment) and had no effect on the rhythm. That is, the stimulus apparently caused an

extra burst in one motor neuron, but the motor burst thereafter was unaffected.

In some preparations (5 of 11), a single 3.5 volt pulse to nerve V_D of ganglion 2, 4, or 5 advanced the next burst in all active segments, but the magnitude of the advances was small relative to advances caused by stimulating ganglion 3. These animals had intact central nervous systems, and motor output was recorded from nerve Vl in several different segments, sometimes simultaneously. Maximum cycle shortening was about 10% when ganglion 2 or ganglion 5 was stimulated, 15% to 20% when ganglion 4 was stimulated, and 50% when ganglion 3 was stimulated. Stimulation of ganglion 1, 6, or 7 had even less effect, so that in animals with cycle-time variability of 10% of the mean unstimulated cycle-time no effect was observed. Furthermore, in the five animals in which a burst advance due to stimulation of ganglion 2, 4, or 5 was demonstrated, not every stimulus in the effective phases advanced a burst. Also, analysis of cycles following shortened ones produced no evidence that the oscillator was reset. This again could have been due to cycle-time variability. Isolating ganglion 2 before stimulating it (so that its own oscillator was responsible for motor output) did not increase its sensitivity to stimulation. As a whole, then, results from these experiments were not nearly as clear as were those from stimulation of ganglion 3. When demonstrated, however, the relationship between stimulus phase and cycle shortening was similar to that seen when stimulating ganglion 3; early stimuli had no effect.

Repeated electrical stimulation likewise was less effective in modulating the rhythm if delivered to a ganglion other than to ganglion 3. Recordings from nerve Vl of ganglion 3 and of the stimulated

ganglion showed that in none of the animals tested could the rhythm be entrained for many cycles by rhythmic pulses to nerve V_D of a ganglion other than ganglion 3. As before, testing various chains of ganglia or ganglion 2 in isolation yielded the same result. In some preparations, the rhythm could be driven at slightly increased frequencies for four to six consecutive cycles by stimulation of afferents to ganglion 4. These results were cases of relative coordination in which entrainment at a preferred phase occurred for a few cycles, after which bursts followed stimuli with increasing latencies until a stimulus was followed by no burst. The next stimulus would then be followed by a burst, initiating the sequence again. The presence of both ganglion 3 and ganglion 4 was necessary to demonstrate this effect of rhythmic pulses to nerve V_D of ganglion 4, and recordings from nerve Vl of other ganglia connected to 3 and 4 showed that their outputs were similarly affected. In summary, the above results indicate that repeated electrical stimulation, like single electrical stimulation, had a much greater efficacy in modulating the rhythm if delivered to ganglion 3.

Discussion

Modulation of the rhythm

Hellgrammite ventilation is a centrally generated, rhythmic motor activity that can be modulated by peripheral input. A properly timed phasic mechanical or electrical stimulus delivered to mechanoreceptors or their afferents in the third abdominal segment shortened the cycle during which the stimulus occurred. Continuous mechanical or repetitive electrical stimulation increased the frequency of the ventilatory rhythm.

It could be argued that antidromic stimulation of motor neurons was responsible for the effects elicited by electrical stimulation. However, stimulating the ventral nerve at a site presumably distal to any motor neurons (that is, distal to branch V4) was effective in modulating the rhythm, whereas stimulating the nerve containing the gill retractor motor neuron (branch V1) was not. These observations show that the effects of electrical stimulation are not caused by antidromic stimulation of motor neurons. Mimicry of electrical stimulation of afferents by mechanical stimulation of corresponding mechanoreceptors is further evidence against antidromic effects.

It is usually the case that, when peripheral input can modulate a rhythm, removal of peripheral feedback generated by the rhythm results in a decrease in frequency (for example grasshopper flight, Wilson and Gettrup 1963; dogfish swimming, Grillner 1974). In these cases, the decrease is thought to result from removal of tonic excitation. In the hellgrammite, there may be no tonic effects of sensory feedback, because removal of input did not reduce the ventilatory frequency. Alternatively, some well-studied preparations involving centrally

generated rhythms require tonic electrical stimulation of interneurons ("command fibers") for the production of motor output (Stein 1978). The hellgrammite requires no such stimulation of the ventral nerve cord; rhythms of high frequency were obtained from unstimulated, isolated ganglia. In some ways, then, the preparation seems less dependent than most on excitatory input. It is possible, however, that removing normal peripheral input did have an effect on frequency, but the effect was masked by the variability of the rhythm since a frequency change that was small relative to the standard deviation of the cycle-time would not have been statistically significant. In addition, factors such as hypoxia have an influence on rhythm frequency (Kinnamon et al. 1981) and the dissection unavoidably involved cutting some tracheae. Stimulation of oxygen receptors by a change in P_{O_2} of the ganglia due to tracheal transection may have produced a small increase in the frequency of the rhythm and thereby obscured a decrease produced by cutting sensory afferents. Attempts to control for effects of hypoxia by aerating the saline during experiments yielded no differences. Although no firm conclusion can be drawn about the influence of peripheral feedback in an intact animal, for dissected preparations it can be concluded that abolition of sensory input has no statistically significant effect on ventilatory frequency. The conclusion seems more plausible for a ventilatory rhythm than it would for a flight or walking rhythm, in which a timing error could have more disasterous consequences.

Although removal of mechanosensory input did not slow the ventilatory rhythm, continuous stimulation of mechanoreceptors sometimes produced an increase in frequency. The stimulus -- saline movement around the gills -- was a tonic, rather than a phasic one. That is, its

magnitude and duration remained constant for a time which was long relative to cycle-time. However, since the effect of a stimulus is termed phasic (as opposed to tonic) if it is seen in the same cycle that the stimulus occurs, it can be argued that tonic mechanical stimulation exerted its effect in a cycle-to-cycle or phasic fashion. This conclusion is based on the following logic: a single mechanical stimulus and a single electrical stimulus have similar effects (Figs. 3 and 5) and are therefore probably acting in the same way. An electrical stimulus advances a burst only if it occurs in the last 70% of the expected cycle (Fig. 6); the same is probably true of a mechanical stimulus. This phase dependence allows the continuous mechanical stimulus, a tonic one, to act as a series of repeated phasic stimuli. The conclusion that the tonic stimulus was exerting its effect in a cycle-to-cycle or phasic fashion is lent further credibility by the finding that a series of repetitive electrical pulses increased frequency by an absolute entrainment of the rhythm, one pulse per cycle. It seems clear in this latter situation that each pulse in the rhythmic series of pulses had an effect analogous to that demonstrated earlier for single pulses.

The magnitude of the maximum possible burst advance in response to a single stimulus in an animal may theoretically be used to predict the limits of stable entrainment to a repeated stimulus (Pinsker 1977). Since neither preferred phase nor percentage of cycle shortening possible varied with unstimulated ventilatory frequency, a single upper and a single lower value (expressed as percentages of unstimulated frequency) should define the limits for all animals regardless of initial frequency. The earliest possible burst-advancing stimulus (at a

phase of 0.3) gave about a 50% cycle shortening; no stimulus delayed a burst. These data lead to the prediction that the lower and upper limits of stable entrainment to repeated stimulation are 100% and 200% of initial frequency. The actual experimental limits were found to be 100% and 135%. Maximum cycle shortening by a single stimulus is possible when the stimulus occurs at a phase of 0.3 (see Fig. 6), but during entrainment stimuli usually occurred at a phase between 0.5 and 0.7. Thus the predicted upper limit of entrainment to a repeated stimulus was not observed.

Comparison with other systems

It is generally agreed that generation of rhythmic motor output is not dependent on peripheral feedback but may be modified by it (Delcomyn 1980). The hellgrammite ventilatory rhythm is no exception to this generalization, but effects of sensory input are in some ways unusual in this animal.

Oscillator resetting by peripheral input has been found in several behaviors, including jellyfish swimming (Horridge 1959), dragonfly larvae ventilation (Mill and Hughes 1966; Mill 1970), and cockroach ventilation (Farley and Case 1968), the latter exhibiting a burst delay rather than an advance. Phase dependency has not been demonstrated in these systems, although it could nonetheless be present. Several systems in which the effect of input on a rhythm is dependent on phase have been well studied (Mohl and Nachtigall 1978; Beltz and Gelperin 1980; Nagy and Moulins 1981). Burst advance or delay through the entire range of possible stimulus phases with phase-dependent magnitude, or burst delay in phases where there is no advance, seem to be common

findings. In the hellgrammite, early stimuli were completely ignored. This result is unusual, although Nagy and Moulins (1981) obtained a similar result with the exception of a slight delay at a phase of about 0.2 in the cape lobster.

In addition to the effects of sensory input on one cycle of a rhythmic behavior, many investigators have attempted to modulate the frequency of a rhythm using sensory stimulation. Frequency modulation has been caused by interfering with periodic limb movements in stepping of the cat (Duysens 1977) and cockroach (Wong and Pearson 1976), and by absolute or relative entrainment to imposition of a different frequency of movement on the body part associated with the behavior (locust flight: Wendler 1974; Mohl and Nachtigall 1978; Limulus ventilation: Wyse and Page 1976; lobster ventilation: Young and Coyer 1979). Other examples of frequency modulation by input acting phasically exist (Horridge 1959; Farley and Case 1968; West et al. 1979). To our knowledge, however, our results provide the first demonstration of an isolated ganglion producing a rhythmic behavior the frequency of which can be modulated by phase-dependent mechanosensory input acting in a cycle-to-cycle fashion.

Understanding frequency modulation of hellgrammite ventilation is complicated by the fact that the rhythm involves appendages on several body segments. More than one segment contains an oscillator, and the outputs of these oscillators must be coordinated in some way during a change in frequency in order to maintain proper intersegmental phase relationships. In the hellgrammite, the oscillator in ganglion 3 nearly always drives the rhythm. If ganglion 3 is not connected to ganglia 1 and 2, the oscillator in ganglion 2 drives these two segments. There is

some evidence that an oscillator in ganglion 4 can drive segments 4-7 when these segments are isolated from ganglion 3. Thus, oscillators in the various body segments differ greatly in their ability to drive the ventilatory rhythm. Effects of peripheral inputs to these various body segments are likewise very different. This result is in contrast to studies on swimmeret beating in crayfish (Ikeda and Wiersma 1964) and ventilation in dragonfly larvae (Mill 1970), in which the justification for calling a particular oscillator dominant is that it is in the segment that leads the rhythm, or swimming in jellyfish (Lerner et al. 1971), in which no segment consistently leads the rhythm. In these systems, effects of peripheral input are relatively independent of stimulus location. There are, however, systems in which stimulus location makes some difference. For example, in locust flight, hindwing and forewing stimulation have somewhat different effects on the flight rhythm (Mohl and Nachtigall 1978). The hellgrammite seems to be at an extreme end of this spectrum of systems having varying sensitivities: it provides a dramatic case of seemingly homologous body segments that have a widely varying range of sensitivities to peripheral input. To our knowledge, no other rhythm involving linked oscillatory centers in homologous body segments has been found which can be reset by sensory input to only one center. More information on other systems is needed before any conclusions can be drawn about the efficacy of a stimulus in modulating a rhythm as a function of the site of stimulation. However, our data suggest the hypothesis that, if rhythms involving chains of segments fall on a continuum having at one end no particular dominant oscillator and having at the other end a specific oscillator that is extremely dominant, a parallel continuum may exist for the importance of

stimulus location in modulating rhythmic output. Phasic input may be best able to modulate a rhythm when applied to the segment containing the oscillator most important for that rhythm.

It makes sense for phasic input to have a greater effect on the oscillator most important for the motor output if cycle-to-cycle modulation is required. In the hellgrammite, peripheral input on ganglion 3 seems in many ways analogous to the input of ganglion 3 on other ganglia. The inability of these other ganglia to respond to peripheral input may aid in intersegmental coordination in that varying amounts of mechanosensory input might cause confusion when mixed with coordinating input from ganglion 3.

Although in the hellgrammite phasic sensory input has little effect on oscillators other than the dominant one, tonic inputs such as hypoxia or exogenous octopamine are effective frequency modulators when applied to "secondary" oscillators as well as to ganglion 3 (Kinnamon et al. 1981). These observations lead to the following hypothesis: In a coordinating system containing a dominant oscillator, phasic input (which has an effect in the same cycle in which it occurs) may act primarily on the dominant oscillator, because the period of any secondary oscillator has already been programmed by the dominant one, whereas tonic input (which lasts for a time that is long relative to cycle-time) can modulate the rhythm when applied to both dominant and secondary oscillators, because the input is integrated over several cycles during which time interactions among oscillators may occur. It remains to be seen if systems other than Corydalus confirm this hypothesis.

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MODULATION OF THE VENTILATORY RHYTHM OF THE HELLGRAMMITE *CORYDALUS CORNUTUS* BY MECHANOSENSORY INPUT

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ABSTRACT

Relatively little is known about the mechanisms by which sensory input influences motor output generated by the central nervous system. This problem was investigated in the hellgrammite, an aquatic insect larva, in which ventilation consists of rhythmically repeated retractions and protractions of seven pairs of abdominal gills. Rhythmic retractions can be initiated by at least two separate neuronal networks, or oscillators, in the central nervous system. Data were obtained by extracellularly recording ventilatory motor output while mechanosensory stimulation was applied.

Mechanosensory input can modulate the ventilatory rhythm. Brief water movements around the gills often causes the next wave of gill retractions to occur earlier than expected. If stimulation is continued for 60 seconds, the stimulus can produce a 35% increase in ventilatory frequency during the stimulated interval. In abdominal segment 3, which houses the dominant oscillator for the rhythm, electrical stimulation of sensory nerves gives results similar to those obtained with mechanical stimulation. A single electrical pulse can shorten the next ventilatory cycle, and repeated pulses can increase ventilatory frequency by as much as 35%. Electrically stimulating segment 2, which contains a subordinate oscillator for the rhythm, yields much less dramatic results. This suggests that a phasic input is better able to modulate the rhythm if it is delivered to the dominant oscillator for the rhythm.