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MODES OF ACTIVATION OF MOTONEURONS CONTROLLING VENTILATORY MOVEMENTS OF THE LOCUST ABDOMEN

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CONTENTS

1. Intracellular recordings have been made from tonic and phasic expiratory, inspiratory and spiracular motoneurons and presumed sensory integrating neurons of the metathoracic ganglion in a locust during rhythmic ventilatory movements of the abdomen. The neurons have somata with diameters of no more than 30 μm situated on the ventral surface of the ganglion.

2. No motoneurons showed an intrinsic rhythmicity, all being driven in the ventilatory rhythm by complex patterns of synaptic inputs in one of the following ways: (*a*) excitation alone during the phase when spikes are produced (spiracle closer and some tonic expiratory motoneurons); (*b*) excitation during one phase and inhibition during the other (some tonic expiratory motoneurons); (*c*) excitation and inhibition during both phases (most motoneurons) in which one type of input dominates a particular phase.

3. The burst of spikes by a particular motoneuron may end because of a lack of excitatory input (spiracle closer motoneurons) or be terminated rapidly by inhibition (inspiratory motoneurons). Inhibition may also precede the main burst of spikes (inspiratory motoneurons) so that any spikes during the opposite phase are abolished. The pattern of synaptic input determines the frequency code of spikes within a burst.

4. Phasic expiratory motoneurons receive an underlying pattern of synaptic inputs in phase with ventilation even when they do not spike. Non-specific excitation (for

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example, a d.c. depolarization of the soma) is able to produce bursts of spikes in the correct phase of ventilation.

5. No direct pathway between any groups of motoneurons was found. Driving by common antecedent interneurons is inferred for those motoneurons which show similar patterns of spikes (inspiratory and spiracle closer motoneurons).

6. Stimulation of descending fibres in the pro-mesothoracic connectives evokes e.p.s.ps in some motoneurons, perhaps monosynaptically. In inspiratory motoneurons these fibres cause e.p.s.ps but will abolish the inspiratory burst of spikes and reset the ventilatory rhythm. All observations imply that the mechanism of the ventilatory rhythm lies among interneurons.

Introduction

Locusts ventilate their tracheal system by regular dorso-ventral pumping movements of their abdomens, with air entering through the thoracic spiracles and leaving through the abdominal ones. The rhythmic abdominal movements require no phasic sensory inflow for their patterning and will proceed, albeit at a reduced frequency, when the central nervous system is completely isolated (Huber 1960; Miller 1966). The whole movement requires the coordinated output of the thoracic and abdominal ganglia, and in isolation any of the abdominal or metathoracic ganglia are capable of producing alternating bursts of motor impulses to the expiratory and inspiratory muscles, but in the intact locust the metathoracic ganglion is the dominant pacemaker. All descriptions to date of the neural control of the ventilatory movements have been based on data collected by extracellular recordings from nerves or muscles. From these studies have come descriptions of the pattern of motor impulses to the thoracic spiracles (Miller 1967), the sequence and pattern of motor impulses which emanate from the abdominal ganglia (Lewis, Miller & Mills 1973), and the implication of a role for interneurons in the coordination of the motor impulses of the abdominal ganglia (Huber 1960; Miller 1966; Farley, Case & Roeder 1967). The descriptions are not complete but now that it is feasible to record intracellularly from the somata of identified insect motoneurons (Hoyle & Burrows 1973) the attempt to understand the neural mechanisms underlying the ventilatory rhythm can progress a stage further. Access to the synaptic inputs to the motoneurons has allowed hypotheses concerning the mechanism for the production of the ventilatory pattern to be tested in this study. The idea that the motoneurons have a rhythmicity or produce the pattern by suitable direct pathways among themselves must now be rejected. No motoneurons showed any inherent rhythmicity and no direct pathways between motoneurons such as reciprocal coupling of antagonists were observed. Interneurons providing a variety of rhythmic, excitatory and inhibitory synaptic inputs to the motoneurons are inferred to produce the rhythm.

Methods

Adult *Schistocerca gregaria* of either sex and obtained from culture were mounted ventral side uppermost with only the thorax restrained and opened ventrally to expose the meso- and metathoracic ganglia. A wax-covered platform slipped between the pro-mesothoracic connectives isolated these ganglia from movements of the body. The tracheal supply to the ganglia was maintained intact and the thorax was perfused with a constant flow of a saline solution (Usherwood & Grundfest 1965). The technique for recording from somata of neurons in the intact ganglia was similar to that used by Hoyle & Burrows (1973). The recording microelectrodes were filled with a 2 M solution of potassium acetate or 4 g/100 ml of Procion Yellow

and had resistances of 50–80 MΩ. Current could be passed through the recording microelectrode by means of a Wheatstone-bridge circuit. Injection of Procion dye at the end of a recording showed the position of the neuron's soma. Pairs of hook electrodes were placed on each of the pro-mesothoracic connectives and suction electrodes were used to record *en passant* from lateral and median nerves of the metathoracic ganglion. Dorso-ventral movements of the abdomen were monitored by a lever attached to the anode of an RCA 5734 tube. The rate of ventilation was modified by changing the concentration of carbon dioxide in the surrounding air.

Results

General features of abdominal ventilatory movements

In a quiescent locust ventilation of the tracheal system is provided by dorso-ventral pumping movements of the abdomen at frequencies of 5–20/min. All the segments of the abdomen move at approximately the same time so that the abdomen acts as a unit (Lewis *et al.* 1973). The movements of a single segment involve activity in some 13 muscles, but the action of each muscle is not known in detail. The expiratory dorso-ventrally situated muscles lift the sternites upwards and into the body cavity while the single inspiratory muscle in each segment pushes them downwards and outwards. The effect is to expand and contract the abdomen and force air through the longitudinal trachea. Both inspiratory and expiratory phases involve active muscular contraction, but the frequency of pumping movements is not constant; there may be periods of inactivity lasting several minutes, or two or more ventilatory strokes may occur rapidly followed by a longer than usual expiratory period before the normal rhythm is resumed. During more stressed ventilation longitudinal telescoping of the abdomen and pumping movements of the neck may be added (Miller 1960*a*). In addition to the pumping movements of the abdomen, the synchronized opening and closing movements of the spiracles permit air to enter or leave the trachea. Spiracles 1 and 2 of the thorax and 3 and 4 of the abdomen open during the inspiratory phase of the abdominal movements while spiracles 5–10 of the abdomen may open during expiration (Miller 1960*b*). Both opening and closing movements of all spiracles except 2 may involve active muscular contraction (Miller 1960*b*).

The metathoracic ganglion is formed by the fusion of the segmental metathoracic ganglion with the first three abdominal ganglia. All supply innervation to muscles involved in ventilation. The expiratory muscles are innervated by lateral nerves, the spiracle and inspiratory muscles by the median nerves and by some lateral nerves. The details of the innervation of the muscles is not known but the complexity may be gauged from the fact that the dorsal longitudinal muscles of the first abdominal segment receive eight motor axons (Tyrer 1971). No attempt has been made here to relate the centrally impaled motoneuron with the muscle it innervates except those innervating the spiracles. During abdominal pumping movements the expiratory motoneurons are recruited in accordance with the diameter of their axons (Hinkle & Camhi 1972). Spikes in the motoneurons with small-diameter axons begin and end an expiratory burst with some of the smaller ones continuing to spike throughout the interburst period. Motoneurons with larger-diameter axons reach a peak frequency midway through the burst while still larger fibres are recruited only during stressed ventilation when the small axons are spiking at their maximum.

The somata of the motoneurons penetrated which control abdominal pumping movements lie on the ventral surface of the ganglion, almost all posterior to the emergence of leg nerve 3, but

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others could occur on the dorsal surface. Unlike limb motoneurons, their somata are not necessarily on the same side of the ganglion as the nerve trunk which contains their axon. The diameter of the somata, as shown by injecting Procion Yellow into a soma from a microelectrode or by electrophoresing cobaltous chloride from the cut axons, varies from 10 to 30 μm. Both staining methods probably result in some swelling of the somata.

The patterns of synaptic activity seen in ventilatory motoneurons will be considered under five headings: tonic expiratory motoneurons; phasic expiratory; inspiratory; spiracular and finally, descending interneuronal inputs to these motoneurons. The categories of tonic or phasic expiratory motoneurons are arbitrary; the responses of some motoneurons cut across the boundary and particular motoneurons may participate in different ways in different movements

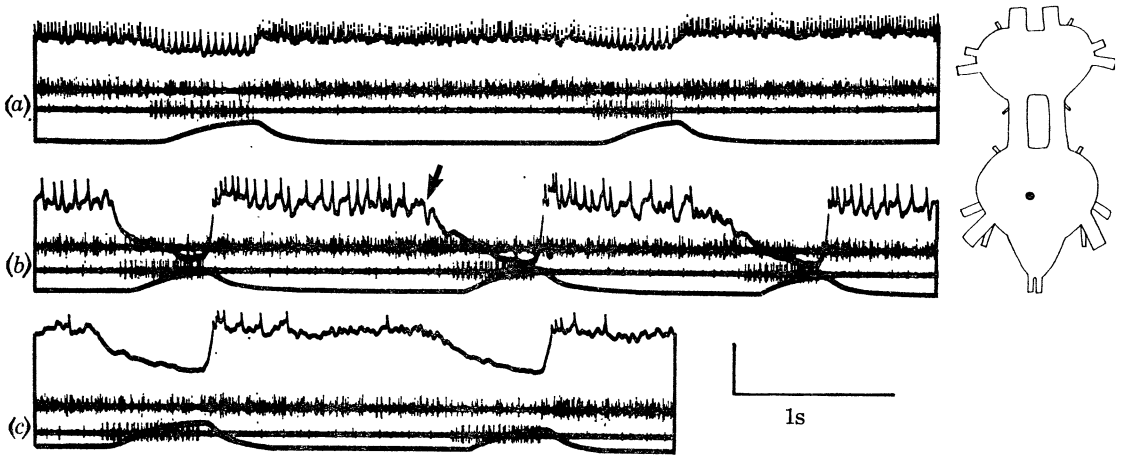

FIGURE 1. A tonic expiratory motoneuron. (*a*) During expiration the tonic frequency is maintained at 40–45 Hz but falls to 26 Hz during inspiration at a ventilatory rate of 22/min. (*b*) Increasing the ventilatory rate to 35/min and applying a d.c. hyperpolarization to the soma reveals an inhibitory synaptic input at the start of inspiration (arrow) and an excitory one during expiration. (*c*) The hyperpolarization is increased to show the excitatory synaptic input during expiration. The traces are: first, intracellular soma; second, extracellular from the lateral nerve containing the axon of this neuron though its spike is too small to be readily distinguished; third, extracellular median nerve recording; fourth, dorso-ventral abdominal movements where an upward deflexion indicates inspiration. The position of the impaled soma on the ventral surface of the metathoracic ganglion is indicated in the diagram on the right. Calibration: vertical (*a*, *b*) 10 mV, (*c*) 20 mV.

of the abdomen. The types of responses reported are the characteristic types seen during ventilatory movements. Where the same motoneuron could be identified, it behaved in the same way in different locusts. The impaled neurons were identified as motoneurons in four ways: (1) by correlating the spikes in the soma with extracellularly recorded spikes in peripheral nerve trunks; (2) by stimulating the peripheral nerves and recording an antidromic spike in the soma; (3) by passing current into the soma to evoke spikes and observing the subsequent movement of the abdomen; (4) by injecting Procion Yellow and histologically verifying that the neuron had a peripheral axon. It was rare that all four critera could be applied to one neuron, and sometimes it was necessary to assume that the impaled neuron was a motoneuron upon evidence previously accumulated for a motoneuron behaving in the same way.

Tonic expiratory motoneurons

Within the normal range of frequencies of dorso-ventral pumping movements, one category of tonic expiratory motoneurons spikes continuously (figure 1*a*). At the onset of expiration the frequency of its spikes is highest at 50–60 Hz but then falls to 40–45 Hz during the remainder

of the expiratory phase. Before the start of an inspiratory burst of spikes by the median nerve motoneurons the frequency falls by half and remains at that level throughout inspiration. The increase in frequency in the next expiratory cycle begins only when the spikes in the median nerve have ceased. To test whether the ventilatory pattern of spikes is inherent to the motoneuron or results from a rhythmic synaptic bombardment, this and subsequent motoneurons were hyperpolarized by d.c. current injected through the recording microelectrode. This is usually successful in blocking the spikes and allows examination of the synaptic inputs. Slowing the frequency of spikes of the tonic expiratory motoneuron by an applied hyperpolarization reveals that the expiratory burst is terminated by an inhibitory input (figure 1*b*). These inhibitory post-synaptic potentials (i.p.s.ps) occur only at the end of the burst and before spikes are recorded in the median nerve and do not persist throughout the entire inspiratory phase. Consequently the membrane potential initially falls quickly but then more gradually and passively in the absence of any discernible input. At the onset of expiration the membrane potential is depolarized rapidly by excitatory potentials which lead to the high frequency of spikes at the start of expiration. Throughout the expiratory phase a high frequency of excitatory potentials of differing time courses and amplitudes, and thus presumably from different antecedent interneurons, maintains the membrane potential depolarized by 10–15 mV above the level during inspiration (figure 1*c*). Blocking the spikes does not advance the onset of the next burst of spikes as would be expected if the motoneuron were its own pacemaker. The ventilatory rhythm in this motoneuron thus derives from an excitatory input during expiration, causing spikes, an inhibitory input at the end of expiration which is not maintained throughout inspiration so that the spike frequency during this phase is merely reduced.

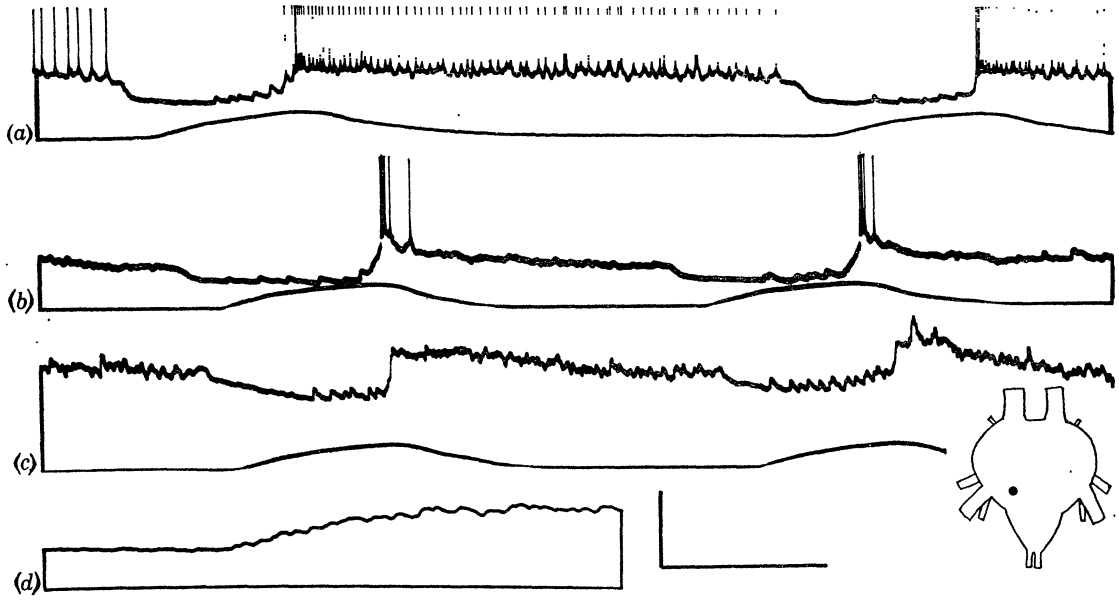

FIGURE 2. A tonic expiratory motoneuron showing a high frequency of spikes at the start of expiration (downward deflexion of the second trace) which then gradually declines. (*a*) The pattern of spike activity during ventilation at 15/min. Spikes cease before the onset of inspiration and an excitory synaptic input begins before it has ended. (*b*, *c*) Hyperpolarizing the motoneuron shows that the rhythmic spike output is caused by a rhythmic synaptic input. In (*b*) the applied hyperpolarization is insufficient to prevent the burst of e.p.s.ps causing the intial high frequency of spikes. Note the absence of synaptic input at the start of inspiration and the gradually declining frequency of input during expiration. (*d*) The initial portion of an expiratory cycle on an expanded time scale to show that the rapid depolarization is caused by a high frequency of e.p.s.ps. Calibration: vertical (*a*, *b*) 30 mV, (*c*, *d*) 15 mV; horizontal (*a*–*c*) 1 s, (*d*) 100 ms.

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A second type of tonic motoneuron again has its highest frequency of spikes (50–60 Hz) during the first 100–200 ms of the expiratory phase (figure 2*a*). Thereafter during expiration the frequency gradually falls so that at the end it may be no more than 10 Hz. Hyperpolarizing the motoneuron shows that the initial high frequency of spikes is caused by a massive and high-frequency excitatory synaptic input (figure 2*b*, *c*, *d*). Only on an expanded time scale can the rapid depolarization be resolved into identifiable excitatory post-synaptic potentials (e.p.s.ps) (figure 2*d*). Throughout expiration the membrane is depolarized by excitatory synaptic inputs, of higher frequency at the start of than at the end (figure 2*c*). The decline in frequency of synaptic input causes a fall in the level of depolarization which in turn is an adequate explanation of the fall in the frequency of the spikes. An inhibitory input does not terminate the burst which

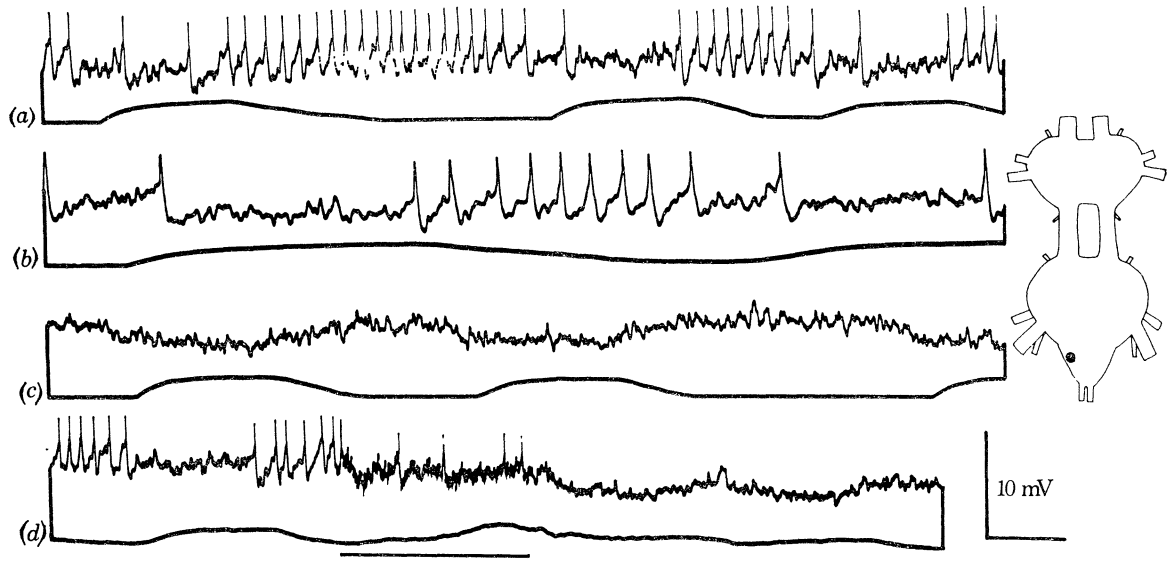

FIGURE 3. An expiratory motoneuron whose tonic activity is either interrupted or reduced in frequency during inspiration (upward movement of second trace). (*a*, *b*) The pattern of synaptic and spike activity at a ventilation rate of 20/min. (*c*) Hyperpolarizing the motoneuron so as to abolish spikes reveals a rhythmic synaptic input with excitation causing depolarization during expiration. There is an inhibitory input during inspiration. (*d*) Stimulation of the pro-mesothoracic connective ipsilateral to this motoneuron's soma at 73 Hz (horizontal bar) evokes e.p.s.ps but abolishes the ventilatory rhythm during the stimulation. Note the increased ventilatory rate upon cessation of stimulation. Calibration: horizontal (*a*, *c*, *d*) 500 ms, (*b*) 250 ms.

thus ends before the onset of inspiration merely from a cessation of the excitatory input. At ventilatory frequencies below 20/min, the first part of inspiration is marked by an absence of any synaptic input to this motoneuron. Then e.p.s.ps, perhaps from one or at most a few sources, arrive and increase in frequency before the massive depolarization at the start of the next expiratory cycle. As the frequency of ventilation increases, the e.p.s.ps occur progressively earlier in the inspiratory phase and may depolarize the motoneuron sufficiently to evoke spikes at a frequency of less than 5 Hz. The ventilatory rhythm of this motoneuron thus results solely from an excitatory synaptic input during expiration.

The frequency of spikes in a third type of tonic expiratory motoneuron gradually rises to reach a peak midway through expiration and then declines once more (figure 3). During ventilation at frequencies of less than 20/min spikes at low frequency may occur during inspiration but during faster ventilation not only does the frequency of spikes during expiration rise but the bursts may become more clearly delineated. The synaptic input underlying the rhythm in

these motoneurons is complex (figure 3*c*). Hyperpolarization reveals mixed excitatory and inhibitory inputs during both phases of the cycle; during expiration, excitation predominates so that the membrane is depolarized; during inspiration, inhibition so that the membrane is hyperpolarized. Sometimes the frequency of excitatory synaptic potentials may be highest both at the start and at the end of expiration so that two bursts of spikes are produced resulting in a frequency doubling of expiratory relative to inspiratory bursts (cf. Lewis *et al.* 1973). The ventilatory rhythm in this motoneuron results from the interplay between tonic excitatory and inhibitory inputs, the relative frequencies of the two changing during the different phases.

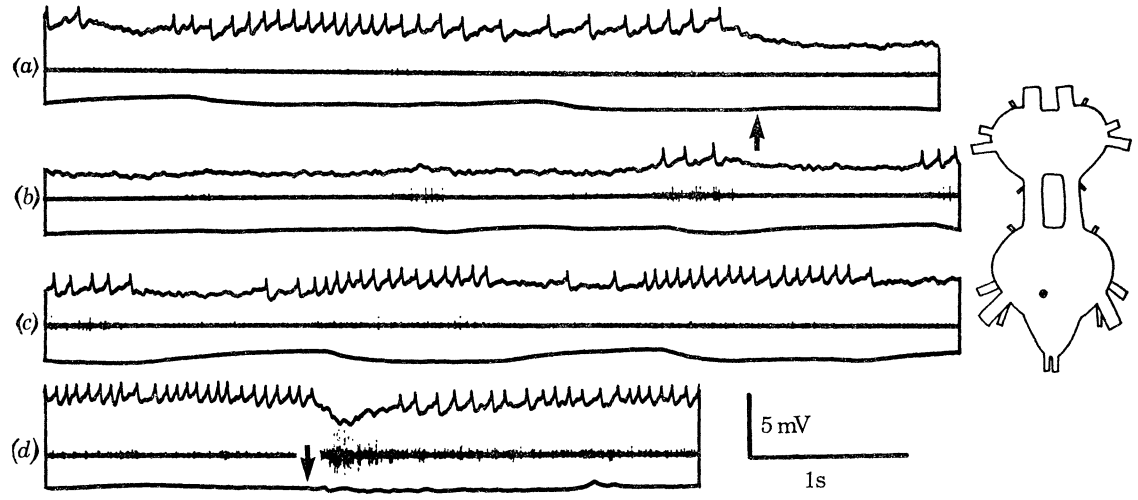

FIGURE 4. A tonic expiratory motoneuron. (*a*) At a ventilation frequency of 24/min its tonic activity is only partially interrupted during inspiration. Upon increasing the carbon dioxide concentration in the air (arrow), spikes are inhibited despite the increased ventilatory rate. (*b*) Gradually the spikes return but only a few occur at each cycle. (*c*) As the ventilatory rate returns to normal so the frequency of spikes increases. (*d*) Inhibition of spikes also results from touching the ventral surface of the abdomen (arrow). Records (*a–c*) are continuous and the traces are: first, intracellular soma recording; second, extracellular recording from a lateral nerve containing expiratory motoneurons but not the impaled one; third, the mechanical movements of the abdomen, where an upward deflexion indicates inspiration.

Not all tonic expiratory motoneurons show a progressive increase in spike frequency with increasing frequencies of ventilation (figure 4). Doubling the ventilatory rate by a large and abrupt increase in the concentration of carbon dioxide in the air initially abolishes the normal tonic spikes of some motoneurons during expiration. Gradually the spikes return but at first only 2–3 at a frequency of 4 Hz occur on each cycle instead of the usual 15–20 at 12 Hz (figure 4*b*). As the ventilatory rate subsides to 30/min, the tonic discharge of spikes reverts to its original level, but the delineation of the expiratory and inspiratory phases is now more precise, there being fewer spikes during inspiration. This could be caused by an increased sensory feedback from the increased amplitude of dorso-ventral abdominal movements at the higher ventilatory rates. That sensory feedback could play a role is shown by touching the ventral surface of the first abdominal segments which causes inhibition of the spikes (figure 4*d*).

Phasic expiratory motoneurons

Most phasic expiratory motoneurons spike only during stressed ventilation (figure 5) or at most give one to three spikes on each cycle (figure 7, 8). Those which do not spike at ventilation rates of 10–15/min received a complex synaptic input derived from many sources which is modulated in phase with the ventilatory rhythm (figures 5*b*, 6*a*). These motoneurons are

therefore always coupled to the ventilatory rhythm although not contributing spikes. Often the relative contribution of inhibition and excitation during the two phases cannot be assessed (figure 5*b*), but at increased ventilatory rates excitation becomes exclusive to the expiratory phase while inhibition occurs during both, being at a higher frequency during inspiration (figure 5*c*). The i.p.s.ps during inspiration appear to derive from only a few presynaptic neurons and occur at frequencies of 40–50 Hz. At still higher ventilatory frequencies the excitatory synaptic input depolarizes the soma by some 5 mV and 2–4 spikes are evoked on each cycle (figure 5*d*). The ventilatory rhythm of this motoneuron is thus seen to be caused by an interplay of excitatory and inhibitory inputs. At low ventilatory rates the excitatory input during expiration is too weak or the inhibition too strong to cause spikes. Only at high ventilatory rates is the excitatory input able to exceed the motoneuron's threshold and cause spikes. At all frequencies of ventilation there is an underlying rhythmic synaptic input and only at the higher ventilatory frequencies do these inputs increase sufficiently in frequency to evoke spikes.

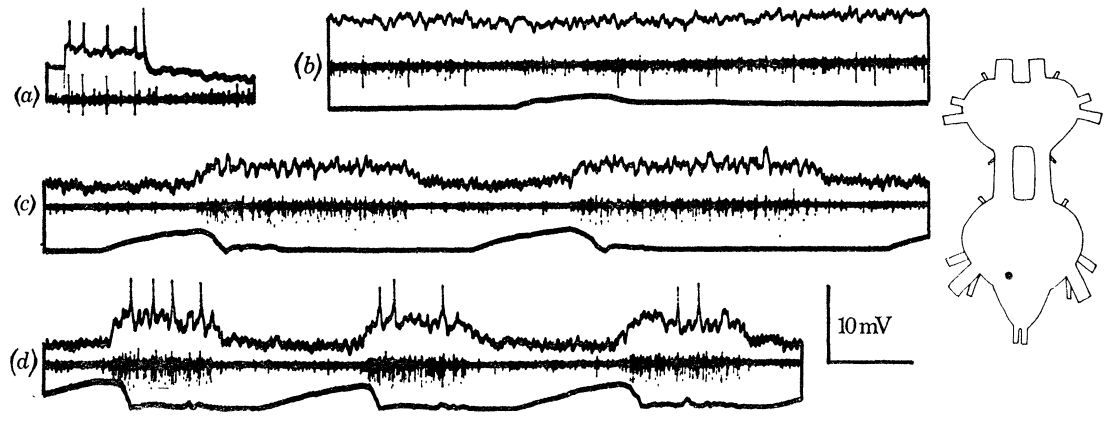

FIGURE 5. A phasic expiratory motoneuron which spikes only during stressed ventilation. (*a*) Depolarizing current injected into the soma evokes spikes (first trace) which can be monitored extracellularly in a lateral nerve (second trace) only in this record. (*b*) During normal ventilation at 12/min the motoneuron receives mixed excitatory and inhibitory synaptic inputs (first trace). (*c*) Increasing the ventilatory rate to 24/min by increasing the carbon dioxide concentration in the air shows a clear inhibitory input during inspiration and excitation during expiration. (*d*) At the still higher ventilatory rate of 40/min, 2–4 spikes may be evoked at each expiration. The third trace monitors the dorso-ventral movements of the first abdominal segment, with an upward deflexion indicating inspiration. Calibration: horizontal (*a*) 360 ms, (*b–d*) 500 ms.

In some motoneurons which do not spike during slow ventilation it is not that the rhythmic synaptic input is insufficient, but that either the threshold of the motoneuron is too high or its general level of excitability is too low (figure 6). The addition of an unpatterned input, such as is provided by an applied d.c. depolarization, is sufficient now to evoke a rhythmic spike discharge in the appropriate expiratory phase of ventilation (figure 6*b*). Raising the level of carbon dioxide in the air now has only a small effect on the rhythmic synaptic input to the motoneuron and on the frequency of spikes within a burst, but increases the ventilatory rate (figure 6*c*). Other phasic expiratory motoneurons which spike only sporadically on each cycle can be induced to produce bursts of spikes if the ventilatory rate is increased and if the general level of excitation of the motoneuron also is raised (figure 7). Depolarizing the soma by some 5 mV is ineffective in evoking spikes, although it does accentuate the underlying ventilatory rhythm of synaptic inputs (figure 7*b*). Increasing the ventilatory rate by raising the carbon dioxide concentration of the air is also ineffective (figure 7*c*). When the two stimuli, d.c.

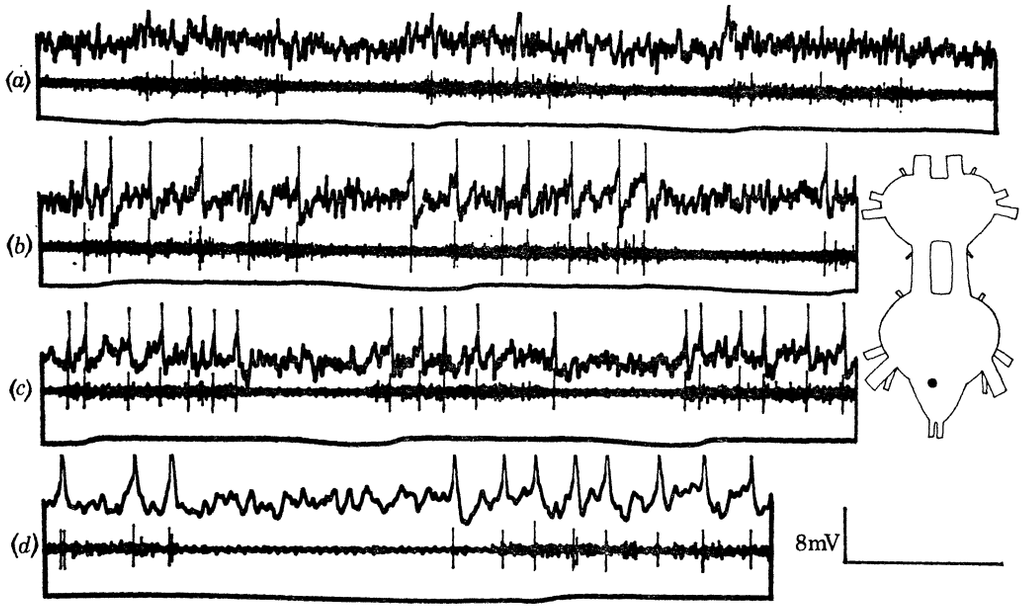

FIGURE 6. A phasic expiratory motoneuron. (*a*) During ventilation at 30/min there is an underlying rhythmic synaptic input but the excitation during expiration is insufficient to evoke spikes. (*b*) Applying d.c. current so as to depolarize the soma by 5 mV now causes rhythmical bursts of spikes. (*c*) Increasing the concentration of carbon dioxide while maintaining the applied depolarization causes a slight increase in ventilatory frequency. (*d*) The synaptic input underlying spike initiation shown on a faster time base. The first trace shows the intracellular activity recorded in the soma, the second the extracellular spike recorded from a lateral nerve, and the third trace the dorso-ventral movements of the abdomen where expiration is upwards. Calibration: horizontal (*a–c*) 1 s, (*d*) 400 ms.

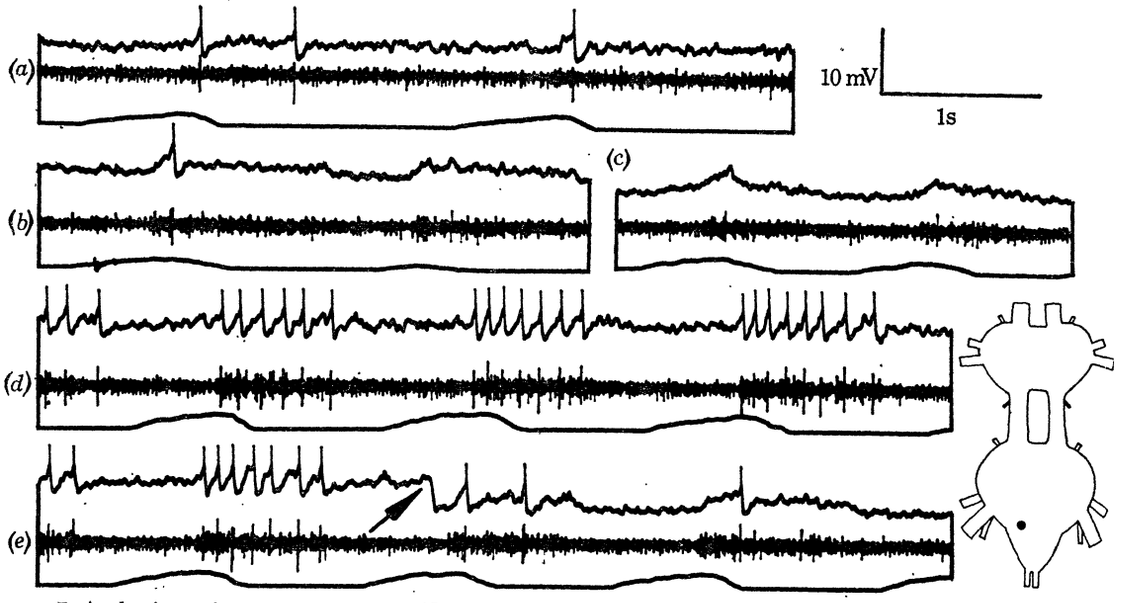

FIGURE 7. A phasic expiratory motoneuron (first trace) showing an underlying rhythmic synaptic input. (*a*) At a ventilatory rate of 24/min one or two spikes (monitored extracellularly from a lateral nerve on the second trace) occur at each expiration (downward deflexion of the third trace). (*b*) Lowering the membrane potential of the soma by 5 mV by applying d.c. current accentuates the rhythmic synaptic input but is ineffective in influencing the frequency of spikes. (*c*) Increasing the ventilatory rate to 40/min by the addition of more carbon dioxide to the air also fails to produced rhythmic bursts of spikes. (*d*) Depolarizing the soma by 5 mV and increasing the concentration of carbon dioxide now causes rhythmic bursts of spikes, with the first spike slightly shifted so that it occurs earlier in the cycle. (*e*) On removing the applied depolarization (arrow) the spike frequency is reduced to the quiescent level.

depolarization and increased CO_2 concentration, are combined, bursts of 6–10 spikes at a frequency of 10 Hz are produced (figure 7*d*). The first spikes of the burst now occur at an earlier phase in the expiratory cycle than in a quiescent locust. That this slight phase shift has resulted from the non-patterned applied depolarization is evident when the depolarization is removed (figure 7*e*); the first spike of the reduced burst now occurs later.

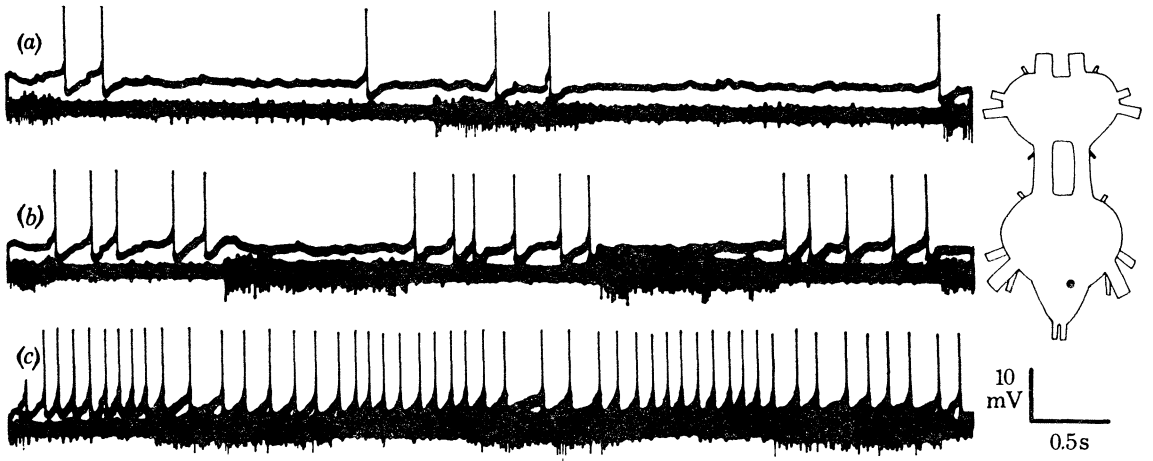

FIGURE 8. A phasic expiratory motoneuron. (*a*) At a ventilatory rate of 18/min it spikes only a few times on each cycle, often coincident with inspiratory bursts by the median nerve motoneurons (second trace). (*b*) At higher ventilatory rates (30/min), spikes occur only during expiration so that a precise ventilatory rhythm is established. (*c*) At a ventilatory rate of 36/min, the frequency of spikes during expiration is increased and spikes may now occur during inspiration but at a lower frequency.

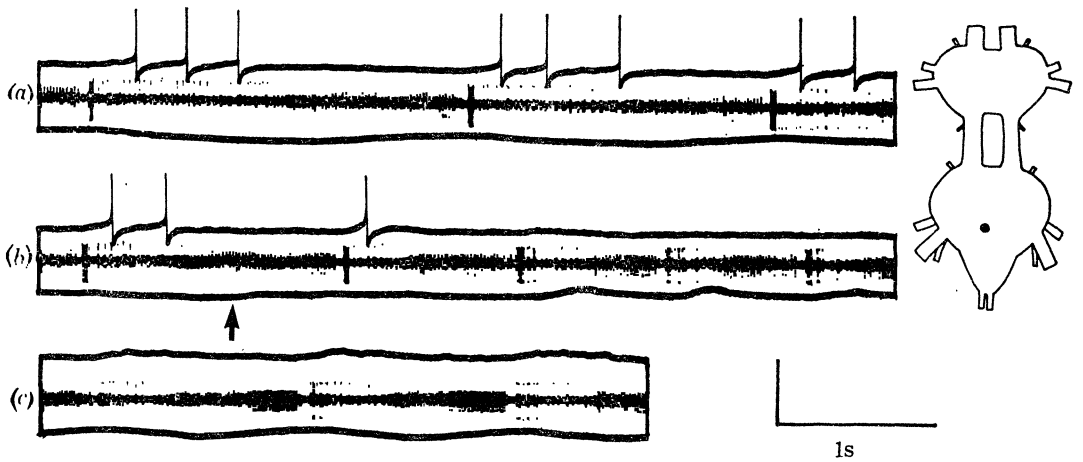

FIGURE 9. A phasic expiratory motoneuron whose spike frequency decreases at high ventilatory rates. (*a*) During ventilation at 25/min this motoneuron gives 3–4 spikes at each expiration (downward deflexion of the third trace). (*b*) When the ventilatory rate is increased by adding more carbon dioxide to the air (arrow) spikes stop. (*c*) A continuation of (*b*). Depolarizing synaptic inputs still occur during expiration but are ineffective in evoking spikes. Calibration: vertical (*a*, *b*) 10 mV, (*c*) 5 mV.

Increasing the ventilatory rate may have different effects on other types of phasic ventilatory motoneurons. One type of motoneuron produces spikes sporadically, at low ventilatory rates, not necessarily linked to expiration; often the spikes may be coincident with inspiration (figure 8*a*). At higher ventilatory rates a precise rhythm is established with the spikes restricted to expiration (figure 8*b*). The synaptic input is of a low amplitude with mixed excitation and

inhibition occurring throughout the cycle. Blocking the spikes with hyperpolarizing current reveals a rhythm linked to ventilation, underlying the synaptic input. Stimulating the median nerve at 100 Hz has no effect on this synaptic activity. At still higher ventilatory rates, the frequency of spikes increases during expiration and spikes spill over into the inspiratory phase (figure 8*c*).

Instead of their spikes becoming coupled to the rhythm at high frequencies of ventilation, those of some motoneurons may become uncoupled (figure 9). At low ventilatory rates 1–3 spikes occur on each expiration, but as the rate increases the spikes fall out (figure 9*b*). The implication is that the form of abdominal movements changes so that the force provided by the motoneuron is no longer required. This result is important for it shows that the recruitment of motoneurons is not merely due to a progressive and non-specific irradiation of excitation from a hypothetical ventilatory pacemaker. Such non-specific irradiation has been implicated to explain the occasional discharge of motoneurons other than ventilatory ones in time with the ventilatory rhythm which produces movements apparently trivial for ventilation (Bentley 1969; Miller 1971).

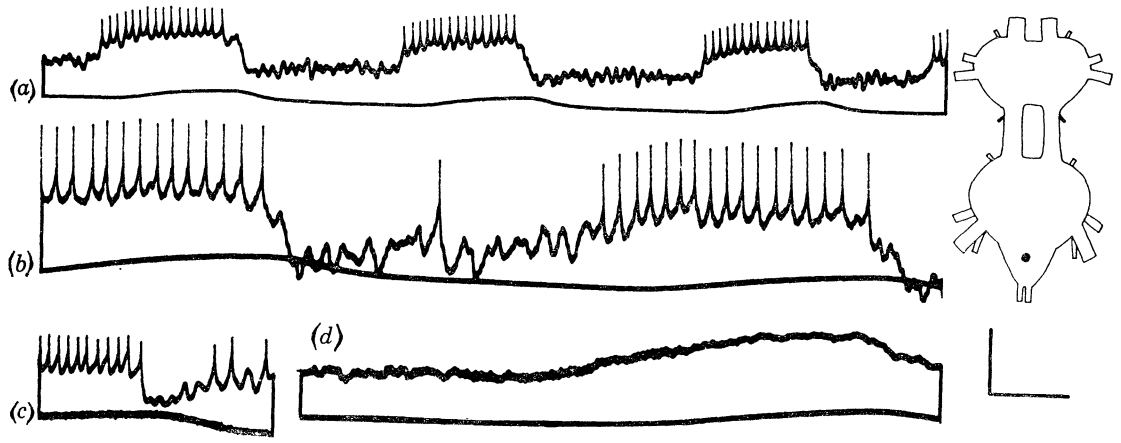

FIGURE 10. An inspiratory motoneuron. (*a*, *b*) Bursts of spikes at 20–22 Hz occur during inspiration (indicated by an upward deflexion of the second trace) and inhibitory synaptic potentials during expiration. (*c*) Active inhibition is responsible for the termination of the burst as shown by depolarizing the neuron with applied d.c. current to cause continuous spike activity; the i.p.s.ps are clearly seen to interrupt the spikes at the end of inspiration. (*d*) That a rhythmic excitory synaptic input underlies the inspiratory burst of spikes is revealed when the spikes are abolished by hyperpolarizing the neuron. Calibration: vertical (*a*, *c*, *d*) 10 mV, (*b*) 4 mV; horizontal (*a*, *c*) 500 ms, (*b*, *d*) 200 ms.

Inspiratory motoneurons

The spike discharge of motoneurons of either the third or fourth median nerves of the metathoracic ganglion is characterized by a steady frequency during inspiration (figures 10, 11). In some locusts the frequency may be 20 Hz at a ventilatory rate of 60/min (figure 10*a*), while in others it may approach 40 Hz though the ventilatory rate may be half (figure 11*a*). The inspiratory burst of spikes is terminated by an inhibitory input, most clearly seen to interrupt the tonic spikes of a motoneuron depolarized by applied d.c. (figure 10*c*) or of a motoneuron showing spontaneous tonic activity during expiration (figures 11*a*, *b*). The inhibition persists during expiration and may (figure 10*a*) or may not (figure 11*a*) be sufficient to suppress spikes. Where ineffective the frequency of spikes is nevertheless a third of that during inspiration. Often the spikes during expiration are inhibited before the onset of inspiration (figures 11*a*, *b*).

The inhibitory potentials occur at a higher frequency than at other times during expiration and cause the membrane potential to be raised by some 5 mV (figures 11*a*, *c*). When two inspirations occur close together (figure 11*b*) the repolarization at the end of the first raises the membrane by 5 mV more than usual and this is maintained throughout the brief expiratory period. The rebound from the inhibition and a high frequency of excitatory synaptic potentials ensures that the depolarization at the start of inspiration is rapid (figure 10*d*). Unlike the spiracle motoneurons, however, the depolarization is not of sufficient amplitude to cause an initial higher frequency of spikes. The excitatory input continues through inspiration maintaining the membrane potential at the soma steadily 10 mV below that during expiration and hence ensuring a steady frequency of spikes. The ventilatory rhythm in these motoneurons thus derives from mixed excitatory and inhibitory inputs during expiration, the balance between the two determining whether there will be a low frequency of spikes. At the end of expiration the inhibitory input increases before the excitatory input at the start of inspiration, which is then maintained during inspiration giving a tonic spike discharge terminated by inhibition.

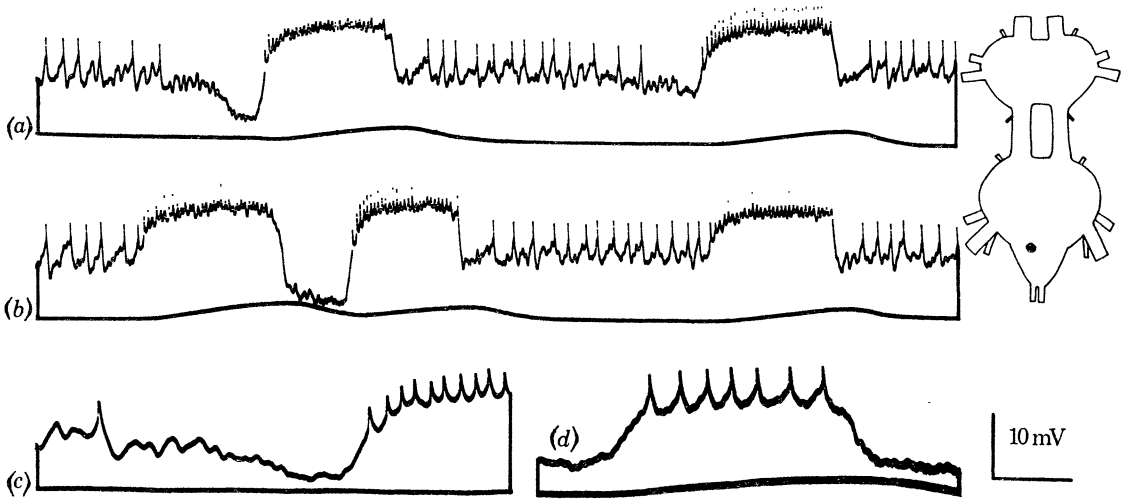

FIGURE 11. An inspiratory motoneuron. Bursts of spikes at 32–36 Hz occur during inspiration (upward deflexion of the second trace) and tonic spikes at 10 Hz during expiration. (*a*) The tonic expiratory activity is inhibited by i.p.s.ps prior to the onset of an inspiratory burst (shown on an expanded time scale in (*c*)). Note the rapid repolarization at the end of inspiration. (*b*) Sometimes two inspiratory strokes occur close together, and the inhibition at the end of the first burst drives the membrane potential some 8 mV below normal. (*d*) Applied hyperpolarization was incapable of suppressing all the spikes but reveals an excitatory synaptic input underlying the production of the burst and an inhibitory input terminating it. Calibration: horizontal, (*a*, *b*) 500 ms, (*c*) 125 ms, (*d*) 250 ms.

Recording from the median nerve motoneurons of the first unfused abdominal ganglion offers the advantage of more readily being able to correlate the spikes in the peripheral nerve with those of the soma. This is because there is one and not four median nerves with which to contend. The largest amplitude spikes recorded extracellularly in the median nerve are from the two motoneurons supplying the dorso-ventral inspiratory muscles (Lewis *et al.* 1973). Recordings from the soma of either of these two motoneurons show bursts of spikes during inspiration of a similar pattern to those of the median nerve inspiratory neurons in the metathoracic ganglia (figure 12*a*). In addition it can be seen that the excitatory synaptic input during inspiration persists after spikes in both the impaled and the other motoneuron have ceased (figure 12*b*). At other times spikes in the impaled neuron may stop towards the end of

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the burst but there is a maintained excitatory synaptic input which is correlated with the spikes in the other neuron (figure 12*c*, *d*). When there is a pause in the spikes of the other neuron, the membrane of the impaled neuron is hyperpolarized but is depolarized again before the occurrence of the next spike (figure 12*c*). This does not imply direct excitatory coupling between the two neurons; there is no apparent potential in one neuron linked to a spike in the other but it does imply similarity in their presynaptic inputs. This can be determined only by simultaneous recordings from the somata of both, which has as yet proved unsuccessful. Pacemaker activity has been attributed to the median nerve inspiratory motoneurons based on the ability to reset the rhythm experimentally with an antidromic spike (Miller 1967; Lewis *et al.* 1973). Blockage of the spikes or an acceleration of the frequency brought about by a hyperpolarization or depolarization at the soma does not affect the frequency of spikes which follow upon cessation of the applied current. A change would be expected if the motoneurons were themselves the pacemakers for their spike discharge.

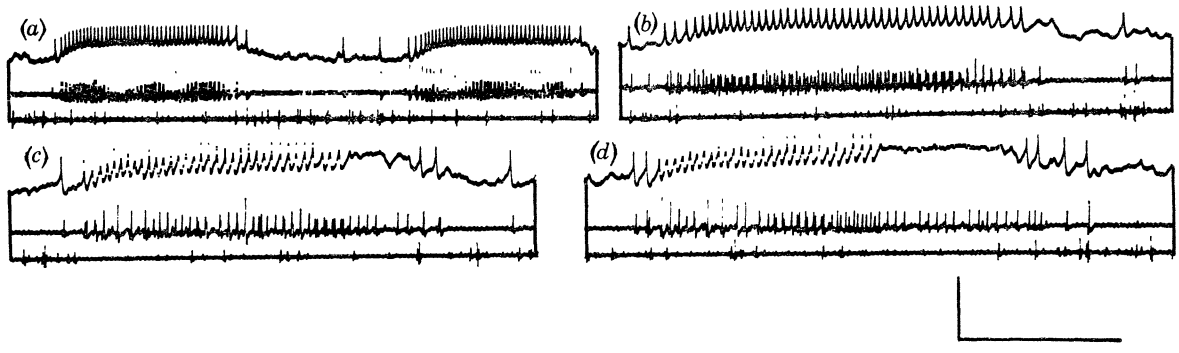

FIGURE 12. A median nerve motoneuron from the first unfused abdominal ganglion. The traces are, first intracellular soma recording, second extracellular from the median nerve in which spikes of a second motoneuron can be seen, third extracellular from lateral nerve 1. (*a*) The pattern of spike and synaptic activity during ventilation at 40/min. (*b*) An inspiratory burst in which the excitatory synaptic input outlasts the spike output of both motoneurons. (*c*, *d*) Towards the end of the burst the impaled motoneuron fails to spike, but the second motoneuron continues to receive an excitatory input. Calibration: vertical (*a*, *b*) 15 mV, (*c*, *d*) 10 mV; horizontal (*a*) 500 ms, (*b–d*) 250 ms.

Spiracular motoneurons

Both the opener and closer muscles of spiracles 3 and 4 are innervated by the median nerves from the metathoracic ganglion. The closer motoneurons have somata with a diameter 25–30 μm compared with 10–15 μm of the opener motoneurons, and produce a spike of larger amplitude in extracellular recordings from the median nerves. Recordings have been made only from the closer motoneurons, with those from median nerves 3 and 4 having similar patterns of synaptic inputs and spike outputs (figure 13). Expiration begins with a high frequency of spikes at 100 Hz which then gradually declines to 20 Hz at the end (figure 13*a*). Throughout expiration the spikes tend to occur in groups of two or three at frequencies of 100 Hz with each group separated from the next by 40–50 ms. At low ventilatory frequencies, spikes may occur sporadically during inspiration, but at higher rates are limited solely to expiration. There are two patterns of spikes to be explained: the basic ventilatory pattern of inspiratory and expiratory phases and the derivation of the frequency code during expiration. The motoneurons were therefore hyperpolarized to block the spikes and reveal the synaptic inputs, which appear the same in both motoneurons (figure 13*b*, *f*), consisting largely of excitation during expiration (figure 13*b–e*). During inspiration the synaptic input is of low amplitude and its contribution

to the absence of spikes is not obvious. The excitatory input during expiration is an adequate explanation of both the ventilatory rhythm and the frequency code within the burst. At the start of expiration the depolarization is rapid, perhaps aided by rebound from inhibition, and leads to a high frequency of spikes. Thereafter the amplitude of the depolarization falls causing a gradual fall in the frequency of spikes. The expiratory phase ends because of a lack of excitatory inputs and not because of an inhibitory input. This is clearly seen in figure 13*f*, where at the end of the excitatory phase the membrane potential falls only gradually until the onset of the next expiration.

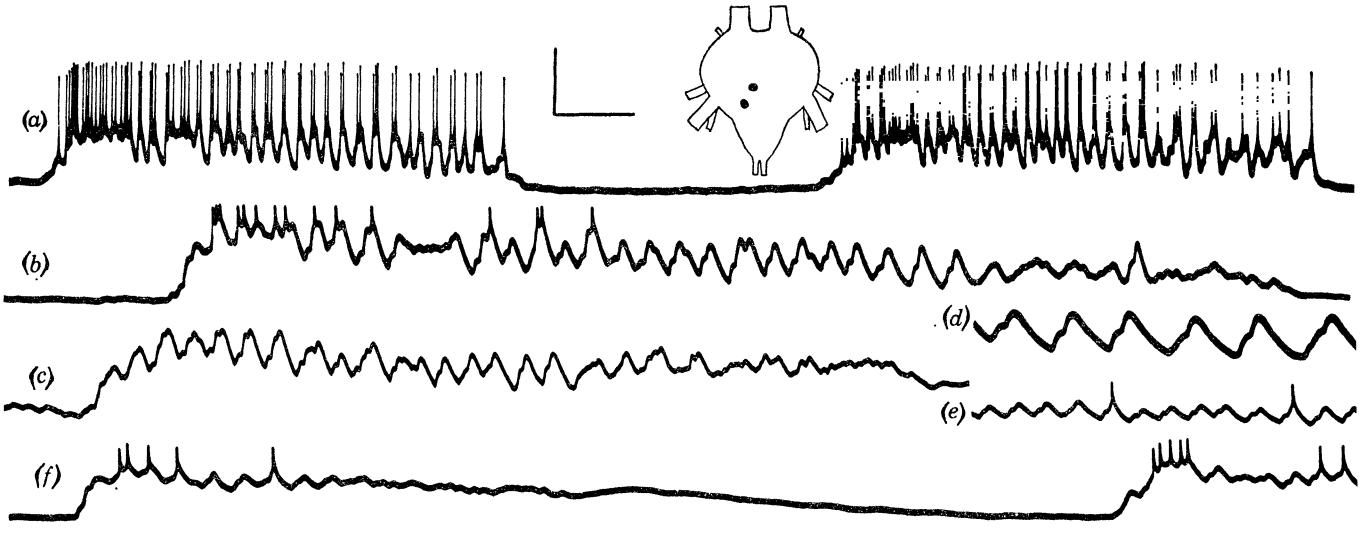

FIGURE 13. Motoneurons to the spiracle closer muscles. Records (*a–d*) are from the motoneuron with the more anterior soma (see inset), (*e*, *f*) from the more posterior and in a different locust. (*a*) The pattern of spike and synaptic activity at a ventilatory rate of 20/min. Note the characteristic high frequency of spikes at the start of the burst, the subsequent declining frequency and the tendency for the spikes to occur in groups of two or three. There is only a low level of synaptic inputs during the interburst period. (*b*, *c*, *f*) The neuron is held hyperpolarized by applied d.c. current. In (*b*, *f*) the hyperpolarization is insufficient to suppress the first few spikes but in (*c*) all spikes are abolished to reveal a patterned excitatory synaptic input. The burst is terminated by a lack of an exitatory input and not by inhibition. (*d*, *e*) A comparison of the synaptic input of the two motoneurons to show their similarity. Calibration: vertical 10 mV; horizontal (*a*) 250 ms, (*b*, *c*, *e*, *f*) 125 ms, (*d*) 63 ms.

The synaptic input is itself patterned and causes the patterned spike output. It consists of groups of two and sometimes three e.p.s.ps which summate and, if the applied hyperpolarization is insufficient, cause a group of spikes (figure 13*b*). In the middle of the burst the interval between the groups of e.p.s.ps is 50 ms (figure 13*d*, *e*) but towards the end the e.p.s.ps become less precisely grouped and the pattern disappears. This also happens at high ventilatory rates so that at both times the spike output is more regular. The changing phase relationships of the e.p.s.ps suggests that they are derived from at least two presynaptic interneurons. At the start of expiration the synaptic input differs only from that which follows in that the total depolarization is larger causing a higher frequency of spikes. Because the pattern of synaptic input is the same at both times there would seem no justification in subdividing the whole burst into two distinct types of burst (type 1, the initial high frequency; and type 2*b*, the patterned spikes which follow, as was done by Miller (1967) for the closer motoneurons to spiracles 1 and 2). The two differ in degree and not in cause. The similarity between the synaptic inputs to the motoneurons of the third and fourth median nerves of different locusts (figure 13*d*, *e*) is remark-

able and suggests that both may be driven by the same presynaptic interneurons. A similar inference was made by Miller (1967) for the closer motoneurons to spiracles 1 and 2 which may show close coupling of their spikes. The synaptic input to either of the motoneurons impaled here is not changed by isolating the metathoracic ganglion from the head ganglia by cutting the pro-mesothoracic connectives, or by isolating it from the abdominal ganglia.

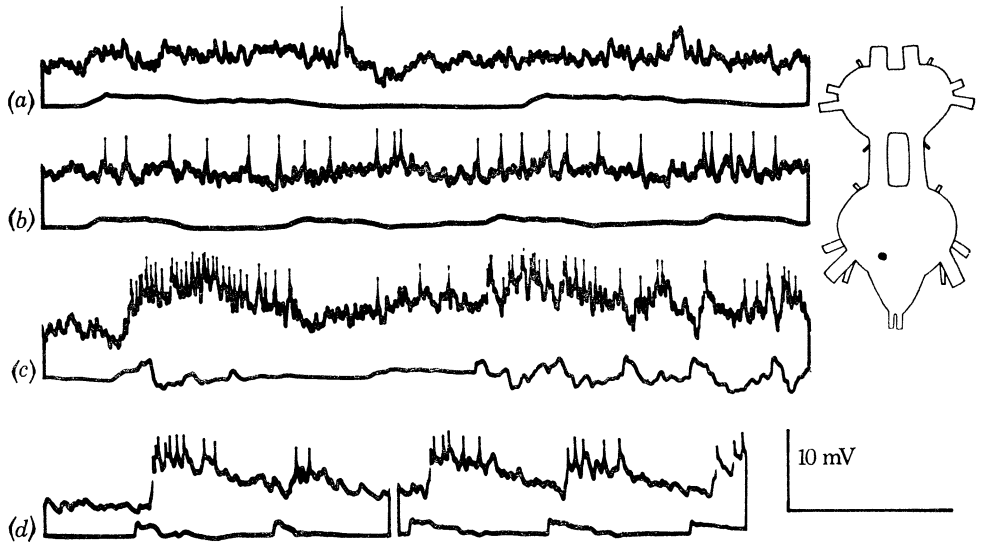

FIGURE 14. A neuron thought to be responsible for the integration of sensory input from the abdomen. (*a*) During ventilation at 24/min it receives a mixed synaptic input and spikes sporadically. (*b*) During more rapid ventilation, the spike frequency increases, typically being highest at the end of inspiration (upward deflexion of second trace). (*c*) During vigorous non-ventilatory movements of the abdomen the neuron is most active. (*d*) Touching the ventral surface of the abdomen evokes spikes. Calibration: horizontal (*a–c*) 1 s, (*d*) 400 ms.

Sensory integrating interneurons

Occasionally neurons were penetrated which failed to produce any noticeable motor effect and staining with Procion Yellow failed to show any peripheral axon. They were thus thought to be interneurons, but as the criteria on which such an assumption is made are negative ones, they must be treated with circumspection; the neurons might well have a weak motor effect, or one which is difficult to detect, and small diameter axons which staining fails to reveal. One of the supposed interneurons most commonly encountered receives a continuous high frequency of mixed synaptic inputs during low rates of ventilation from which spikes arise as the ventilation becomes more stressed (figure 14*a*, *b*). The peak frequency of spikes occurs at the maximum dorso-ventral distension of the abdomen during inspiration, but higher frequencies of spikes occur during vigorous spontaneous and non-ventilatory movements (figure 14*c*). Lightly touching the ventral surface of the abdomen also causes excitation (figure 14*d*). Air movements, either caused by loud sounds or directly by an air stream and detected by the abdomen, cause excitation.

Descending interneuron inputs to motoneurons

Removal of the brain causes a decline in the ventilation frequency, one reason being that low threshold carbon dioxide receptors believed to be in that region have been removed (Miller 1960*a*). Stimulation of regions within the brain can lead to an increase or decrease in the ventilatory rate (Huber 1960). To test if the interneurons which convey this information to the

thorax influence the motoneurons directly, intracellular recordings were made from the motoneurons while stimulating a whole pro-mesothoracic connective. Such stimulation activates many fibres so that the effect produced may be general rather than specific. E.p.s.ps occur in some tonic expiratory motoneurons as a result of connective stimulation (figure 15*d*). These are often compound and follow the stimuli 1:1 at 50 Hz, but at higher frequencies may drop out, indicating that the connexion is likely to be monosynaptic. Stimulation at 50 Hz may abolish spikes in these motoneurons, yet still cause the production of e.p.s.ps (figure 3*d*), indicating that the source of the excitation which leads to the rhythm of ventilatory spikes has been inhibited. In other motoneurons the tonic frequency of spikes may be increased by stimulation (figure 15); if maintained the ventilatory rhythm is abolished, but after a few seconds of persistent stimulation begins to reassert itself as shown by the rhythmic modulation of the spikes. Upon cessation of stimulation the locust hyperventilates (figure 15*b*, *c*) and the excitation normally confined to the expiratory phase spills over into inspiration.

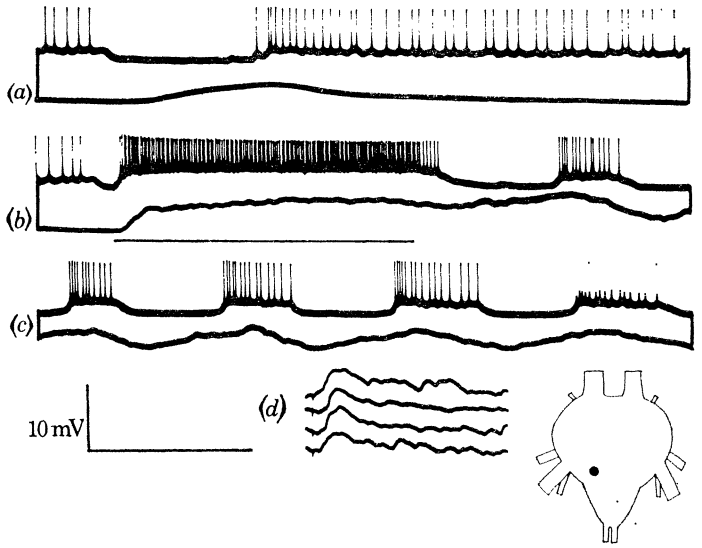

FIGURE 15. Descending interneuronal inputs to a tonic expiratory motoneuron. (*a*) Normal ventilatory activity (inspiration is indicated by an upward deflexion of the second trace). (*b*) The pro-mesothoracic connective ipsilateral to the impaled soma is stimulated at 50 Hz (horizontal bar) causing high frequency spike activity and a cessation of the ventilatory rhythm. (*c*) When the stimulation stops ventilation resumes at a frequency four times higher than before. Records (*a*–*c*) are continuous. (*d*) Multiple sweeps showing that single shocks to the connective evoke e.p.s.ps in this motoneuron, which is the same one as shown in figure 2. Calibration: horizontal (*a*–*c*) 1 s, (*d*) 130 ms.

Connective stimulation also evokes e.p.s.ps in inspiratory motoneurons, which follow 1:1 at frequencies of 100 Hz, suggesting that monosynaptic pathways are activated (figure 16*a*, *b*, *c*). The stimuli are effective in producing e.p.s.ps no matter at what phase of the ventilatory cycle they arrive. If a brief burst of stimuli at 50 Hz is delivered during the expiratory phase, when the motoneurons are tonically active at 10 Hz, there is little change in the frequency of spikes (figure 16*d*). There may be a fall in the membrane potential, however, despite the occurrence of the e.p.s.p after each stimulus. This indicates two effects of the stimulation, the second being an inhibition of an excitatory input normally present during expiration. If the burst of stimuli are now shifted to occur at the end of inspiration, then the inspiratory burst of spikes is rapidly terminated, though again e.p.s.ps occur in the motoneuron (figure 16*e*). Moving the burst of

stimuli to occur earlier terminates the inspiratory burst progressively earlier. If, however, the burst is terminated after only a few spikes, then a second full inspiratory burst follows after a shorter than usual interval with subsequent bursts at usual intervals (figure 16*f*). The ventilatory rhythm has thus been reset.

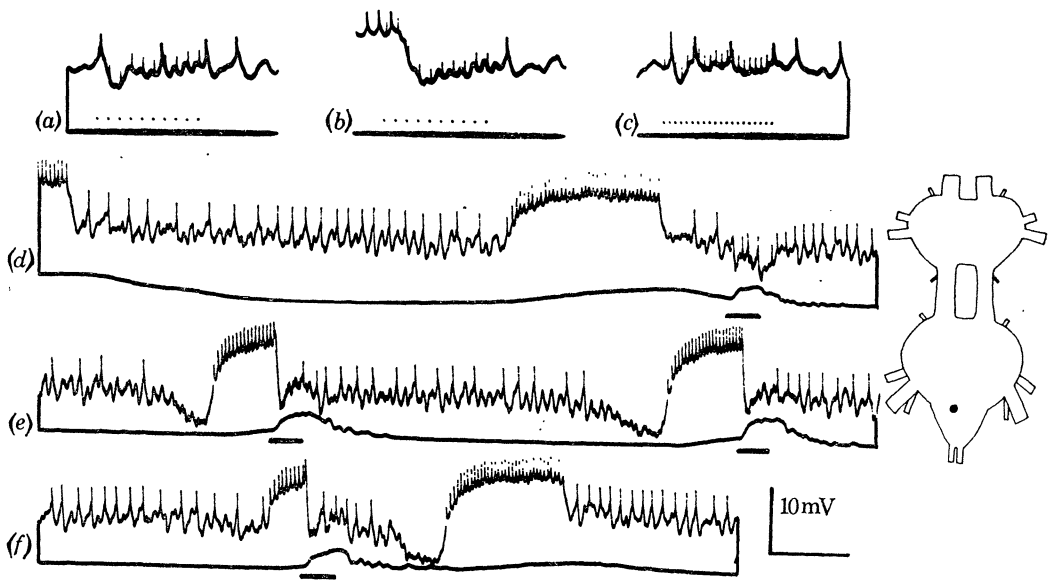

FIGURE 16. Descending interneuronal inputs to an inspiratory motoneuron. (*a*, *b*) Stimulating the pro-mesothoracic connective ipsilateral to the soma of the impaled neuron at 50 Hz evokes e.p.s.ps at any phase of the cycle. (*c*) The e.p.s.ps follow 1:1 at a stimulation frequency of 100 Hz. (*d–f*) Brief bursts of stimuli at 50 Hz are given at various times during the ventilatory cycle (horizontal bars). (*d*) When the stimuli occur during expiration (downward deflexion of second trace) they are without effect on the rhythm, merely evoking e.p.s.ps. (*e*) When they occur during inspiration, the burst of spikes is rapidly terminated. (*f*) When the burst is terminated after only a few spikes, the ventilatory rhythm is reset and the onset of the next inspiratory cycle is advanced. This motoneuron is the same one as shown in figure 11. Calibration: horizontal (*a–c*) 180 ms, (*d–f*) 500 ms.

DISCUSSION

Origin of the ventilatory rhythm

The evidence that the motoneurons receive synaptic inputs which impose the ventilatory rhythm upon them is that depolarizing or hyperpolarizing current injected into a motoneuron does not alter the ventilatory rhythm itself or have an effect on the pattern of spikes in a burst upon cessation of the stimulus. While this finding may not conclusively rule out motoneuron autorhythmicity there seems no need to invoke it in an explanation of the ventilatory rhythm. Closer motoneurons of spiracles 1 and 2, however, have been shown experimentally to produce a rhythm of their own (Miller 1967). When isolated from the metathoracic ganglia these closer motoneurons, with somata in the pro- or mesothoracic ganglia respectively, spike tonically. An antidromic spike is capable of resetting the rhythm of the tonic spikes. When the anterior ganglia are connected to the metathoracic ganglion, the spikes occur in bursts at the ventilatory rhythm and antidromic spikes are unable now to reset the pattern of spikes within a burst. In median nerve motoneurons of the abdominal ganglia which produce ventilatory bursts even when isolated from the metathoracic ganglion the situation is apparently different; antidromic stimulation is reported to 'reset the phase of spikes in the train' (Lewis *et al.* 1973). These types of experiments give information only about the derivation of the frequency code within a burst

and not about the formation of the burst itself. Moreover resetting of the rhythm of a sequence of spikes by an antidromic spike does not distinguish between an autoactive neuron and one whose membrane potential is maintained at or close to spike threshold by a tonic synaptic input, but merely between an autoactive neuron and one driven by a patterned synaptic input. If the antidromic spike fails to invade all regions of the neuron, as is known to happen in other systems (Mulloney & Selverston 1972), this experiment fails to distinguish between all three possibilities. Thus the difference between the discharge of the median nerve motoneurons in isolated, as opposed to ganglia connected with the metathoracic ganglion, may be that they now receive a tonic instead of a patterned synaptic input.

In a proposed model for the production of ventilatory bursts, Lewis *et al.* (1973) envisage a pacemaker whose discharge phase inhibits two tonically active interneurons. These spike during expiration exciting the expiratory neurons and inhibiting the inspiratory motoneurons. Release from this inhibition would be sufficient to allow the inspiratory motoneurons to express their innate rhythmicity. Intracellular recordings from the motoneurons suggest that the motoneurons are not themselves pacemakers and disagree with other aspects of the proposed model. The inspiratory bursts are terminated by inhibition and in some inspiratory motoneurons the inhibition may be maintained throughout expiration. Often, however, there is an excitatory input at this time so that the motoneurons fire tonically but at a lower frequency than during inspiration. Excitation during inspiration is essential for the production of spikes. In the spiracle closer motoneurons studied here the excitatory synaptic input is patterned and provides an adequate explanation of the patterned spike output so that motoneuron autorhythmicity need not be invoked. The model is thus an oversimplification. Observations on the pattern of motor or sensory impulses are many stages removed from the mechanisms which actually produce those patterns. Hence inferences about the mechanism so derived will provide many models which are plausible at that level of analysis. Central recordings are as yet too few to be the basis of a new generation of models which will replace those based on peripheral recordings.

The synaptic input to the motoneurons appears to be the important factor determining the ventilatory rhythm and in some instances the frequency code of the spikes within a burst. What is the source of the rhythmic synaptic inputs? The first possibility is that the rhythm derives from reciprocal coupling among synergists. To show unequivocally that there are direct pathways between motoneurons requires simultaneous recordings from pairs. Strong reciprocal coupling is not implicated; for example, there is considerable variability between the onset of spikes in inspiratory motoneurons and inhibitory inputs to the antagonistic expiratory motoneurons, the latter often occurring before the former. Similarly the inhibitory input to the inspiratory motoneurons at the end of inspiration may begin before spikes occur in the smallest expiratory motoneurons (smallness being judged from spike height in extracellular nerve records (cf. Hinkle & Camhi 1972)). Extracellularly recorded bursts of expiratory and inspiratory spikes may overlap but usually do not (Lewis *et al.* 1973) but this apparent evidence for lack of reciprocal coupling between the motoneurons themselves could result from slowly developing inhibition of the antagonist at the start of the agonist's burst. In an isolated abdominal ganglion stimulation of the median nerve may cause a 'temporary' inhibition of expiratory motoneuron spikes (Lewis *et al.* 1973). Where tested such stimulation produced no direct synaptic effect on impaled motoneurons. The lack of an observed synaptic input to spiracle closer motoneurons during opening means that the rhythm of spiracle movements does not derive from reciprocal coupling of motoneurons. If there is direct coupling between the two spiracle motoneurons,

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abolition of the spikes in one should decrease the synaptic input to the other and hence that motoneuron's spike frequency. Finally the synaptic input to the impaled motoneuron should be reduced as a result of the reduced frequency of spikes in the other. Hyperpolarizing a spiracle closer motoneuron progressively to abolish its spikes does not alter the synaptic input which it receives.

The two motoneurons to inspiratory muscles which emerge through the median nerves of abdominal ganglia usually spike together but sometimes spikes in one may cease while the others continue. The silent motoneuron continues to receive excitation which falls only when spikes in the other motoneuron also stop. This would argue in favour of some form of direct coupling were it not for the observation that any renewed excitation of the impaled motoneuron begins before spikes are recorded in the other. Common synaptic inputs to both motoneurons are most likely to be implicated.

The best evidence for the derivation of the ventilatory rhythm from neurons other than the motoneurons comes from experiments in which descending interneurons are stimulated. Some of these neurons produce e.p.s.ps in inspiratory motoneurons, and yet when bursts of stimuli are applied during the inspiratory phase the spikes are abruptly curtailed. If the stimuli occur early in the inspiratory phase then the ventilatory rhythm is reset and the next inspiration occurs earlier than expected. This effect is not produced by hyperpolarizing the motoneuron itself and abolishing its spikes.

The types of results reported here go some way towards providing a firmer basis of knowledge upon which an understanding of a rhythmic behaviour may be based. The results and the conclusions reached are similar to those reported by Wyse (1973) on ventilatory motoneurons of *Limulus*. Further progress on the control of ventilation will only come with the more precise definition of the role of the interneurons. Recording intracellularly from these will be difficult; somata of motoneurons probably account for all the larger ones within a ganglion so that the size of interneuronal somata may be expected to be small. The connexions of the pre-motor interneurons at least can be determined by multiple and simultaneous intracellular recordings from motoneurons in a way that is proving feasible for those concerned with the control of flight (Burrows 1973) or leg movements (Burrows & Horridge 1974).

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