NEURAL PATTERNS ASSOCIATED WITH VENTILATORY MOVEMENTS IN DRAGONFLY LARVAE

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INTRODUCTION

Rhythmic motor activity is a widespread phenomenon which has received considerable attention in arthropods in recent years. Insect respiration, insect flight, swimmeret movements and heart beat in decapod crustaceans in particular have been studied in some detail. Beating of the heart in decapods is initiated by the so-called cardiac ganglion, which consists of a peripheral pool of neurones in close association with the heart and is capable of sustaining rhythmic activity (e.g. Maynard, 1955; Bullock & Terzuolo, 1957). This rhythmicity can be modified by activity originating in the central nervous system via cardiac accelerator and inhibitor axons (e.g. Maynard, 1953). In the other systems mentioned the rhythm is initiated from within the central nervous system. Peripheral receptors tend to play a comparatively small role, modifying rather than initiating the rhythm. The extent of their influence varies in the different systems.

In the crayfish the pattern of activity controlling swimmeret beating is produced in the abdominal ganglia and phased by a pacemaker which is normally situated in the penultimate ganglion. The rhythm can be maintained by the isolated abdominal nerve cord in the absence of any peripheral input (Ikeda & Wiersma, 1964). Normally the release of this patterned activity is under the control of higher centres via 'command' interneurones (Hughes & Wiersma, 1960; Wiersma & Ikeda, 1964). Similar rhythmic activity occurs in the lobster (Davis, 1969). Peripheral control is apparently of greater importance in the control of locust flight but, although the receptors which monitor wing-beat provide the central nervous system with information on the phase, frequency and amplitude of the wing-beat, it is the general level of activity in these sensory axons that appears to be utilized to maintain the frequency of wing-beat, and the phase component is filtered out. The rhythmic pattern still occurs in the absence of this sensory input, but the wing-beat is of lower frequency (Wilson & Gettrup, 1963). However, various sensory inputs are of considerable importance in correcting any asymmetry which exists in the central programme (Wilson, 1968).

Research on insect respiration has been concentrated on the control of ventilation in the anisopteran dragonfly larva (Mill & Hughes, 1966; Mill, 1968), the locust (Miller, 1960a) and the cockroach (Farley, Case & Roeder, 1967; Farley & Case, 1968); and on spiracular control in the locust (Hoyle, 1959, 1960; Miller, 1960b) and adult dragonfly (Miller, 1962). In all these cases the respiratory rhythm originates centrally, and control via peripheral receptors is limited. For instance, sensory inputs can reset
the ventilatory rhythm under certain prescribed conditions (Mill & Hughes, 1966; Farley & Case, 1968) and this will be discussed later. Respiratory rhythms in general may be modified by the tension of carbon dioxide in the environment and by the state of activity of the animal. Although the former could conceivably be effected through peripheral receptors it seems more likely to be a direct effect on the central nervous system (Miller, 1960a). The contractions of some spiracular muscles may be modified by the effect of local tensions of carbon dioxide on the muscles themselves (Hoyle, 1960), but this is a rather different situation since it does not involve feedback via the central nervous system.

In the locust the metathoracic ganglion appears to be the site of the principal ventilatory pacemaker (Miller, 1960a) and the spiracles are all synchronized with ventilatory movements (McArthur, 1929). However, in adult dragonflies rhythmically firing ventilation centres are only found in the abdomen, although higher centres do have considerable influence. In some species of dragonfly movements of the thoracic spiracles are at times synchronized with the ventilatory rhythm and the latter acts as a pacemaker for them.

In a previous paper Mill & Hughes (1966) described the motor pattern to the principal segmental expiratory muscles and to the two main inspiratory muscles, and inferred the presence of a rhythmically firing pacemaker in the last abdominal ganglion. In the present investigation some differences have been found between two closely related genera—*Aeshna* and *Anax*—in the expiratory phase and these, together with some further details of the system in general, are described.

**MATERIALS AND METHODS**

The majority of experiments were performed on the penultimate larval instar of the emperor dragonfly *Anax imperator*. Large larvae of *Aeshna* spp. were also used in a number of experiments.

Dorsal preparations were made as outlined in Mill & Hughes (1966). In an attempt to produce conditions more akin to those in the intact animal ventral preparations were also utilized. The animal was secured ventral side uppermost on wax in a dish, and the sternum over one of the posterior segments was carefully removed to expose the ventral nerve cord and the bases of the three pairs of lateral segmental nerves. This method of approach has two main advantages over dorsal preparations. First, the amount of dissection, and hence damage to the preparations, was minimal and, secondly, the relationships of the sclerites to one another were not distorted in any way. On the other hand respiratory movements tended to disturb the recording conditions and it was not possible to make a visual check on which muscles were contracting or to record from the dorso-ventral muscles. Furthermore, the pressures exerted in the abdominal cavity almost invariably had the effect of extruding some of the abdominal organs between the nerve cord and the longitudinal sternal muscles thereby seriously interfering with the recording conditions and often making it impossible to distinguish the nerve roots. In all preparations chilled dragonfly Ringer was used (Fielden & Hughes, 1962).

Fine platinum electrodes were used for stimulating and recording. The nervous activity was amplified and displayed, using Tektronix pre-amplifiers and oscilloscope;
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and permanent records were obtained with a Grass oscilloscope camera. Audio
monitors were also used. Stimulation was carried out with a Grass S4 stimulator
and isolation unit.

RESULTS

The general anatomy of the respiratory system, its mode of operation and the
rhythmic nervous activity associated with normal ventilation in larvae of the genus
_Aeshna_ have been described in a number of previous papers (Mill, 1963; Mill, 1965;
Hughes & Mill, 1966; Mill & Hughes, 1966). Part of the hind gut is modified to form
a respiratory or branchial chamber and ventilation is concerned with the tidal move-
ment of water in and out of this chamber via the anus. In normal ventilation the first
phase is expiration. This involves raising the floor of the abdomen and contracting the
branchial chamber to increase the pressure in the latter. Subsequently the anal valves
are opened and the increased internal pressure forces water out of the respiratory

![Fig. 1. Recording to show the alternation of expiratory and inspiratory activity. The upper
trace (n2) is recorded from a second lateral nerve (expiratory) of the seventh abdominal ganglion.
The lower trace (sit) is from the subintestinal transverse muscle (inspiratory). Dorsal prepara-
tion. The expiratory bursts are typical of _Aeshna_.](image)

![Fig. 2. Bursts of rhythmic expiratory activity recorded from a second lateral nerve of the
sixth abdominal segment. This was from a ventral preparation of _Anax_. Note the presence of
at least three different motor units.](image)

system. Following this phase inspiration occurs immediately. This is effected by
contraction of dilator muscles at the extreme hind end of the gut (vestibule), by
contraction of two large abdominal muscles (the diaphragm and the subintestinal
muscle) which force the floor of the abdomen down, and by the natural elasticity of
the abdominal sclerites which has a similar effect. The net result is to lower the pressure
in the branchial chamber below that of the surrounding water and this causes water
to be drawn in. There may then be a pause before the next cycle begins.

The sterna are lifted during expiration by the respiratory dorso-ventral muscles
(Mill, 1965; Mill & Hughes, 1966). The pattern of activity in the second segmental
nerves, which innervates these muscles, has been confirmed in _Aeshna_ in dorsal
preparations and is essentially the same in ventral ones. Figure 1 shows the alternation
of activity in the second nerves (expiratory) and the subintestinal transverse muscle
(inspiratory). There is normally only a single unit in the expiratory burst, but a second
unit firing at this time is sometimes also observed. However, in _Anax_, the muscular
system of which is virtually identical with that of _Aeshna_ (Mill, 1965), there are almost
invariably three units involved in the expiratory bursts (Fig. 2). One of these is
probably homologous with the single unit usually found in _Aeshna_ preparations, and
can readily be identified by its characteristic firing pattern. It gradually increases in frequency to a maximum, which may be maintained for a short period of time before the frequency decreases somewhat and the unit is cut off abruptly. Furthermore, the relationship between this unit and activity in the respiratory dorso-ventral muscle has been confirmed by recording simultaneously from a second segmental nerve and from the respiratory dorso-ventral muscle which it innervates. The usual 1:1 relationship is observed between nerve and muscle action potentials and the extracellularly recorded muscle action potentials increase in amplitude as the frequency increases (Fig. 3).

This latter phenomenon may be due to facilitation. Of the other motor units one is small and commences firing well before the main unit, and there is some indication that there are really two such units. The other is much larger, fires only a few times, and occurs towards the end of the expiratory burst. In some preparations this large unit is absent; in others it is present in some expiratory bursts but not in all. Neither of these units can readily be correlated with any extracellular action potentials in the dorso-ventral muscles, or in any other muscles for that matter. No obvious differences are apparent between dorsal and ventral preparations in Anax.

Other lateral segmental nerves may also exhibit motor activity during expiration, and this invariably seems to be the case in Anax at least. Figure 4 shows such synchronous activity in a first (Fig. 4a) and in a third (Fig. 4b) lateral nerve of segment seven. The fifth lateral nerves of the eighth (last) abdominal ganglion innervate most of the
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musculature of the branchial chamber and vestibule and of the valves; and recordings from these nerves show an alternation of expiratory and inspiratory activity (Fig. 4c, d). Motoneurones firing during expiration are not restricted to the lateral nerves. The last (tenth) of the unpaired segmental nerves, which arises at the hind end of the last abdominal ganglion (Mill, 1965), contains motor activity synchronized with expiration (Fig. 4d). It is thought to innervate some of the intrinsic musculature of the branchial chamber or vestibule.

Mill & Hughes (1966) reported that stimulation of a first lateral segmental nerve from one of the more posterior ganglia, during the period between two expiratory bursts, elicited an expiratory burst in the second nerves and thus re-set the rhythm. The duration of the inter-burst period between the elicited burst and the one following it was the same as that prior to stimulation and so the frequency was not affected.

(a)

(b)

Fig. 5. The effect on the ventilatory frequency of volleys of electrical stimuli to a first lateral nerve. (a) and (b) are continuous. In this preparation the recording is from a second lateral nerve of the sixth abdominal ganglion. Stimuli were applied to the contralateral first lateral nerve of the same ganglion. The lower marker indicates the duration of stimulation. Ventral preparation of Anax.

This is of particular interest in the light of a recent paper by Farley & Case (1968) on the ventilatory system of the cockroach, Periplaneta americana. A single burst of electrical stimuli to one of the segmental nerves in this animal had the effect of delaying the start of the next ventilatory burst rather than of initiating it but, as in the case of Aeshna, had no effect on the subsequent ventilatory frequency. A few experiments were carried out in the present investigation in which segmental nerves were electrically stimulated, and these confirmed the earlier findings of Mill and Hughes in that, whenever an effect was produced by such stimulation of a first segmental nerve, it was in the nature of a decrease in the inter-burst period not an increase. On a single occasion stimulation of a second segmental nerve towards the end of an inter-burst period was observed to produce a slight delay in the occurrence of the next expiratory burst in the contralateral second nerve. This could have been due to antidromic stimulation of the motor neurones.

Figure 5 shows the results of an experiment in which a burst of electrical shocks was applied repetitively to one of the first segmental nerves of segment six cut distal to the stimulating electrodes. The recording electrodes were placed on the contralateral second nerve root of the same segment. The effect of the first volley in this short series was to re-set the rhythm by initiating an expiratory burst; and the rhythm immediately became phase-locked with the volley frequency. On cessation of stimulation the original ventilatory frequency was immediately resumed. In this experiment the ventilatory frequency was 24/minute and the volley frequency was about 33/minute.
This phase-locking of the ventilatory with the volley frequency also occurs in the cockroach (Farley & Case, 1968), but in this animal is caused by a delaying rather than an accelerating of motor activity.

**DISCUSSION**

The differences between the expiratory burst in the second lateral segmental nerves in *Aeshna* and *Anax* are of interest. A single unit is typical of these rhythmic bursts in *Aeshna*, although a second one sometimes occurs; whereas in *Anax* there are always at least two and normally three (or possibly four). In each case the available evidence indicates that only one unit innervates the respiratory dorso-ventral muscle (Fig. 3). This is further supported by a preliminary study of the pattern of innervation of the abdominal muscles which indicates that only a single axon may innervate the respiratory dorso-ventral muscle (P. J. Mill, unpub.). There are no obvious contractions associated with these extra units in *Anax* in any of the segmental muscles, and this also applies to the apparently expiratory units observed in the other lateral nerves. Slight contractions could occur in some of the smaller muscles where they may not readily be observed, or they may be obscured by the nature of the preparations. Thus, to observe the muscles, dorsal preparations had to be used and this involved a considerable amount of dissection as well as some restraint of the preparation. However, there are other possible explanations. It may be that some of these units are inhibitory and innervate muscles which could otherwise interfere with the expiratory movement. Alternatively some may serve to increase the tone in the other dorso-ventral muscles and thus act synergistically. The extra units in the second segmental nerve presumably act in this manner since the muscles which this nerve innervates could only enhance the expiratory movement by their contraction. This would also apply to the motor units in the third segmental nerves, since recordings of these were always distal to the branch leading to the sternal muscles (Mill, 1965). Thus the anterior and posterior dorso-ventral muscles may well aid expiration under certain conditions. There is reason to believe that the oblique tergo-pleural muscle is also involved since in the larva of *Libellula* (one of the dorso-ventrally flattened dragonfly larvae) the oblique tergo-pleural muscle is oriented rather more dorso-ventrally and contracts synchronously with the respiratory dorso-ventral muscle (Mill, 1968). Like the respiratory dorso-ventral muscle it receives a rich tracheal supply in *Libellula* (but not in *Aeshna* or *Anax*). In so far as the fifth paired nerves and the tenth unpaired nerve are concerned the expiratory activity in these will be associated with movements of the branchial chamber and vestibule and of the valves.

In the dragonfly larva the respiratory rhythm can be re-set by stimulation of a first segmental nerve. This is caused by the reflex production of an expiratory burst in the second segmental nerves (Mill & Hughes, 1966; and this paper). Furthermore, there is evidence to suggest that the rhythm can be immediately phase-locked to repetitive volleys of electrical stimuli to a first segmental nerve, when the volley frequency is higher than that of the naturally occurring rhythm. Thus the frequency of ventilation is increased. On cessation of stimulation the original frequency is resumed at once.

Farley & Case (1968) have demonstrated a similar re-setting of the ventilatory rhythm in the cockroach when the abdominal segmental nerves are stimulated, but in
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This case it is effected by a delay in the production of the next motor burst. Again phase-locking occurs to repetitive volleys of electrical stimuli but, because of the nature of the effect—one of delay rather than initiation—this has been achieved by using a volley rate (120/min.) below that of the naturally occurring rhythm (180/min.), thus producing a lower frequency of ventilation.

It could be argued that in the cockroach antidromic stimulation of the expiratory motor neurones causes this delay by having a refractory effect on them since, although Farley & Case stimulated and recorded from different nerves, the nerves which were stimulated did contain expiratory motor fibres. This explanation would be supported by the isolated observation in the dragonfly larva in which a second segmental nerve (which contains the expiratory motor axons) was stimulated and produced a slight delay in the initiation of the next burst. However, Farley & Case produced identical results using deformation of the cuticle as a stimulus instead of electrical stimulation; and this presumably precludes any possibility of antidromic stimulation. Further elucidation of this process must await a more detailed investigation into the effects of peripheral stimuli on the ventilatory rhythm.

In dragonfly larvae rhythmic expiratory activity occurs in the segmental nerves of abdominal ganglia 5–8 at least. The rhythm starts in the last (eighth) abdominal ganglion and spreads anteriorly; that is to say the primary pacemaker is located in the last ganglion. This ventilatory system closely resembles the swimmeret control system in the crayfish (Ikeda & Wiersma, 1964; Wiersma & Ikeda, 1964) and lobster (Davis, 1969), with the proviso that in the crayfish at least the last (sixth) abdominal ganglion is not involved (the sixth segment does not bear any swimmerets) and the rhythm starts in the fifth ganglion. In the crayfish sectioning of the ventral nerve cord immediately in front of the fifth abdominal ganglion leads to the rhythmic centre in the fourth ganglion taking over the function of pacemaker. In turn, removal of the fourth ganglion sometimes causes the third to take over this role, whereas the second and first never act as pacemakers (Ikeda & Wiersma, 1964). In dragonfly larvae details of pacemaker control by individual ganglia have not been resolved as yet; although the experiments on re-setting the rhythm do indicate that posterior ganglia other than the eighth abdominal may act as pacemakers. Wiersma & Ikeda (1964) demonstrated the presence of five interneurones in the crayfish ventral nerve cord which, when stimulated at a frequency of between 20 and 100/sec., elicited bilateral rhythmic swimmeret movements. Rhythmical activity associated with these movements has not been reported anterior to the abdomen. In the adult dragonfly rhythmically firing ventilation centres are confined to the abdomen (Miller, 1962), and this is probably the case in the larva also.

However, in the locust (Miller, 1962) and cockroach (Farley et al. 1967) the primary ventilatory pacemaker is situated in the metathoracic ganglion, and from here rhythmical activity is conveyed to the other thoracic ganglia and to the abdomen. All of the abdominal ganglia in these animals contain centres capable of eliciting rhythmical activity, that is to say contain secondary pacemakers, and in some instances a pacemaker in the second abdominal ganglion of the cockroach may become dominant.
SUMMARY

1. Rhythmic bursts of motor activity associated with the expiratory phase of ventilation have been recorded from the second lateral segmental nerves of posterior abdominal ganglia in *Aeshna* and *Anax* larvae.

2. In *Aeshna* the rhythmic expiratory bursts contain one, or sometimes two, motor units; whereas in *Anax* there are almost invariably three units. In both animals only one unit is associated with action potentials in the respiratory dorso-ventral muscle.

3. Motor activity synchronized with the expiratory bursts in the second nerves has been recorded from the other lateral nerves and from the last unpaired nerve. In addition the fifth lateral nerves carry inspiratory bursts.

4. It has been confirmed that stimulation of a first segmental nerve can re-set the ventilatory rhythm by initiating an expiratory burst in the second nerves. The original frequency is immediately resumed on cessation of stimulation.

5. The nature of the ventilatory control system in dragonfly larvae is discussed in relation to other rhythmic systems in the arthropods.

REFERENCES


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