THE ACTIVITY OF SINGLE MOTOR FIBRES IN ARTHROPODS

I. THE DRAGONFLY NYMPH

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(Received 8 October 1964)

INTRODUCTION

Although myoneural mechanisms of arthropods have been extensively studied, comparatively little work has so far been carried out on the central connexions of motor units. The effect of motor discharges has usually been investigated with nerves isolated from their ganglia, and hence the role of the efferent synapses in modifying the information transmitted to the muscle is relatively unknown. On the other hand the synapses relaying sensory input to the central nervous system have received considerable attention both in crustaceans (Preston & Kennedy, 1960; Hughes & Wiersma, 1960) and insects (Roeder, 1948; Fielden, 1960, 1963a; Fielden & Hughes, 1962). It therefore appeared a profitable approach to employ the dissection technique for isolating single fibres in studying some of the synaptic junctions of arthropod motor neurones. The work covered by this paper concerns the responses of efferent fibres in nymphs of the dragonflies, Anax imperator and Aeschna brevistyla. A subsequent article will describe motor fibre activity in a crustacean, the Australian yabbie, Cherax destructor.

Relatively few studies have involved the reflex responses of motor fibres in insects. Discharges of motor neurones have been investigated by Hagiwara & Watanabe (1956) in the cicada, and by Luco (1963) in the cockroach. In addition the activity of efferent fibres in whole nerves has been studied in the cockroach and mantis (Roeder, Tozian & Weiant, 1960) and in the dragonfly nymph (Mill, 1963). Some observations have also been made on the responses of motor fibres, with intact central connexions, in the control of leg muscles (Pringle, 1940; Hoyle, 1957; Usherwood, 1962), of flight muscles (Wilson, 1961, 1964; Neville, 1963) and of spiracular muscles (Hoyle, 1960; Miller, 1960). However, the main emphasis of these workers has been on the neuromuscular events and there is comparatively little reference to the properties of motor neurone synapses. In the present work responses to both mechanically and electrically induced afferent volleys have been studied in single units isolated from the segmental nerves.

The results show that both inhibition and temporal summation/facilitation are important at motor junctions and these are discussed in relation to the control of insect muscle.

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METHODS

Single-unit responses were obtained using the dissection techniques described in a previous article (Fielden & Hughes, 1962). Briefly, whole dragonfly nymphs were placed ventral side uppermost in physiological saline. The exoskeleton was removed in order to expose the terminal segments of the ventral nerve cord and the segmental nerves were then dissected using electrolytically tapered tungsten needles. The nerve was split repeatedly into smaller bundles whose activity was monitored by a fine platinum electrode. The paring process was continued until it was possible clearly to recognize single responses produced either by mechanical stimulation of receptors or by electrical stimulation of a connective or of another segmental nerve. Fine paint brushes were used for stimulating hairs, and needles for compressing or telescoping segments. For electrical stimulation, square wave pulses of 0.1 - 1.0 msec. duration were delivered through silver/silver chloride electrodes. Potentials were displayed on a cathode-ray oscilloscope after amplification by a Tektronix pre-amplifier and were simultaneously made audible through a loud-speaker unit.

The above approach suffers from two disadvantages; first, it was rarely possible to identify the same fibres in different preparations, and second, since pre-ganglionic recording shows the afferent response as a compound action potential, the precise number of afferent fibres synapsing with a given efferent fibre was unknown. An attempt was made to overcome the former difficulty and pin-point certain motor fibres by recording from some of the finer terminal branches of the caudal nerves. The efferent nature of these fibres was confirmed by recording where possible from the muscle concerned and by demonstrating the absence of activity on mechanical stimulation combined with appropriate cutting. This eliminated the possibility of any antidromic stimulation of sensory fibres. The results are compiled from a study of 70-80 units.

RESULTS

(a) Preparation

The activity of single motor fibres was studied in last instar nymphs of Anax imperator and Aeschna brevistyla, a smaller Australian species. The majority of work was on the latter, and very little difference was noted between the two species. In both, the fifth pair of nerves (N5) from the last abdominal ganglion was most often used for the dissection of fibre bundles. These nerves are composed of approximately four hundred axons which show structural differences in their origin resulting in the localization of predominantly sensory fibres in the ventral part, and predominantly motor fibres in the dorsal part of the root (Fielden, 1963b). The motor axons, which are thick walled, range up to 10-14 µ in diameter and can be traced to groups of ganglion cells lying ventrally between the origins of both ipsi- and contra-lateral segmental nerves. A large proportion of these fibres arise from cells situated between the fourth and fifth roots, but occasionally motor cells appear to be separated by a considerable distance from the nerves which they supply.

The peripheral distribution of the major branches of N5 to the muscles of segment 10, the paraprocts and epiproct is shown in Text-fig. 1. The fifth nerve also innervates part of segment 9 and the respiratory chamber extending as far forward as segment 5.
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It shows an anastomosis with the fourth nerve and anastomoses occur between several branches. The occurrence of fusions between nerves, apparently a feature of the insect nervous system, was noted several times and must be borne in mind during physiological investigations. The smaller and more ventral of the two main branches of N5 shown in Text-fig. 1 is largely sensory in function and arises from the inner surface of the ipsilateral paraproct. The larger branch divides into two main trunks one of which receives sensory fibres from the tergite and supplies the dorsal segmental muscles, and is also joined by a large branch from the median spine. The other trunk appears to be largely motor in function and supplies the rectal dilator muscles, sphincter and paraproct muscle. In segment 10, therefore, longitudinal sternal and tergal muscles, dorso-ventral oblique muscles, abductors of the paraprocts, retractor muscles of the anus, rectal and sphincter muscles are all supplied by motor fibres in branches of N5. N5 also receives sensory fibres from the sternite, pleurite and tergite of segment 10, paraproct, lateral and median spines. The activity of single efferent fibres could sometimes be recognized in the motor branches enabling certain efferent units to be characterized. More often, however, several units were active even in the smaller branches which proved very difficult to dissect into finer bundles.

Text-fig. 1. Ventral view of the main branches of the fifth nerve (N5) in segment 10 and the paraproct of a nymph of Anax imperator. The ventral longitudinal muscles and some of the rectal dilator muscles have been removed. m., muscle.
Approximately 30–40% of the efferent fibres isolated in the fifth nerve possessed a background discharge or spontaneous activity. This activity, which in most cases could be affected by stimulation, was apparently endogenous to the last ganglion, as it occurred after complete de-afferentation. The discharges were independent of the age or condition of the preparation and consisted of rhythmic bursts or continuous firing at varying rates, comparable to those described for interneurones dissected in

Text-fig. 2. Spontaneous activity of single efferent fibres dissected in the fifth nerve of *Aeschna* nymph. (a) Endogenous bursts in the absence of stimulation. (b) Inhibition of the resting discharge of one unit and excitation of a second on touching the inner hairs of the contra-lateral paraproct. Time scale 100 msec. (c) Inhibition of the resting discharge of another unit produced by repetitive stimulation of the ipsi-lateral connective.

the abdominal connectives (Fielden & Hughes, 1962). Continuous tonic firing was common and ranged from a regular rhythm of impulses to less regular activity with variable intervals between impulses. In both types of unit the discharge rates were normally between 2/sec. and 30/sec., though occasionally higher frequencies were recorded (Text-fig. 2). Paired impulses in the same axon were only rarely seen. Activity characterized by intermittent bursts of impulses occurred frequently and sometimes two or three units showed synchronization of firing. The frequency of bursts ranged from 5/min. to 30/min. and, although these often showed no apparent relationship to any breathing or heart rhythm, in some preparations they could be related to respiratory activity. Many of the motor fibres were obviously concerned with phasic activity of the muscles underlying respiratory and jet-propulsive movements as they were excited by manipulation of the tactile hairs bordering the paraprocts. Hence, although their intermittent activity was apparently endogenous to the last ganglion, additional activity could be triggered by a small afferent source.

Occasionally some ‘silent’ efferent fibres began firing spontaneously upon repetitive stimulation of an afferent fibre tract, as if this produced some priming action or possibly removed inhibition. It may well be that these effects were normally produced
by a higher centre such as the brain or suboesophageal ganglion, as in these experiments the connectives were usually severed between the sixth and seventh ganglia. However, the opposite effect was also noted, that of the removal of inhibition following transection of tracts from higher centres. This phenomenon has been observed in motor nerves of the mantis and cockroach by Roeder et al. (1960), who discuss the behavioural significance of differential inhibition of local efferent activity in insects. Comparable results have been found in the dragonfly nymph, where the background discharge of a few fibres was increased by cutting the connectives, and three units started firing spontaneously subsequent to this procedure. Increases in firing frequency were normally seen within 5 min. of transection and discharges persisted at this higher rate during the period of recording. The majority of efferent fibres, however, showed only a transient burst of impulses on cutting.

(c) Responses to natural stimulation

Sensory fibres enter the last ganglion through all except the third segmental nerve and their tactile and proprioceptive endings can be readily excited by brushing hairs or manipulating the terminal abdominal segments. Pre-synaptic afferent stimulation, evoked either in this manner or arising from electrically induced volleys, produced a variety of responses in the majority of both spontaneously active and quiescent motor fibres. Although it is difficult to generalize, these were on the whole less predictable and more intermittent than those recorded from interneurones. In addition the post-synaptic motor responses often appeared to be considerably affected by any prior activity in the stimulated pathway. This emphasizes the difficulty of attributing properties to the motor junctions, since it is not possible to determine the number of intervening synapses between afferents and efferents and hence the role played by interneurones is unknown. Most motor neurones responded to either proprioceptive or tactile stimulation and a few examples were found of fibres responding to both modalities. The peripheral areas producing excitation were occasionally very restricted. For example, the unit shown in Text-fig. 26 was fired only by stimulation of a few hairs on the inner side of the contra-lateral paraproct. This type of fibre was relatively rare, however, and more commonly units responded to several inputs, though response proved more difficult to elicit by natural stimulation than by electrically evoked volleys. Convergence of excitation from both contra- and ipsi-lateral sensory endings has been demonstrated in many motor units, and this could sometimes be strictly localized by cutting all other inputs.

Excitation in spontaneously active fibres was usually shown by an acceleration of their discharge rate which was often followed by a post-burst depression. Repetitive trains were common and the frequency of impulses within these trains was sometimes extremely high (up to 200/sec.). Comparable high-frequency bursts of impulses were also of frequent occurrence in fibres without a background discharge. These showed varying degrees of adaptation and after-discharge, the rate of adaptation usually being considerably slower than that found for sensory fibres (Fielden & Hughes, 1962). In other fibres the spontaneous activity could be inhibited by peripheral stimulation and the inhibitory effects often outlasted the period of afferent discharge. Cases of inhibition of one unit and concurrent excitation of a second unit in the dissected bundle have also been recorded (Text-fig. 2b).
(d) **Responses to electrically evoked afferent volleys**

Electrical stimulation of afferent pathways also produced excitation and inhibition of tonic motor discharges. Excitation was shown either by an acceleration of the discharge with multiple firing to a single stimulus, or by the interpolation of single or double evoked impulses within the spontaneous rhythm. Whereas in the yabbie synchronization of this rhythm with repetitive afferent volleys was common, this was extremely rare in the dragonfly nymph and was only found in four fibres. In a few other fibres, which initially followed the applied rate, the rhythm rapidly reverted to its own frequency although afferent stimulation continued.

Central inhibition of motor discharges commonly occurred in the dragonfly nymph, in contrast to the crustacean. Spontaneous activity could be reduced or interrupted by varying rates of repetitive afferent stimulation in the range of 5–50/sec., 20–30/sec. proving the most effective (Text-fig. 2). Inhibitory effects depended on the sensory pathway excited and on the strength of the applied shocks as in the case of excitation, but sometimes inhibition occurred only on stimulation of a restricted number of inputs. For example, the spontaneous discharge of a unit in one of the branches of N4 was inhibited only by repetitive stimulation (20/sec.) of the ipsi-lateral N5. In this case the unit was also inhibited by pressure on the ventral surface of ipsi-lateral segments 9 and 10. Inhibition was also frequently found on stimulation of either intact or transected connectives and in the latter case it simulated descending inhibition from higher centres. As in interneurones the effects of inhibition were often found to outlast the period of stimulation, and it is not known whether it can be attributed here to the motor synapse or to some intermediate link.

In fibres which were not spontaneously active, responses to afferent volleys varied from single spikes to repetitive trains of impulses. The number of discharges exceeded ten in some units but usually five to seven impulses were evoked by a single volley. Multiple discharges appeared to occur less frequently at the motor synapses of *Aeschna* than at the interneuronal junctions of either species. They closely resembled those of interneurones, however, and the duration and frequency within the train depended on both the source and the magnitude of the afferent volley (Fielden, 1963a). An increase in the intensity of the volley increased the length of the train and sometimes shortened its latency, while an increase of repetition rate reduced the number of impulses until normally, at rates above 10/sec., only single-spike responses were seen. Similar observations have also been described by Hagiwara & Watanabe (1956) for motor neurones of the cicada, where changes in shock strength altered the number of impulses set up by the shock. In the dragonfly nymph raising the frequency of stimulation often shortened the latency of the train more significantly than an increase in size of the volley. Delays for the first impulse were reduced by as much as 0.8–1.0 msec. before they subsequently lengthened with fatigue. This was also seen in single-spike responses, where the delay became less intermittent at certain frequencies (Text-fig. 3). These observations contrast with those on interneurones, suggesting that temporal summation or facilitation is more important at motor junctions than spatial summation. In addition, although efferent responses were on the whole readily fatigued and labile, they were normally very dependent on the frequency of successive volleys, often indicating an enhanced responsiveness of the
motor fibre after repetitive stimulation. This effect is described in more detail in the next section.

The diffuse nature of the connexions between neurones in the dragonfly ganglion has been demonstrated previously by tracing pathways between connectives and intact segmental nerves (Fielden, 1960; Mill, 1963). In the current experiments this

![Text Fig 3](image-url)

Text-fig. 3. The effects of frequency of stimulation on the response of efferent neurones in N4 and N5 of Anax nymph. (a) Recording from the intact N5 (proximal to the last ganglion) (upper trace) and from a dissected bundle of the branch of N5 which passes over the longitudinal sternal muscles (lower trace). A single shock to the distal end of the same N5 produces an afferent response seen in the upper record but no post-ganglionic discharge. (b) Same preparation. Increasing the frequency of stimulation does not change the afferent discharge but elicits a post-ganglionic response seen in both recordings. (c) and (d) Responses of the same unit in N4 to two frequencies of stimulation of the ipsilateral N5 showing that shorter and more constant delays are obtained at the higher frequency. (c) 10/sec., (d) 40/sec. Time scale msec.

has been shown even more conclusively by placing stimulating electrodes on all the ganglionic inputs and monitoring the efferent response in a dissected bundle containing a readily identifiable unit. A large proportion (60%) of fibres responded at various intensities to most of the afferent pathways and, as expected, their responses varied in type, delay, and regularity with stimulation of different inputs. Several motor fibres were found which gave a multiple discharge on stimulation of one nerve
root and a single impulse to another, implying different synaptic links in the two cases. The latter feature is also apparent from measurements of ganglionic delays which were estimated at between 1.5 and 10-15 msec. for different routes to efferent neurones. As in interneurones, shorter delays were associated with fibres which followed higher frequencies and longer delays with those which fatigued more readily. Some units responded synchronously to 100/sec. but normally responses failed at 30-50/sec. or less. From tracing these multiple pathways within the ganglion it was obvious that a single motor fibre plays an important role as the final common path and that, in addition, some complex connexions between afferent and efferent neurones may be involved in some cases. This is substantiated histologically from both the methylene blue studies of Zawarzin (1924) on Aeschna nymphs and from tracing tracts in silver-impregnated preparations of the last ganglion of Anax.

(e) Effects of repetitive stimulation on the responsiveness of motor synapses

Initial experiments on the dragonfly nymph demonstrated an enhanced responsiveness of motor junctions dependent on repetitive stimulation, since some efferent neurones were excited by subthreshold volleys when the pulse repetition rate was increased (Fielden, 1960). This was illustrated more clearly in recordings from single units where a large proportion (50-60%) showed a marked increase in response on raising the rate of afferent excitation. Hence in these cases frequency seems to have effects other than fatiguing the junctions. Pre-ganglionic recording indicated that the effects could be attributed to events at the intervening synaptic junction, since no increase in the size of the afferent volley was detectable when the frequency was raised (Text-fig. 3). This was also substantiated by stimulating the motor axon directly where 1:1 responses were normally obtained to stimuli above threshold. Furthermore, it seems almost certain that the frequency sensitivity is a property of the motor synapse itself since it has been shown to occur at junctions believed to be mono-synaptic; e.g. the response shown in Text-fig. 3 where a large efferent fibre in a branch of N5 crossing the longitudinal sternal muscles was excited, normally after a short delay (1.5-3.0 msec.), by sensory fibres of the same root.

An investigation of these post-synaptic responses suffered from the disadvantage that any previous activity at the efferent junction often affected its responsiveness, sometimes quite considerably, even though frequent rests were given. The types of responses described here were, however, independent of the age of the preparation since the effects of frequency were as significant in fresh preparations as in those which were dissected but left for 2-3 hr. before use.

Motor fibres affected by increase in the repetition rate of the afferent volley could broadly be classified into the following categories:

(1) Units which were excited by sub-threshold volleys when the frequency of stimulation was increased. At the intensities used these fibres did not respond at repetition rates of 1/sec., but showed a distinct facilitatory effect of frequencies higher than 8-10/sec., when they responded in a 1:1 manner with the stimulating volley until the responses became intermittent with fatigue. Post-activation potentiation was often shown by these units in that they were excited by previously subthreshold volleys for a short period of time following the high-frequency burst (Pl. 1a). Fibres
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showing this interesting effect of frequency were by far the most common (30 units) and their responses are discussed in more detail below.

(2) Units which showed an increased excitability as the stimulation rate was raised but responded with a discharge that was not 1:1 with the afferent volley. This group includes a wide variety of fibres some of which produced responses of higher frequency than the stimulating pulse, some which responded at a lower frequency and others which fired only a few impulses at increased frequencies (Pl. 1b). Units of the latter type often did not respond to any intensity of stimulation at the lower end of the frequency range and appeared to be dependent on a considerably increased rate of afferent excitation to achieve their firing level.

(3) Units which produced intermittent burst activity on increasing the frequency of stimulation. Occasionally a unit could be made to respond rhythmically in bursts to a continued stimulus (Pl. 1c). These fibres were rare and have only been encountered in five preparations. The burst activity could normally be triggered by frequencies of 10–20/sec., and stopped on cessation of the stimulus. Similar fibres have been observed amongst the interneurones of the connectives which respond in an intermittent manner to continued mechanical stimulation (Fielden & Hughes, 1962).

(4) Units which produced a distinct after-discharge following repetitive trains of stimuli. These fibres were quite common (12 units) and varied from those in which a response was seen only after repetitive stimulation was terminated to those in which after-discharges followed induced spikes. In some cases the latter were directly related to the frequency of stimulation whilst in others no relationship was apparent. After-discharges lasted up to 2 or 3 sec. and occasionally longer, and often resembled those observed in cockroach motor fibres by Luco (1963). The peculiar activity shown in Pl. 1d for an Anax motor fibre was rare, but the unit showed a readily reproducible response. The fibre showed only an occasional response at low rates of stimulation, and was totally inhibited at higher frequencies, though these produced an after-discharge. Continued low-frequency stimulation during the after-discharge produced a single response per stimulus followed by a period of inhibition before the normal pattern was resumed after several seconds. This may indicate the presence of multiple junctions interacting upon a single efferent fibre, producing mixtures of inhibition and excitation.

To summarize, therefore, many efferent synapses in the dragonfly nymph show an enhanced responsiveness during, or subsequent to, repetitive afferent activity. The latter was most effective in the range 10–100/sec.; at lower frequencies the facilitatory effect was not usually seen and at higher frequencies the responses become intermittent with fatigue. It was apparent that in many efferent neurones increments in intensity and frequency were almost interchangeable in exciting a response, implying that both spatial summation and temporal summation/facilitation are important.

(f) The relationship between intensity and frequency of stimulation

Changes in the parameters of stimulation were investigated in more detail for units of group (1) where it was clear that at different intensities of the stimulating volley there were different frequencies at which the maximum response occurred. However, an attempt to study these parameters quantitatively met with the difficulty that any previous activity altered the excitability of the motor junction. Conditions
therefore were maintained as standard as possible and the relationship between frequency and intensity was plotted for several units (Text-fig. 5). To obtain these graphs a known intensity of stimulation was used and the frequency then increased by increments of 10/sec. until a consistent reproducible efferent response was obtained.

Text-fig. 4. Responses of an efferent fibre dissected in N5 of Aeschna nymph to different rates of stimulation applied to the ipsilateral connective. (a) 20/sec., (b) 30/sec., (c) 40/sec., (d) 50/sec. Stimuli are recorded on the lower trace.

A standard period of rest was given between the stimulating bursts. This was found to give more dependable results than the alternative procedure of determining the intensity to produce a response at a known frequency. From the curves it can be seen that at high intensities the responses occurred at low repetition rates, whereas at lower intensities a very much higher frequency was necessary to elicit a post-synaptic response. There was very little reduction of the intensity threshold at
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frequencies below 10/sec. while at higher frequencies (above 40/sec.) a minimum intensity for excitation existed.

In the above experiments, because of the difficulties of measuring all variables for one unit, a response was judged present or absent for a standard period of stimulation, and its latency at the different frequencies was not taken into account. The term

Text-fig. 5. Plot of relative intensity against frequency of stimulation to produce a response in four different units dissected in N5 of Aeschna nymphs.

Text-fig. 6. Plot of latency against frequency of stimulation for four units dissected in N5 of Aeschna nymphs.
latency here refers to the time from the onset of stimulation to the appearance of the first impulse at the different frequencies tested (Text-fig. 4). This time interval will obviously vary with the size and repetition rate of the stimulating volley. A graphical representation of the relationship between frequency and latency for several units shows that the time interval shortens to a minimum as the frequency of the afferent volley increases above 50-60/sec. (Text-fig. 6). Latencies were considerably longer at the lower frequencies and often very variable. Responses at these frequencies were also sometimes intermittent, presumably due to varying thresholds at the junctions responding to the combined effects of spatial and temporal summation or facilitation.

Latencies are perhaps more informative when expressed in terms of the number of afferent volleys before the appearance of a response at the different frequencies used (Text-fig. 4). In a few units it was possible to plot this relationship between frequency and number of stimuli for different intensities of stimulation. An example is shown in Text-fig. 7, where the curves all represent the activity of one unit. They were constructed using four known stimulation intensities and recording the effects of 10/sec. increments in frequency at each of these intensities. It is obvious that at any given frequency the number of pulses required to elicit a response varies inversely with the stimulus strength. On the other hand, for any given intensity there is a less-marked variation in the number of pulses required for a fairly large change in the frequency of stimulation, especially at higher repetition rates. At lower frequencies it seems probable that the apparent exception shown in curve 3 would occur at all intensities if stimulation were continued for long enough.

These curves demonstrate more clearly the combined effects of spatial and temporal summation and/or facilitation in eliciting responses at motor junctions. However,
at the present time it does not appear possible to distinguish between the last two of these alternatives in assessing the role of frequency. The initial part of the response pattern is typically preceded by different number of stimuli at each frequency and intensity of stimulation, which seems to indicate the importance of temporal summation. On the other hand the fact that many units show effects of post-activation potentiation on cessation of repetitive stimulation implies that a long-lasting facilitation may be involved. Most probably a mixture of these two phenomena is operative at these efferent junctions resulting in their peculiar responsiveness to increases in the rate of efferent excitation.

DISCUSSION

This investigation shows that the salient properties of insect motor neurones include the presence of a background discharge, the occurrence of convergence, and the importance of both inhibition and temporal summation/facilitation. Many of these properties are also common to interneurones but these are dependent on spatial summation, and the peculiar effects attributed to the timing of afferent impulses seem to be particularly characteristic of the efferent junctions. This has been noted in earlier work on the cockroach and dragonfly nymph (Roeder, 1948; Fielden, 1960), where the major physiological control of escape responses appeared to occur at the frequency-dependent, labile synapses between interneurones and motor efferents. No evidence for facilitation or temporal summation was found at the afferent/giant-fibre junction in the nymph, but in the cockroach Pumphrey & Rawdon-Smith (1937) found that the post-ganglionic response could be elicited either by raising the intensity or the frequency of stimulation. Comparable effects of increases in frequency have been described at other insect junctions. Huber (1960) found an optimum frequency range for the behavioural responses produced by implanted electrodes in the brains of gryllids which is possibly significant in this context. In cockroach motor neurones Luco (1963) has described a 'natural response' similar to the after-discharge of some dragonfly efferents and dependent on a frequency threshold in the same way.

A reciprocal relationship of frequency and intensity of stimulation has been observed in the control of locust flight muscle (Wilson 1961). Here it was found by recording muscle action potentials that the response normally followed a fraction of the stimulus frequency which could, within limits, be set by the intensity of the stimulus. The initial part of the response train was often comparable to that found for the dragonfly nymph and indicated the importance of temporal summation. The subsequent regular oscillation of the response showing a distinct phase relationship to the stimulus was not observed in Aeschna, unless the triggering of burst activity is an indication of this effect. Wilson (1961, 1964) attributes this regular intermittency of response to an inherent driving oscillator within the ganglion and suggests that refractoriness of the motor neurone may also play a part. No evidence for prolonged refractory periods was found in the current work either at the central junction or in the motor axon itself, and responses in the dragonfly appear to be dependent on the combined effects of temporal summation and facilitation. A microelectrode study of synaptic events is necessary to assess the relative importance of these two phenomena.

Changes in pulse repetition rate have been shown to be capable of producing excitation, inhibition, after-discharge and potentiation at different motor junctions...
in the dragonfly nymph. The functional significance of such a frequency-sensitive system is not immediately obvious, but it appears to provide for a considerable variation in efferent responses. The properties of the synapses appear to be ideally suited to the pattern of pre-synaptic impulses whether this arises in sensory neurones or in interneurones. If the motor neurone synapses directly with sensory fibres severe stimulation of receptors can result in a high-frequency discharge which may excite or inhibit efferent fibres unaffected at lower frequencies. On the other hand if the afferent impulses excite interneurones these have been shown to depend on the number of fibres active and respond characteristically with a repetitive train of impulses, the frequency of which will govern the efferent response. Interneurones therefore appear to play a part as a ‘matching device’ in coding spatial information into a temporal pattern suited to the motor neurone.

Finally, the role of these motor junctions in the control of insect muscle must be considered. The variability of the relationship between the efferent response and the frequency, intensity and pathway of stimulation possibly has its significance in allowing for a wide range of signals in the very few fibres which typically supply arthropod muscle. In addition peripheral inhibition does not appear to occur in insects and it is therefore of interest to find that central inhibition of motor discharges is of common occurrence in the dragonfly nymph. From work on other insects (Hoyle, 1957) it seems probable that in the execution of natural movements bursts of fast-fibre activity are normally brought in to reinforce the background slow-fibre activity. In the current observations the rates of discharge found for spontaneously active units are comparable with those found to maintain tonic contraction of the slow system, whereas the frequency of impulses within mechanically or electrically induced bursts is in the range known to produce strong muscular movement. The fast-fibre system appears to have the higher threshold of activation (Hughes, 1952; Hoyle, 1957) and it seems probable that the frequency-sensitive neurones of the dragonfly are components of this system, since they are dependent on a high intensity of afferent stimulation. So far the nature of the neuro-muscular control of contraction in abdominal muscles of the nymph is unknown and it is obviously essential to determine whether fast or slow fibres are differentially affected by changes in the frequency of stimulation. One may conclude, however, that the properties of both neural and myoneural junctions contribute to an extremely efficient system capable of functioning with considerably fewer nerve units than does that of the vertebrate.

**SUMMARY**

1. Responses to mechanical and electrical stimulation have been investigated in single motor fibres dissected in the segmental nerves of the dragonfly nymph.
2. A large proportion of fibres possessed a background discharge which was often accelerated or inhibited on stimulation. Examples of central inhibition were common.
3. Efferent responses varied in type, delay and regularity, both with the input under stimulation and with the frequency and intensity of the volley. The majority of fibres responded to stimulation of more than one nerve root.
4. In many motor fibres changes in the parameters of stimulation demonstrated a reciprocal relationship between intensity and frequency. An enhanced responsive-
ness occurred with frequency increases in the range of 10–100/sec. indicating a considerable importance of temporal summation/facilitation.

5. The characteristic frequency-sensitivity of motor fibres and the variability of their response patterns are discussed in relation to the control of insect muscle.

I would like to thank Prof. A. K. McIntyre for his constructive criticism of the manuscript.

REFERENCES


EXPLANATION OF PLATE

The effects of increased pulse repetition rate on efferent neurone responses in N4 and N5 of *Anax imperator* nymph. (a) Excitation and post-activation potentiation. (b) Facilitation of two units which respond to increased frequency but not in a 1:1 manner. (c) Intermittent activity in a previously silent unit on increasing the frequency. (d) After-discharge and inhibition. Further explanation in text. Stimuli are recorded on the lower trace.