PROPERTIES OF INTERNEURONES IN THE ABDOMINAL NERVE CORD OF A DRAGONFLY NYMPH

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INTRODUCTION

Previous investigations have shown that the nymph of the dragonfly, *Anax imperator*, provides certain advantages for studying the properties and organization of neuronal units in an insect (Fielden, 1960; Fielden & Hughes, 1962). The activity of single units has been recorded in bundles split off from the abdominal connectives, and different patterns of resting discharge and responses to natural stimulation have been described. Descriptions of the responses of these units to tactile and proprioceptive stimulation are valuable in providing information of direct physiological significance as they indicate the ways in which the sensory input may be distinguished by the central nervous system. It is difficult, however, to relate this approach to a survey of synaptic pathways (Fielden, 1960) since it does not provide the necessary information on the properties of the junctions with the central units. Hence the present paper continues the investigation by describing the activity of interneurones which is evoked by electrically induced volleys.

Comparable work to that described here for the dragonfly nymph has been done on the crayfish (Kennedy & Preston, 1960). *Aplysia* (Hughes & Tauc, 1962) and to some extent the cockroach (Yamasaki & Narahashi, 1960; Boistel, 1960). Many of the properties of interneurones in the dragonfly nymph appear to be very similar to those described for spinal interneurones. Among these are the frequent occurrence of repetitive discharge to single afferent volleys, the presence of a background discharge and the occurrence of convergence from multiple afferent sources upon individual units. These features of interneurones have been noted in the responses to natural stimulation of single units in the *Anax* cord. Although the relevance of such a broad comparison with the vertebrate spinal cord is questionable the results are related to the work of Frank & Fuortes (1956), Hunt & Kuno (1959) and McIntyre & Mark (1960) on the cat.

METHODS

The methods employed for obtaining single-unit responses have been described in detail in a previous article (Fielden & Hughes, 1962). Briefly, last instar nymphs of *A. imperator* were pinned ventral side uppermost in physiological saline. The terminal segments of the ventral nerve cord were exposed by removing the sternum, and the connectives between the sixth and seventh ganglia were dissected with the use of electrolytically tapered tungsten needles. The connective was split repeatedly into

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smaller bundles. Activity in these bundles was monitored using a platinum electrode, and the paring process was continued until, when the bundles were sufficiently small, responses of single units could be readily distinguished. This proved difficult since the final strands of fibres were even smaller than those used in studying responses to natural stimulation. In the latter case a more specific afferent source excites fibres selectively but electrical stimuli produce a diffuse sensory input. This is relatively unselective and hence the bundles have to be smaller to give identifiable responses. Pre-ganglionic recording was used where possible but an electrical pulse stimulates an unknown number of fibres in a nerve and hence the afferent input to the interneurones in the connectives is virtually unknown. Ascending fibres in the connectives were stimulated via the roots of the last ganglion and descending fibres via the roots of the sixth ganglion. Square-wave pulses of 0.1-1.0 msec. duration were delivered to the segmental nerves through silver/silver chloride electrodes. The impulses were displayed on a cathode-ray oscilloscope after amplification by a Tektronix pre-amplifier and were heard simultaneously through a loud-speaker unit. Viability of preparations was good (1-3 hr.) at room temperatures. The results are compiled from a study of over 80 units.

RESULTS

Types of response in interneurones

All the interneuronal responses were recorded from the connectives between the sixth and seventh ganglia and in most experiments electrically induced volleys were applied to the paraproct nerves. A large proportion of the fibres isolated showed a background discharge (Fielden & Hughes, 1962) which was usually affected by nerve volleys but sometimes remained unaltered. A few units could be identified as primary sensory fibres, but the great majority of responses described below arose from fibres which could with some certainty be identified as interneurones. The latter were distinguished by their repetitive responses to nerve volleys usually in more than one segmental nerve, and also by the presence of 'synaptic' properties. These consist of appreciable and variable delays together with blocking of the response at relatively low transmission frequencies (150-200/sec.) Primary afferent fibres could be identified by single impulses evoked by stimulation of a particular segmental nerve and by the absence of any synaptic properties. Mechanical stimulation was sometimes used to check this identification since responses in primary sensory fibres are affected by stimulation of more localized areas and the frequency of discharge is higher and more rapidly adapting than that of interneurones (Fielden & Hughes, 1962).

Many of the responses of interneurones to nerve volleys were very similar to those arising from mechanical stimulation. It was found that afferent volleys could produce excitation and inhibition in interneurones with a background discharge. Excitation in these fibres was sometimes shown by an acceleration of their discharge rate and produced a burst of impulses. A common, though not universal, feature was a post-burst depression of the background discharge of these units which sometimes lasted several seconds (Fig. 1a). Occasionally this depression of activity was the most prominent feature of the response. Inhibition was shown by a reduction or interruption of the background discharge and was very dependent on the frequency of the stimulating pulses (Fig. 1b). It was rarely achieved with frequencies below 5/sec.
While frequencies of 30–40/sec. were the most effective. Inhibition was also dependent on the nerve root under stimulation and two units were found in the 6–7 connective which could be excited by one pathway and inhibited by another. Another feature of inhibition in these interneurones was that the effects sometimes outlasted the period of stimulation. The unit illustrated in Fig. 1b is of this type.

Fig. 1. Responses of two interneurones in a 6–7 connective to electrical stimulation of the ipsilateral fifth nerve. (a) Excitation produced by a single volley and followed by a post-excitatory depression of the resting discharge. (b) Inhibition of the resting discharge produced by repetitive stimulation (20/sec.). The interval between pulses is plotted and lines drawn between the average of three successive intervals.
A few units were found which could be inhibited by both natural and electrical stimulation. For example, the discharge of a fibre dissected in the connective between ganglia 6 and 7 was inhibited by dorso-ventral pressure on ipsi-lateral segments 9 and 10 and by repetitive stimulation of the ipsi-lateral fifth nerve. Occasionally it was possible to inhibit the background discharge of some of these units by stimulation of both ascending and descending inputs. In some experiments inhibition of the discharge of interneurones in the terminal connectives was achieved by stimulating the
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cervical connectives. In most experiments, however, the connectives were cut anterior
to the sixth ganglion and hence the responses were normally free of any cephalic
influences. Most interneurones were affected by stimulation of at least one of the
paths used, but in a few cases the resting discharge was completely unaffected by
stimulation of any of the segmental nerves of the sixth and seventh ganglia. Some of
this activity was detected in descending fibres but some also appeared to be ascending
in the cord and could be attributed to the isolated last ganglion.

Fig. 3. (a) Repetitive firing to a single nerve volley in a unit dissected in a 6–7 connective.
(b) Plot of a consecutive series of these responses to a constant volley. The method of plotting
is described in the text.

Responses were investigated in 'silent' units which did not show a background
discharge. These interneurones usually responded with a train of impulses to a volley
in a segmental nerve (Fig. 2). The repetitive trains were comparable to those which
have been recorded in spinal interneurones (Frank & Fuortes, 1956; Hunt & Kuno,
and, as in the case of these interneurones, the number and frequency of impulses varied both among different units and with the source and intensity of stimulation. A few fibres were found which never fired repetitively under the conditions of stimulation used, whilst other units fired repetitively to one input but not to another. The trains of impulses produced by a constant stimulating volley were remarkably similar and an example is represented graphically in Fig. 3b. For the sake of comparison this plot has been made using the method of Hunt & Kuno (1959) in which each impulse is plotted as a point on the graph, the abscissa indicating the latency from the stimulus (or pre-synaptic response) and the ordinate the reciprocal of the interval between impulses (frequency). It can be seen that while the latency of the first impulse remains almost constant over a series of trials, subsequent impulse intervals show some variation. The pattern of response shown in Fig. 3 of an initial spike followed by a delayed burst was quite common in the interneurones studied and has been described in units in the vertebrate reticular neurones (Amassian & Devito, 1954) and spinal cord (Hunt & Kuno, 1959). The length of the response trains varied considerably between units and with the source of the afferent volley. The latter feature possibly indicates an inherent lability of the interneurones or, more likely, the presence of a variable number of junctions between the afferent input and the central interneurones. Some response trains were remarkably long and had a duration of 0·5 sec. or more. Even these prolonged bursts showed very similar impulse patterns in response to a constant volley. These discharge patterns have been observed in response to natural stimulation in the dragonfly nymph (Fielden & Hughes, 1962) and to nerve volleys in other invertebrates (Yamasaki & Narahashi, 1960; Preston & Kennedy, 1960; Fuortes & Mantegazzini, 1962). Responses to natural stimulation were generally at a lower frequency than those arising from nerve volleys.

Synaptic delays for interneurone responses could be estimated by recording pre-ganglionically from the nerve root under stimulation and post-ganglionically from the dissected bundle. Delays are not very significant when the relative conduction velocities of the fibres concerned are unknown but they provide a comparison with those measured for the intact connectives (Fielden, 1960). Following stimulation of the fifth nerve the delays for single units in the 6–7 connective ranged from 1·5 to 7·5 msec. Delays around 2–3 msec. were the most common and these often shortened on increasing the intensity of the stimulating volley. Occasionally it was difficult to decide whether some of the units with the shorter delays were primary sensory fibres or interneurones, the latter presumably excited monosynaptically. It is possible that some of these units, which also transmitted high frequencies synchronously, were primary sensory fibres passing straight through the ganglion. However, they were very few in number (four) and the great majority of units showed synaptic properties when stimulated via afferents in the paraproct nerves and hence confirm the evidence of the initial physiological survey.

**Effects of the frequency and intensity of stimulation**

The form of the repetitive response described above was dependent both on the size and frequency of the stimulating volley. Changes in intensity produced significant effects at the afferent synapses connecting with interneurones, implying that spatial summation is of some importance. In most interneurones as the size of the stimulating
volley was increased the delay for the first impulse was shortened and the frequency of discharge during the response train increased. Fig. 4 illustrates the responses of an interneurone in a 6–7 connective to graded volleys recorded in the ipsi-lateral fifth nerve. It can be seen that an increase in stimulus strength excited higher threshold afferent fibres and it appeared that summation was required for the initiation of further spikes in the interneuronal response. These responses to changes in the intensity of both mechanical and electrical stimulation have been reported in other interneurones (Kennedy & Preston, 1960; Hunt & Kuno, 1959). The graphical representation of

![Graphical representation](image)

Fig. 4. The effects of the intensity of the stimulating pulse on the response of an interneurone. In (a) to (d) the intensity of stimulation of the ipsi-lateral fifth nerve was steadily increased and an increase in the number of spikes and a shortening in the delay of the post-ganglionic response can be seen. Upper trace recording from the fifth nerve, lower trace from an interneurone in the connective between ganglia 6 and 7. Time scale in msec.

Fig. 5 shows that the latency of all the spikes evoked by increasing the intensity shortened as the strength of stimulation was raised. Normally the effect was most marked for the first impulses of the train where the latency sometimes shortened by as much as 0.8 msec. (Fig. 6). Latencies also shortened for single spike responses which could seldom be provoked to fire repetitively on increasing the afferent volley. Precisely similar results have been described for spinal interneurones and in particular for neurones of the dorso-lateral tract investigated by McIntyre & Mark (1960). As these workers point out, there must be convergence of several afferent fibres upon single interneurones since the amplitude of the afferent volley can determine the latency and number of the post-synaptic impulses.
Fig. 5. Plot of the responses of an interneurone in a 6–7 connective to electrical volleys of increasing intensity (2–5 arbitrary units) in the ipsi-lateral fifth nerve.

Fig. 6. Plot of latency against intensity of stimulation for a unit in a 6–7 connective following stimulation of the ipsi-lateral fifth nerve.
In most interneurones the number of impulses in a repetitive burst can therefore be varied by changing the intensity of the stimulating pulse. The number can be reduced by increasing the pulse repetition rate. It was found that as the rate of stimulation was raised above 2.5/sec. the earliest impulse persisted with little change in latency but later impulses became intermittent and finally dropped out. Interneurones varied considerably as to the frequencies at which these changes occurred. In the majority the repetitive spikes became intermittent at frequencies of 5-20/sec. while the initial spike dropped out at higher frequencies of 30-40/sec. In many the synapse followed to 80-100/sec. and occasionally fibres were found that apparently transmitted synchronously to frequencies of 200/sec. and higher. In general, shorter delays were associated with units which followed higher frequencies and longer delays with units which readily became intermittent. This is correlated with the length of the intra-central pathways as the longer ones obviously involve more synaptic junctions. Most junctions with interneurones showed higher frequency limits, however, than did those with efferent neurones in the segmental nerves and did not show the peculiar facilitatory effects of frequency seen at the latter.

Receptive fields and connexions of interneurones

The results of this aspect of the investigation are closely comparable to those obtained using natural stimulation to map the central representation of peripheral areas (Fielden & Hughes, 1962). The majority of the units responded to the stimulation of more than one of the segmental nerves and more were excited by ipsi-lateral than by contra-lateral stimulation. Most responded to stimulation of the paraproct nerves; these are the largest of the nerves of the last ganglion and comprise over 400 axons. Many of the interneurones possessed very diffuse connexions similar to those shown for the intact preparation. It is not known how physiologically significant these are; electrical stimulation was employed in both cases. The different afferent (or interneuronal) connexions of an interneurone showed distinct variations in synaptic properties in terms of thresholds, delays and transmission frequencies, in addition to differences in the length of the response train. Some of the units investigated responded to stimulation of the nerves of the sixth as well as the seventh ganglia, i.e. they were multi-segmental and could conduct in ascending and descending directions in the abdominal nerve cord. There were many similar examples of the fact that the initiation of spikes can take place at different locations. Often these could be shown to be of the multi-segmental type by the use of mechanical stimulation. For example, a unit which produced a train of impulses on stimulation of all the ipsi-lateral first nerves of the abdominal ganglia also responded to stroking the ipsi-lateral edge of the abdominal segments. It does appear that the Anax cord could produce a very useful preparation for a study of the convergence and interaction of responses from different inputs, comparable to that of Preston & Kennedy (1960) on the caudal ganglion of the crayfish. So far preliminary experiments utilizing two sources of stimulation have not given any indication of spatial summation from two different inputs.

Some unsuspected connexions of interneurones have been demonstrated by the use of electrical stimulation. In one experiment small bundles of fibres were dissected in both connectives between the sixth and seventh ganglia. Recording from these bundles and stimulating a fifth nerve showed a repetitive burst of spikes in the ipsi-lateral
connective which was followed in a 1:1 manner by a descending burst in the contralateral bundle. Many neuronal connexions of similar types were found and despite the difficulties of the dissection, this method, combined with the use of repetitive electrical volleys, could enable some of the physiological anatomy of the dragonfly cord to be mapped as in *Aplysia* (Hughes & Tauc, 1962).

**DISCUSSION**

Interneurones have been the least studied elements within the nervous system of both vertebrates and invertebrates. The scarcity of information possibly arises from the fact that they pose more difficulties in terms of isolation, identification and stimulation than do other nervous units. However, certain properties of interneurones appear to be common to the mammal, crayfish and now the insect. These include the repetitive response of interneurones to a single nerve volley, the presence of a background discharge and the occurrence of convergence. Repetitive discharge appears to be particularly characteristic of interneurones and it has been suggested that this may be an important factor in prolonging activity in chains of interneurones, i.e. a discharge in the first interneurone may excite a longer discharge in the next, increasing progressively down the chain (Hunt & Kuno, 1959). This could mean that prolonged activity might occur in a relatively short number of interneurones and hence effect a certain degree of economy. The functional significance of this repetitive firing may also lie in the excitation of motor neurones which have been shown to be dependent to some extent on facilitation or temporal summation (Fielden, 1960). An interneurone will therefore prolong the effect of a sensory discharge at an efferent junction but the subsequent discharge will be at a lower frequency.

The mechanisms of repetitive firing have been attributed either to long-lasting pre-synaptic excitation or to post-synaptic events, the latter possibly being inherent in the post-junctional cells themselves or in the form of reverberating circuits (Wall, 1959; McIntyre & Mark, 1960). In the dragonfly nymph the second alternative is eliminated, since it can be shown that the mechanism is pre-synaptic by the fact that the number of impulses in the response train varies both with the afferent input and the strength of the stimulating volley. Workers on spinal interneurones have also concluded that their repetitive firing is due to prolonged pre-synaptic activity (Hunt & Kuno, 1959; Wall, 1959). Three possible factors responsible for prolonging this activity have been discussed by McIntyre & Mark (1960) in relation to the repetitive firing of dorsolateral tract neurones. Prolonged excitation may arise first from temporally dispersed pre-synaptic impulses due to differences in conduction velocity, or, secondly, from pre-synaptic reverberating circuits, or, thirdly, from prolonged transmitter action at individual afferent terminals. From the present evidence it is impossible to deduce which mechanism may be responsible in the dragonfly nymph but some information has been interpreted as supporting these suggestions in spinal neurones (Wall, 1959).

It is interesting to demonstrate the effect of spatial summation on the response of a single interneurone in the abdominal connective of the dragonfly nymph. Increasing the intensity of stimulation excites higher threshold afferent fibres and some of these synapse either directly or indirectly with the post-ganglionic fibre. Their summed
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effect produces a larger response with a shorter delay. Preston & Kennedy (1960) have suggested that this effect is due to a graded longitudinal threshold of the post-fibre but it seems more likely that additional afferents contribute to a more steeply rising synaptic potential. Spatial summation may protect the central nervous system from continual sensory bombardment. In addition, these interneuronal junctions show a frequency limitation to repetitive volleys which could also be protective in avoiding over-stimulation and fatigue.

The significance of spinal interneurones in modifying the afferent message to a motor neurone and in setting 'tone' for neurone activity has been stressed by Hunt & Kuno (1959). Interneurones have been shown to play a comparable role and exhibit similar properties in the dragonfly. Vowles (1961) has contended that insect nerve cells are potentially less efficient as integrating units than are vertebrate neurones, this argument being based on the fact that insect neurones offer a much smaller surface area for synaptic contact than do those of the vertebrate because the cell body is not involved and the cells possess fewer dendritic branches. It has been pointed out (Hughes, personal communication) that this may be disputed as many interneurones have synaptic connexions in several ganglia and individual insect neurones may perform functions which are normally ascribed to several vertebrate neurones. Furthermore, this feature should be taken into account in discussions of the 'economy' of neurones as it will help to compensate for a deficiency in the number of nerve cells. The present work adds further support to this view as multiple connexions and extensive branching have been demonstrated using both afferent volleys and sensory stimulation (Fielden & Hughes, 1962). It appears unlikely that insect neurones should be less efficient since they are few in number and are capable of producing varied behaviour patterns though these may be much more stereotyped than in the vertebrate. Possibly these patterns are brought about by simple control circuits as Vowles has suggested and possibly the insect neurone possesses different and simpler properties than that of the vertebrate. Present knowledge of insect nerve cells is very inadequate but the demonstration of widespread convergence and the occurrence of inhibition, often combined with excitation, indicates the complexity of response which can occur in these cells.

SUMMARY

1. Responses of single interneurones in the abdominal connectives of the dragonfly nymph following electrical stimulation of the segmental nerves were investigated.
2. Excitation and inhibition were seen in fibres with a resting discharge. Repetitive trains of impulses, varying between units and with the source of the afferent volley, were common.
3. Changes in the intensity and frequency of stimulation affected the pattern of the repetitive responses. An increase in the intensity of the volley shortened the latency and increased the length of the train, while an increase in rate reduced the number of impulses.
4. Most interneurones responded to stimulation of more than one afferent pathway. Multisegmental fibres and multiple connexions of fibres were common.
5. These responses are compared with those of spinal interneurones and discussed in relation to their function in the animal.
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REFERENCES


