MECHANORECEPTORS, PHOTORECEPTORS AND RAPID CONDUCTION PATHWAYS IN THE LEECH, HIRUDO MEDICINALIS

By M. S. LAVERACK

Gatty Marine Laboratory and Department of Natural History, University of St Andrews, Fife, Scotland

(Received 27 May 1968)

INTRODUCTION

The central nervous system of leeches has received less attention than that of the other annelid classes, the Oligochaeta and the Polychaeta. Knowledge of the morphology of the group has advanced little since the review of Scriban & Autrum (1934), though Hagadorn (1958) has added some details in dealing with the neurosecretory properties of the leeches. Physiological studies have been almost entirely confined to the giant cells of Retzius (Hagiwara & Morita, 1962; Eckert, 1963; Kerkut, Seddon & Walker, 1966) but a little is now known of other cells of the ventral cord (Baylor Nicholls & Stuart, 1966; Bianchi, 1967). The connexions and pathways of these cells with others in the nervous system have not been demonstrated.

The properties of sensory cells in the leech are almost unknown. Walther (1966) has presented some details of the photoreceptor cells of the anterior regions, and Bullock & Horridge (1965) quote some observations made by Hagiwara and Morita. The only information available on interneurones in the Hirudinea is contained in this work. Hagiwara (personal communication) has indicated that these studies are no longer in progress. Wilson (1960) has shown that the segmental nerves carry motor neurones.

The fine structure of the nerve cords, and of the nerve cell somata, has received excellent treatment from Gray & Guillery (1963), Coggeshall & Fawcett (1964) and Coggeshall (1966), and consequently there is a wealth of information on synapses, fibre diameters, cellular inclusions and sheath structure. Some of the physiological properties of the glial cells have been examined by Kuffler & Potter (1964) and Kuffler (1967).

The present paper presents results that expand those of Hagiwara and Morita on the mechanoreceptors of the body wall, and the interneurones of the cord, especially when stimulated via photoreceptors.

MATERIALS AND METHODS

Large specimens of Hirudo medicinalis were obtained from L. Haig and Co. Ltd., Newdigate, Surrey. They were kept in glass jars containing a small amount of water in a cool place.

The nervous system of the animal was exposed by dissection from the dorsal side and by removal of the gut. Blood was removed from the exposed area by pipette or
filter paper. The sheath covering the connectives and ganglia of the nervous system was stripped off with fine forceps. The preparation was flooded with liquid paraffin to prevent desiccation, since drying disrupted transmission along the connectives and peripheral nerves. When the anterior portions of the nervous system were investigated the cerebral ganglion was carefully exposed and the first, second and third pairs of nerves were traced from the ganglion to the periphery. These nerves could be used for recording purposes providing the mobile anterior segments were securely pinned.

Records of nervous activity were obtained using conventional means (platinum hook electrodes, a.c.-coupled pre-amplifier) with a Tektronix 565 oscilloscope with two 3 A 3 pre-amplifiers for display of up to four channels.

Mechanical stimulation was effected using a Southern Instruments pen unit carrying a small probe, driven by a Servomex waveform generator. Light flashes were obtained by movements of a small card interposed between the light source (focusing microscope lamp) and the animal. Movement was driven by the waveform generator. Heat produced by this lamp was minimized by a heat filter. Control experiments on heat stimulation were carried out by holding heated objects (test-tubes, matches, investigator’s finger) close to the preparation.

RESULTS

Mechanoreceptors of the body wall

The first three nerves of either the right or the left side were used for the following observations. The units involved usually serve considerable areas, and there is some overlap in the areas served by each nerve.

Mechanoreceptor units are readily seen in these nerves since they produce the largest potentials. Impulses occur in the absence of imposed stimulation, but it is notable that they fire when the body wall moves during muscular contraction and most of the background activity is probably due to this cause. A record taken over a period of several minutes with the body wall intact shows considerable fluctuation in the activity of such units.

The location of the sensory endings appears to be in the superficial layers of the body wall. Light manual stimulation as well as more pronounced deformation of the body wall evokes activity. The sensory dendrites presumably extend over wide areas, as reflected by the activity of single units when stimulated at widely separate points. There is no indication that these receptors are associated with the sensory hillocks that occur on one annulus in each segment.

Figure 1 shows responses of a mechanoreceptor to different types of stimulation. Figure 1a is the response to a sinusoidally applied depression of the body wall at the anterior border of the head of the leech. Firing commences when a small amount of indentation has taken place, the first few impulses being of lower frequency. Frequency falls slightly towards the end of the cycle, the unit thus being velocity-sensitive. In this case a small response also occurs on the reverse phase of the stimulus (release of indentation).

The same unit is represented in Fig. 1b. The waveform in this case is of constant velocity. Under velocity of deformation the frequency of discharge is relatively constant. This is also shown in Fig. 1c in which unit one fires regularly throughout the cycle of indentation.
indentation, and unit 2 is recruited as the deformation reaches a maximum and spreads over a greater area.

The firing frequency of the mechanoreceptor rises as the rate of the body-wall deformation increases. A long slow deformation invokes a constant-frequency response and rapid cessation of the stimulus is followed by a rapid discharge in the receptor. In

![Fig. 1. The response of a mechanoreceptor in the body wall of Hirudo. These units provide the largest potentials recordable from the segmental nerves and their ramifications extend over large areas of the body surface. (a) Response to a sinusoidally applied indentation of the body wall, (b) to a wave form of constant velocity, (c) to a slow indentation, rapid release, and (d) to a rapid indentation followed by slow release.](image)

![Fig. 2. (a) Constant velocity stimulus applied to body wall at 2.5 cyc./sec. Response is a short burst of potentials, on indentation of the body wall. (b) Onset and ensuing few cycles at 25/sec. First burst of activity is of 4-5 spikes followed by two potentials per cycle (the phase relationship is reversed compared with (a)).](image)

the reverse case (Fig. 1 d) the rapid stimulus gives rise to rapid firing, but the slower release invokes no activity at all. The absolute velocity is thus important in determining the characteristics of the response.

If the frequency of the stimulus is increased the mechanoreceptor follows with
shorter and shorter bursts (in terms of number of spikes per burst) until each cycle of stimulation is monitored by only one or two impulses. Figure 2 shows frequencies of 2·5/sec. (ramp) (Fig. 2a) and 25/sec. (sinusoidal) (Fig. 2b). The phase relationship is inverted between these two figures so that in Fig. 2a indentation occurs on the upward movement and in Fig. 2b on the downward movement. Figure 2b also shows that the first cycle or two invoke more activity than succeeding cycles. An upper vibration-frequency limit of about 50–60/sec. seems typical of these units.

Through-conduction pathways

In the course of observations on responses of units in the ventral nerve cord in relation to the peripheral input provided by the mechanical deformation described above, it was soon noted that light flashes also stimulate activity. When records were made from connectives between any two ganglia along the length of the ventral chain it was found that activity could be invoked by photo-stimulation of most parts of the body wall. No specific properties of the photoreceptors can be deduced from these observations, only that they provide an input for the central nervous system.

If the light from a focusing microscope lamp falls on the body wall of *Hirudo*, impulses occur in the connectives of the ventral cord, providing that a short dark period had preceded the stimulus. The response accommodated fairly rapidly. That it was light and not the heat emitted from the lamp that was important was indicated by the lack of any response to a source of heat (up to 40° C.) placed close to the body wall and by virtue of the fact that dark adaptation took place so that responses after 5 min. in dim lighting were considerably greater than those to rapidly repeated flashes. Continual bright illumination evoked no response yet could provide continual heat if this were the stimulant (though a heat filter was interposed in the light beam).

It was possible to record from several successive interganglionic connectives simultaneously, the number of such recordings being limited by the number of available channels of recording facility.

The response recorded in the c.n.s. to a light flash at either the anterior or posterior end of the animal was characteristically a short burst in an interneurone (Fig. 3a). Responses of similar type and duration occurred if the frequency of stimulation was less than once every 10 sec. Stimulation at more frequent intervals led to diminution of the response (Fig. 3b), and eventual adaptation (Fig. 3c) in which only the first of several flashes evokes a response. This adaptation takes place at the receptor-to-interneurone synapse, as is shown by Fig. 4, which demonstrates that when the effect of photic stimulation had adapted out, the interneurone still responded to mechanical input. If mechanical stimulation is repeated many times, the mechanoreceptors continue to discharge (see above), but the interneurone fails after two or three cycles, but can be restimulated via a fresh sensory pathway.

Figure 5 is a typical record obtained while recording from three connectives with a fourth beam monitoring the stimulus. In Fig. 5a light fell on the head and recordings were made from connectives between the ganglia of segments 15–16, 16–17, 17–18. It is evident that impulses originated in a fast pathway that extends at least from the anterior end to this level. By stimulating the rear end of the body (Fig. 5b) it could be shown that impulses occurred in the same unit and passed forward. The velocity of conduction is about 1 m./sec. in this system.
A single large response appeared in these central responses but some interesting variations were seen. In some cases it is likely that activity is in several units firing more or less synchronously (e.g. Fig. 5c). Figure 5c shows the same preparation as in Fig. 5a, b. The preparation was stimulated anteriorly in this case. The first impulses arrived first in connective 15–16 then at the subsequent connectives. The impulse, however, took longer to traverse the first ganglion in the chain than the second, as shown by conduction time and by comparison with Fig. 5a, b. It should also be noted in Fig. 5c that four impulses occurred in connective 15–16 but only three in the later connectives. One thus dropped out at segmental ganglion 16, but is is possible that
Fig. 5. Response of an interneurone to light. Records from connectives 15–16, 16–17, 16–18 on bottom, middle, and top beams respectively. (a) Response to stimulation anteriorly, (b) stimulation posteriorly, (c) stimulation anteriorly, but note slight delay in transmission from 15–16 to 16–17 (see text).

Fig. 6. Connectives 10–11 (bottom), 11–22 (mid), 12–13 (top). (a) All connectives intact, stimulation anteriorly, (b) right connective between 12–13 cut, (c) left connective 11–12 cut as well, (d) right connective 10–11 cut. None of these cuts interfered with the transmission of the interneurone.
this is activity in a second unit. Records from this preparation also showed that impulses originating posteriorly also took longer to cross ganglion 16.

The location of the fast conducting pathway in the connective was shown as follows. The connectives of the right and left side in any particular segment can be cut to interfere with conduction between ganglia on that side. If a unit could not be recorded distal to a cut then it was assumed that the pathway had been interrupted. Figure 6 shows the results of one such experiment. The first trace shows the usual response (Fig. 6a), the second the response after the right connective was cut between segments 12–13 (6b), the third record shows the response after also cutting the left connective in segments 11–12 (6c), and in the fourth trace the right connective 10–11 is also severed. In all cases the unit still fired synchronously in each connective. Destruction of the response occurred only if the cut was carried across the mid line to sever part of the contralateral connective.

Leeches possess a small median connective, Faivre's nerve. This nerve is not easy to see with a dissecting microscope, but can be destroyed by longitudinally separating the two major connectives with a scalpel. Incision in one small part is sufficient. Figure 7a shows the usual control response in three contiguous connectives with anterior photic stimulation. Figure 7b shows the responses in the same three connectives after section of Faivre's nerve in connective 16–17. The anterior connective still conducted the train of impulses, the second (electrode placed distal to the cut) recorded no potentials, and the third in line recorded a single impulse that bore no apparent relation to the events further up the cord. If the second electrode was placed...
anterior to the cut (Fig. 7c) then impulses were seen in both connectives 1 and 2, but not in 3. Thus the through-conducting pathway was cut by severing Faivre's nerve.

Photoreceptors are present in each segment of the body, and input to the fast pathway may occur in any segmental ganglion. Impulses in the pathway are synchronized if either extremity of the body is stimulated from several receptors. Input must be summed and at what level synchronization occurs remains obscure. Figure 8 shows the responses in connectives anterior and posterior to the destroyed Faivre's nerve when the preparation was stimulated in the mid region at the recording site. When the second electrode is positioned anterior to the cut, impulses in the first and second connective (in this case 15-16, 16-17) begin synchronously, but 16-17 then lags slightly behind. Connective 17-18 carried totally unrelated potentials, presumably generated in ganglion 17 and proceeding posteriorly. If the second pair of electrodes was posterior to the cut (Fig. 8b) and stimulation was carried out in the same segments then connectives 15-16 and 17-18 carried impulses which appeared independent, and 16-17 carried two small potentials bearing some relation to those in 17-18, but the first preceded that in the latter connective and the second lagged behind. The interneurone system can thus be stimulated in any segment.

The connexion with motor fibres in the segmental nerves

It can readily be shown that leeches shorten rapidly when stimulated mechanically, or by light flashes falling on the body (Mann, 1962). The fast responses of the through-conduction pathway are thus presumably transmitted to the muscle fibres of the body wall via motor nerves. Wilson (1960) demonstrated that motor fibres exist in the segmental nerves of the leech, and when these were stimulated contraction of muscle fibres followed. Experiments were consequently carried out to determine what motor
patterns were generated in the through-conduction pathway. Figure 9 shows the only successful experiment. Pinning out the animal for dissection and cutting the body wall usually fatigues the fast muscle responses, and rapid contraction of the body-wall muscle ceases. In the present experiment recordings were taken from the connective posterior to a segmental ganglion, and the first left segmental nerve of that ganglion. A one-to-one correlation exists between the impulses in the interneurone and the motor fibre. Since in this case the interneurone recording site lies posterior to the ganglion, delay times cannot be precisely determined; but it seems probable that a single synapse is interposed between the fast pathway and the motor neurone, as the delay is less than 1 msec.

Fig. 9. The top line of this record is taken from a connective distal to a ganglion, the second beam is from the first left segmental nerve of that ganglion, third beam is from second right segmental nerve. Activity in the interneurone evoked by anterior light stimulation. Passage of impulse in interneurone slightly precedes motor unit of segmental nerve, so probably a single synapse is interposed between the two. Any motor units in 2nd right segmental nerve are fatigued at the synapse.

DISCUSSION

It has been known for many years that the leech is capable of rapid longitudinal contraction (Gee, 1912; Bethe, 1908), in response to touch. The only information available on the physiology of sensory mechanisms and pathways involved appears to be the summary in Bullock & Horridge (1965) of the work of Hagiwara and Morita, who showed that mechanoreceptors were present in the body wall of Hirudo and that through-conduction pathways exist in the ventral nerve-cord; but no experimental details have ever been published.

The mechanoreceptors of the body wall of the leech are superficially placed, and are stimulated by body movement as well as by imposed mechanical stimulation. Their role in the economy of the animal may be partly in reception of touch stimuli and partly in monitoring the shape and disposition of the body wall. The frequency response and sensitivity to external sources of vibration in the watery medium may be very limited unless distortion of the body wall takes place. Vibration receptors may exist elsewhere on the surface of the animal.

Photoreceptors have been described in leeches (Hesse, 1897), but little is known of their properties apart from the work of Walther (1966) and the electron-microscope studies of Röhlich & Török (1964) and Clark (1967). No information is yet available on spectral sensitivities and required stimulus energy levels. It is apparent, however, that there are photoreceptors scattered over the body of the leech. They may be more numerous anteriorly and posteriorly, but also exist along the length of the body. A
flash of light from a microscope lamp or from an electronic flash source are both potent sources of illumination.

Information from the mechanoreceptors and the photoreceptors may enter a fast-conduction pathway. The velocity in this system is about 1 m./sec., which agrees with the figure given by Bullock & Horridge (1965) but which is greater than that given by Bethe (1908). The receptors may synapse directly into the fast interneurone, with massive input summing to drive the interneurone, or via a collector neurone that serves to synchronize the many inputs before synapsing on to the fast pathway. There is no anatomical information available on these points.

Adaptation of the fast-pathway reflex occurs at the receptor–interneurone junction (assuming the simplest case). Serial repetition of one stimulus modality is followed by cessation of the fast-pathway response within two or three cycles, but further stimulation via a fresh input invokes interneurone activity. The mechanoreceptors are known to respond to continued stimulation, and photoreceptors are assumed to also. Thus the crucial link is the synapse between the two components. If a collector neurone is involved then the failure may occur at either synapse receptor–collector, or collector–fast pathway.

The motor response is more difficult to demonstrate. It is probable that fatigue of the interneurone–motor junction occurs during the preliminaries to the experiment. In this respect, and respect of the adaptation of the receptor–interneurone synapse, the hirudinean shows similarities with other annelids. In oligochaetes the same effects have been shown by Horridge & Roberts (1960) and Roberts (1960, 1962) and in polychaetes by Horridge (1959). Wilson (1960) showed that motor fibres carry excitation to the muscles after dissection, so the final pathway is functional under such conditions.

The interneurone of Hirudo shares with the giant fibres of oligochaetes the ability to conduct information in both directions along the cord. It differs in that in earthworms sensory input to the giant fibres confers polarity of conduction; anterior receptors feeding into the median giant, and posterior receptors into the lateral giants. In leeches the major pathway receives input all along the length of the animal, and transmits in both directions, bringing to mind the function of certain interneurones in crustacea (Hughes & Wiersma, 1960) and echiuroids (Lawry, 1966).

The interneurone potentials are the largest recordable from the ventral cord and are located within the fastest conducting unit. There are no giant fibres in the leech nervous system. Faivre's nerve, the median connective, contains the largest fibre in the nervous system (Rohde's fibre), some 3 μ in diameter (Coggeshall & Fawcett, 1964), and as section of the median connective interrupts the fast pathway it is reasonable to suggest that this large fibre is involved. It should also be noted, however, that in some cases the records of fast activity appear to be of compound spikes (see Fig. 5 b). It is therefore possible that several units are involved, normally firing synchronously and only resolvable under certain conditions. It is also possible that the fast pathway is not a single simple unit that extends from end to end of the leech nerve cord. It may be segmentally arranged, as is indicated by the sometimes variable conduction time through the interposed ganglion (see Fig. 5 c), but directly synapsing on to the contiguous fibre, thus providing for a minimal delay in conduction.

Each section of the fast pathway can operate independently. That is to say input may
Function in leech nerve system

occur at any segmental ganglion. In cases where inputs are multiple, collision of spikes in the fast pathway when potentials are travelling in both directions must occur but has not been demonstrated (Hughes & Wiersma, 1960).

If the fast pathway is cut and stimulation is applied distal to that cut the largest potential disappears, but is sometimes replaced by a smaller one (see Fig. 8). This suggests that other pathways can be involved in the response, presumably located in the lateral connectives. It is probable that the fast pathway not only synapses with the motor fibres of the segmental ganglion, but also with other interganglionic fibres carrying slower information.

SUMMARY

1. Mechanoreceptors in the body wall of the leech Hirudo are stimulated by deformation of the animal's surface. They respond at all frequencies of stimulation up to about 50–60 Hz.

2. Light flashes, from a microscope lamp or an electronic flash source, are also a potent means of peripheral stimulation.

3. After peripheral stimulation impulses can be recorded in a fast central pathway. This pathway conducts equally well in the posterior to anterior and in the opposite directions.

4. Interference with either the right or left connective linking any two segmental ganglia does not interrupt the rapid conduction of these impulses.

5. Severance of the median connective or Faivre’s nerve interrupts conduction. This seems to implicate at least one, and possibly more, of the nerve fibres of this median connective in the rapid transmission of information from the extremities of the body.

6. A slower conducting pathway also exists in the nerve cord.

REFERENCES


