QUANTITATIVE MAPPING OF CUTANEOUS RECEPTIVE FIELDS IN NORMAL AND OPERATED LEECHES, LIMNOBDELLA

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(Received 24 January 1978)

SUMMARY

1. The receptive fields and physiological properties of the sensitive cutaneous mechanoreceptive neurones in the leech Limnobdella australis were found to be very similar to those previously described in Hirudo medicinalis.

2. Following separation from the central nervous system (C.N.S.), the distal dendrite stump and cutaneous receptive field remained unchanged for at least 160 days.

3. There was little spreading of receptive fields into regions of skin isolated from the C.N.S. for at least 184 days.

4. Cutting one dendrite of a mechanoreceptive neurone which has two major dendrites produced little change in the receptive field of the intact dendrite.

5. Abnormalities were found in most of the receptive fields of operated leeches, irrespective of the site of operation. These abnormalities were not seen in normal leeches.

INTRODUCTION

In the leech Hirudo medicinalis Van Essen & Jansen (1977) have shown that, following nerve section, distal dendrite branches of mechanoreceptive neurones survive and retain their function for many months. They also found that receptive fields adjacent to the denervated areas spread by small amounts into these areas.

In this report a quantitative method of mapping cutaneous receptive fields is described. It is used to confirm the persistence of normal function in mechanoreceptive dendrites isolated from their cell bodies in the leech Limnobdella australis. In addition, the spreading of receptive fields described by Van Essen & Jansen (1977) has been found to occur at sites far removed from the location of the operated nerves and the areas of skin they innervate. Two other types of receptive field abnormality in operated leeches and the presence of non- innervated areas in a normal leech are also described.
METHODS

Locally obtained leeches (*Lirnnoedella australis*, Williams, 1968) between 5 and 8 cm long were used for all experiments. Feeding was not necessary, survival for many months requiring only occasional replenishment of the water in which the animals were kept. Temperature was constant at 20 °C.

In the early experiments the leech saline contained 20 mM-Mg$^{2+}$ ions to block synaptic transmission which could otherwise lead to body wall contraction, so disrupting the mapping of receptive fields (see below) and causing activation of motorneurones by self-stimulation. However, this solution caused a decrease in cutaneous mechanoreceptive sensitivity of about 25% per hour. Once this was realized, saline was used without Mg$^{2+}$ ions. This greatly reduced the rate of deterioration of the preparation. The self-stimulation of motorneurones was overcome by increasing the tension in the stretched body wall preparation and wherever possible, removing the central ganglia from the preparation.

Composition of saline was (after Nicholls & Baylor, 1968): NaCl, 115 mM; KCl, 4 mM; CaCl$_2$, 1.8 mM; tris buffer at pH 7.4, 10 mM; glucose, 10 mM.

For operations to section the nerves animals were anaesthetized in 10% ethanol in water and pinned out on silicone rubber. A longitudinal incision was made on the side of the animal opposite to the region of skin supplied by the nerves which were to be cut. The nerves were then exposed by removal of small portions of the alimentary tract. Following operation, the leeches were kept in saline at 4 °C for about 1 week, then transferred to individual perforated containers in a large tank of water kept at 20 °C. The survival rate of operated leeches was above 90%, and all of the ganglionectomized leeches survived.

In the acute experiments leeches were anaesthetized, pinned out and longitudinally
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incised. Once the incision had penetrated the alimentary tract, the leech was pinned out flat, with the skin facing downwards. The viscera and internal muscles were removed. The rostral and caudal extremities of the animal were then removed and the remaining rectangle of body wall strung into a metal frame which fitted into the preparation bath (Fig. 1). When positioned, the outer surface (skin) of the body wall was horizontal and faced downward, with the peripheral nerves projecting from the upper surface. The upper surface of the saline in the bath was kept at the level of the body wall during recording, and at all other times was kept above that level. Action potentials were recorded in peripheral nerves using fine platinum hook electrodes. The underside of the body wall (the skin) was observed via two mirrors with a Zeiss binocular dissecting microscope. Drawings of the skin were made with a camera lucida.

The annular rings of the body wall were used as landmarks (see Nicholls & Baylor, 1968). The skin was stimulated by moving a stiff nylon hair with a rounded tip (diam. 300 µm) along the skin. The hair was mounted on the end of a Perspex rod which was freely hinged in a frame attached to a micromanipulator. The upward buoyancy force of the saline on the Perspex arm applied the tip of the nylon hair to the surface

Fig. 2. (a) The rapid adaptation of the response evoked in the dorsal nerve by step increase and decrease in force against the skin. Upper trace, record from the dorsal nerve; lower trace, potential difference across the solenoid with the arm in contact with the skin. (b) Two populations of action potentials recorded from the posterior root (upper trace) and the anterior root during mechanical stimulation along the skin. Third trace monitors the position of the hair tip. Action potentials are evoked only during movement of hair tip.
Fig. 3. Sequence (a): sensitivity at repeated stimulation along a line through a receptive field. Repeated stimulation leads to declining sensitivity with plateaus. Sequence (b): sensitivity levels with repeated stimulation along a different line through the same receptive field immediately after completion of sequence (a). Little decline in sensitivity is seen.

of the leech skin with a constant vertical force of 25 mg when the Perspex arm was horizontal. The hair was moved horizontally by manual operation of the manipulator. This was monitored with the aid of a potentiometer attached to the control knob.

RESULTS

1. Touch cells in unoperated animals

The properties of touch sensitive neurones (T-cells) of Limnobdella australis appeared to be very similar to those of Hirudo medicinalis (Nicholls & Baylor, 1968). A step increase or decrease in force applied to the skin evoked several action potentials in dendrites of T-cells (Fig. 2a). The response was rapidly adapting. Moving the stimulating hair across the skin produced a continuous response (Fig. 2b), suggesting every T-cell receives effective input from many points in the skin.

Repeated runs of the stimulator along one line through a receptive field evoked successively smaller numbers of action potentials from each annulus (Fig. 3a). The decay with repetition was approximately uniform at all points along the line mapped.
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Fig. 4. This figure shows the effect of velocity of scrape. Speed of skin stimulator hair tip across the most sensitive region of a dorsal receptive field plotted against number of impulses per scrape recorded in the dorsal nerve. Speeds used during receptive field mapping range from 0.25 to 0.55 cm/s. (indicated by dotted lines).

Fig. 5. Reproducibility of mapping technique shown by a single dorsal receptive field mapped twice. Mean number of action potentials per annulus traverse plotted. DM, Dorsal midline.
Fig. 6. Dorsal, lateral and ventral receptive fields of one side of a segment of a normal leech. Inset shows the outlines of receptive fields drawn on the surface of the skin. Fine vertical lines, interannular grooves; DM and VM, dorsal and ventral midlines; MsB, midsegmental boundary: the boundary between the anterior and the posterior root receptive fields; Ant., anterior. Contours on enlarged receptive field maps drawn at 0, 3.5 and 7 action potentials evoked per annulus traversed.

After 16 runs (i.e. four batches of four runs) further stimulation produced only very little further decline in the magnitude of the response. If stimulation was ceased for several minutes and then resumed, the response was found to have returned to the initial level. If the skin along one line was repeatedly stimulated so that this slow adaptation proceeded to the stage where the responses levelled out, and then the stimulation run along another line in the same receptive field, no further decline in
Sensitivity was seen during stimulation of this second line (Fig. 3b). Probably this is due to spread of the slow adaptation throughout the entire field (Van Essen, 1973).

Receptive fields were mapped by moving the hair along longitudinal lines in steps of one annulus. Velocities of the movements used ranged from 0.25 to 0.55 cm/s, because over this range the maximum variation of spike numbers in the response (counted on filmed records) was less than 15% of total spike number (Fig. 4). The variation in evoked activity with different stimulus speeds was calibrated before the most suitable speed was selected. The relevant innervation area was traversed four times along each line - twice in each direction. To prevent effects of adaptation on receptive field mapping intervals of 3 min between mappings along each line were used. The sensitivity (mean number of action potentials evoked per annulus traverse) at each annulus along every line was calculated and then marked on a camera lucida drawing of the skin as contour lines of action potential numbers (Fig. 5). These contours of sensitivity are used in all subsegment receptive field maps.

Three T-cell receptive fields were found on each side of every segment (Fig. 6). They are roughly elliptical in outline (major axis longitudinal) and they overlap extensively. The most dorsal receptive field is supplied by a single dendrite in the dorsal branch of the posterior root. The lateral and ventral receptive fields are each innervated by two major dendrites, one running in the anterior root (supplying the anterior half of the receptive field) and the other in the posterior root (supplying the posterior half). Sensitivity declines gradually away from the middle of the segment and there is extensive overlap with receptive fields of adjacent segments. At the
boundary between anterior and posterior root receptive fields, sensitivity declines very abruptly and there is no overlap of the receptive fields.

In two animals (2 out of 59 pairs of fields mapped) an anterior root receptive field finished one annulus more anterior than expected in the region of the central receptive field (Fig. 7 a). This was probably a deficiency of the ventral receptive field. Furthermore, in three leeches regions of skin were found from which no activity could be evoked while all the surrounding region was innervated normally. Two non-innervated regions were associated with the one annulus deficiency in the anterior root ventral receptive field. In another leech the non-innervated region was surrounded by dorsal nerve receptive fields (Fig. 7 b).

2. **T-cells in animals with peripheral nerves isolated from the C.N.S.**

(a) **Response of peripheral nerves to isolation from the C.N.S.**

In one animal three consecutive ganglia were removed from the C.N.S. The *dorsal* receptive fields were mapped in both ganglioneumctomized and normal regions 150 days post-operatively. The outlines of the receptive fields in all cases were normal (Fig. 8). In five of the six severed dorsal nerves, the responses of the T-cells throughout their receptive fields were also normal. Since the C.N.S. was removed, no regeneration could have occurred. These observations suggest a perfect survival of the severed
nerve branch of the T-cell. Similar observations were obtained in several animals over shorter times. A similar survival was observed in two posterior roots in another animal.

(b) *Response of one root to cutting of another root of the same hemiganglion*

In five animals anterior or posterior roots were cut. After a variety of post-operative periods the receptive fields of anterior root and posterior root T-cell fibres were mapped. Regeneration of proximal nerve stumps did not occur.

Animals in which two posterior roots of different ganglia had been cut were mapped 28 days and 184 days after the operation. In both animals anterior root receptive fields of the operated hemiganglia were slightly abnormal. They extended one annulus posteriorward over the midsegmental boundary in the region of the lateral receptive field (Fig. 9). Animals in which anterior roots were cut were examined 166 and 167 days post-operatively. In both animals the receptive field of the corresponding posterior roots extended one annulus anterior to the midsegmental boundary along most of its length.

In many animals operated on, abnormalities were found in the receptive fields of the anterior and posterior roots, even many segments from the site of operation. The most common abnormality (four out of five animals) was irregularity in the midsegmental boundary (Figs. 9, 10). In one case an insensitive region was found.
Fig. 10. Maps of anterior and posterior root receptive fields from an animal operated on 178 days previously, separated into two diagrams for clarity. Cut nerves have not been mapped. The anterior root (lower diagram) and posterior root receptive field maps of four consecutive ganglia have been separated for clarity. The receptive fields of ganglion (1) terminate abruptly at the incision. No growth across the incision has occurred. Abnormalities are shown in all midsegmental boundaries except for ganglion (3). There is overlap of anterior and posterior root receptive fields in ganglion (4). Contours drawn at 0, 3·5, 7 and 10·5 action potentials per annulus traversed. DM, Dorsal midline; VM, ventral midline.

The next most common abnormality was an anterior or posterior root receptive field that was smaller than usual (Fig. 9). In two of four cases the field was adjacent to an incision. In only two cases, however, was there overlap of the posterior and anterior root receptive fields (Fig. 10). As these abnormalities have never been found in normal leeches they were probably induced by the operation.

These results suggest that following root section the receptive field of the intact root of the pair of anterior and posterior roots spreads into what is normally the receptive field of the operated root, but no further than one annulus beyond the midsegmental boundary. No enlargement of other adjacent receptive fields into the region supplied by the cut nerve was observed. If the enlargements were small they would be difficult to detect due to the natural variability of receptive field size and shape.
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3. Responses of receptive fields to interruption by body wall incisions

In five leeches with body wall incisions the adjacent receptive fields were mapped. In every case in which an incision had interrupted a receptive field, the receptive field ended at the edge of the incision. Neither the scars nor the regions beyond the scars were sensitive to light touch (Fig. 10). All animals were at least 140 days post-operative. None of the receptive fields which abutted upon an incision showed an unusually high sensitivity in the region which pre-operatively would have been part of the contralateral receptive field. It is therefore unlikely that any compensatory growth of other T-cells occurs in response to removal of part of the sensory innervation of a region.

DISCUSSION

In 1968 Nicholls and Baylor identified 14 sensory cells within each ganglion of the leech Hirudo medicinalis. Three cells on each side of each ganglion were sensitive to very light mechanical stimulation (the T-cells), two were sensitive to more intense mechanical stimulation (P-cells) and two required very intense stimulation (N-cells). All of these neurones have centrally located perikarya and dendrites that can be traced for some distance along the peripheral nerves (Van Essen, 1973). Lent (1973) showed that two neurones have very similar properties in four species studied. In view of this, it is not surprising that the light mechanoreceptive fibres and their receptive fields identified in Limnoldella australis in this report are organized in exactly the same way as the receptive fields and fibres of the T-cells of Nicholls & Baylor (1968). No attempt was made to evoke responses from less sensitive mechanoreceptive neurones. On the basis of this evidence, it is highly likely that the fibres recorded from in the experiments comprising this report are T-cells with cell bodies located in the C.N.S.

1. Effect of cutting nerves

(a) Absence of degeneration

In the leech, cutting peripheral nerves so that the peripheral dendrite and sensory endings are separated from the cell body seemed to have no effect on the functioning of the receptors or dendrite up to at least 160 days following nerve section (Fig. 8). Nerve regeneration of T-cells was excluded by removing the C.N.S. ganglia. There are several interpretations.

Leech dendrites and cutaneous receptors may be quite unlike those in any other animal in so far as they are able to survive without any changes for very long times without a cell body. This seems improbable, and axonal degeneration has been seen in the leech C.N.S., although the central and peripheral nerves may differ in this respect (Frank, Jansen & Rinvik, 1975). The second possibility is that the distal dendrite stump and receptors are being maintained by one or more peripheral cell bodies. Neuronal cell bodies in the body wall of the leech were described by Bristol (1898), and Wilkinson & Coggeshall (1975) proposed that most of the fibres in the peripheral nerves arise from peripheral cell bodies, suggesting that there could be a great number of them. On the other hand, degeneration of an axon stump separated from its cell body does not occur in the leech C.N.S. (Frank et al. 1975). In that case the axon stump is perhaps supplied with the unidentified substances required for its
survival by a cell body with which the axon makes an electrical synapse in the adjacent ganglion. It is possible that such a mechanism is enabling the distal T-cell dendrite to survive.

(b) Response of one T-cell receptive field to severing of other axon

As shown more fully in Results, when an anterior (or posterior) root is cut, the receptive field of the intact posterior (or anterior) root of the hemiganglion persists. In four of five animals the only abnormality in these receptive fields was a small enlargement of the receptive field across the midsegmental boundary. Fig. 9 shows two ganglia in which the right posterior root has been cut 184 days previously. The anterior root fields of ganglion (1) are very similar, especially in amount of spread of the midsegmental boundary, although the corresponding posterior root of only one of them has been cut. Thus the slight spreading of the unoperated dendrite receptive field is probably not a direct consequence of cutting of the T-cell's other dendrite.

2. Absence of regeneration across the incision

T-cell axons are known to be capable of regeneration in the central and peripheral nervous systems (Jansen & Nicholls, 1972; Van Essen & Jansen, 1977). In the present study no regeneration of T-cell fibres across incisions was seen in any animal mapped in the region of an incision (Fig. 10). Also, the incisions themselves showed no sensitivity to light touch, in disagreement with Van Essen & Jansen (1977). Le Gore & Sparks (1971) found that body wall incisions in one species of leech were ultimately repaired by filling of the incision with fibrous connective tissue. Possibly such a connective tissue wall prevents regenerating T-cell dendrites from penetrating the denervated region. Prevention of regeneration by scar tissue has been reported for the leech c.N.s. (Jansen & Nicholls, 1972).

3. Spreading of receptive fields

In three situations discussed so far, regions of skin have been isolated from the c.N.s.: (1) when three consecutive c.N.s. ganglia were removed and the dorsal receptive fields mapped some time later; (2) where an incision cut a receptive field in two so that some of the nerve fibres of the receptive field were isolated from the centripetal dendrite; (3) where an anterior or posterior root was cut. In none of these cases did adjacent receptive fields spread across more than one annulus into these areas, and spreading of receptive fields by this amount occurred in unoperated areas. The absence of extensive spreading of adjacent receptive fields into skin isolated from the c.N.s. was not unexpected. The lack of degeneration of dendrites supplying these areas meant that they were not denervated and would not be expected to stimulate dendrite sprouting.

The finding of non-innervated regions in normal leeches suggests that either the developmental mechanisms that ensure total innervation of the skin are not maintained after the initial formation of the nervous system, or that the innervation pattern is also influenced by non-nervous structures in the body wall. The region surrounded by dorsal receptive fields (Fig. 7b) is associated with an abnormality in the organization of the annuli, making it very probable that this is a developmental
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4. Indirect field alterations

Although there appear to be only a few basic types of receptive field abnormality in operated leeches, the abnormalities bear no relation to the site of the body wall incision or to the lesion in the nervous system. Abnormalities occur in receptive fields far removed from the site of operation, and receptive fields which occupy the same relationship to the site of operation are abnormal in quite different ways. Since the location of these abnormalities may be some distance from the site of the lesion, they are called 'indirect' receptive field changes.

Although the indirect receptive field changes were variable, close similarity was found between the receptive fields of operated and unoperated T-cells (Fig. 9). This strongly suggests that cutting T-cell dendrites has no direct effect on the receptive fields of those dendrites and is not the stimulus which leads to the one annulus spreading of adjacent receptive fields.

This project was financed by the National Health and Medical Research Council of Australia, and the Departments of Applied Mathematics and Neurobiology of the Australian National University.

I am grateful to Professors R. Porter and A. K. McIntyre (Monash University), and R. F. Mark and G. A. Horridge (A.N.U.) for their support of this project.

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