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EXPANDED RECEPTIVE FIELDS OF CUTANEOUS MECHANORECEPTOR CELLS AFTER SINGLE NEURONE DELETION IN LEECH CENTRAL NERVOUS SYSTEM

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SUMMARY

1. Individual sensory neurones responding to touch (T) and to noxious (N) stimuli applied to the skin of the leech were killed by injecting pronase into their cell bodies, situated within the C.N.S. This procedure destroys one neurone in its entirety without damaging other cells.

2. When three out of four N cells within a ganglion have been killed, the receptive field of the remaining N sensory cell expands to cover the denervated area of skin. Similarly the field of the touch cell that innervates dorsal skin spreads across the mid line to innervate contralateral skin after the three touch cells on that side have been deleted.

3. The spread is graded and develops with time. The earliest effects appear within 4 weeks and the full spread develops by 3 months.

4. No detectable spread of receptive fields occurs if only two N cells, one on each side, are killed.

5. Following deletion of N cells, the receptive fields of T and pressure sensory cells are unaffected. Similarly, if T cells have been killed, the fields of N cells or pressure cells do not become enlarged.

6. These results represent a modality-specific mechanism by which one sensory cell can be influenced to extend the territory it supplies in the periphery in response to a minimal lesion without its own terminals having been damaged.

INTRODUCTION

A remarkable degree of precision is apparent in the way that individual sensory cells innervate the skin of the leech. A limited number of neurones of a given modality, touch (T), pressure (P) and nociceptive (N) with their cell bodies situated in the ganglia, supply the skin extending over more than one segment on the same side of the animal. The endings of each of these cells cover a well-demarcated area, defined by clear landmarks such as pigment bands and the borders of circumferential annuli (Nicholls & Baylor, 1968; Yau, 1976*a*, *b*; Blackshaw, 1981*a*, *b*). For example, the

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three T cells in a ganglion on one side have fields on the skin situated dorsally, laterally, and ventrally with only a limited degree of overlap. For the two ipsilateral N cells, a greater overlap exists, and their field extends from ventral mid line to dorsal mid line (Blackshaw, Nicholls & Parnas, 1982). Thus the skin resembles a patchwork quilt of overlapping receptive fields with different patterns for the modalities of touch and nociception. Interestingly the dorsal and ventral mid lines constitute extremely sharp boundaries where one can detect no sign of overlap: the T, P and N cells on one side of the ganglion cannot be activated by stimuli, no matter how strong, applied to the contralateral skin.

Questions that arise from this regular organization of the sensory endings in the skin concern factors responsible for determining the territory supplied by one particular cell. If an area of skin is deprived of its sensory innervation, will the fields of other neurones expand to take over the vacant territory as in salamanders (Aguilar, Bisby, Cooper & Diamond, 1973)? Can sprouting be induced in cells that have not themselves been damaged? And will denervation with respect to a single modality cause sprouting by cells of the same modality, or of different modalities, or both? Unlike the situation in vertebrates, section of peripheral nerves or destruction of cell bodies is not effective for depriving the skin of its sensory innervation, since distal processes of leech neurones can survive on their own. Instead, in the present experiments we have injected individual identified T or N cells in the C.N.S. with a mixture of proteolytic enzymes, pronase (Parnas & Bowling, 1977; Bowling, Nicholls & Parnas, 1978). This procedure allows a single neurone to be deleted in its entirety without damaging other cells. Accordingly, touch and nociceptive cells have been killed in intact leeches and the receptive fields of the remaining neurones examined several weeks or months later. A brief account of some of these experiments has been presented elsewhere (Blackshaw, Nicholls & Parnas, 1981).

METHODS

The techniques for recording from and stimulating N and T cells have been described elsewhere, as has the method for injecting pronase (Bowling, Nicholls & Parnas, 1978). In brief, electrodes filled with 4 M-potassium acetate were used to impale T, P or N cells. To inject pronase, electrodes were filled with 0·5 % pronase and 0·4 % fast green dye and 50 mM-KCl. Leeches were anaesthetized with chlorobutanol (0·15 %) and a cut approximately 1 mm long was made in the central annulus to expose the ganglion in which cells were to be injected. Several weeks or months later the preparation was dissected as shown in Fig. 4 of Blackshaw *et al.* (1982), removing the ganglion together with skin. The extent of the skin left on either side of the ganglion naturally depended upon the position of the cuts. On the side of the animal in which the fields of the remaining sensory cell or cells were to be explored, the skin extended across the dorsal mid line up to the laterally situated black stripe on the other side of the animal. Thus, the receptive field of a T, P or N cell remaining in the ganglion could be mapped extensively into the contralateral side. Inevitably, the extent of expansion across the ventral mid line could not be explored as effectively owing to the incision necessary for exposing the ganglion. At the time of the experiment, detailed exploration was made of the ganglion by visual inspection and by penetrating repeatedly with micro-electrodes to ascertain exactly which cells had been killed by pronase injection at the time of the operation. Ganglia that appeared otherwise damaged were not used for mapping fields.

To activate N cells, the skin was pinched with forceps or stimulated electrically by a suction electrode. Pulses of about 1 msec duration and 10 V, about three times stronger than that required for T cell activation, were adequate for stimulating N cells (Blackshaw *et al.* 1982). In unoperated animals, pulses of more than 50 V were quite without effect immediately on the other side of the

dorsal mid line and even severe mechanical stimuli applied there did not give rise to discharges in the N cell. For T cell activation, a piezo-electric crystal was used. Once again, no matter how strong the stimulus, T cells could not be activated from contralateral skin. The terms 'medial' N cell and 'lateral' N cell refer to the positions of these cells in the ganglion, not to their receptive fields (Fig. 1). In all experiments in which three N cells had been killed it was a laterally situated N cell that was spared. Each of the two N cells on one side supplies the ipsilateral skin from the dorsal mid line to the ventral mid line (Blackshaw *et al.* 1982). Hence, removal of a single N cell on either side does not effectively denervate skin.

RESULTS

Effects of killing N cells

To assess whether changes had occurred in the receptive fields of N cells remaining in the ganglion after others had been killed, the dorsal mid line was used as a convenient index. In control experiments, the field of the medial N cell was never seen to cross the dorsal mid line (twenty experiments); the field of the lateral N cell occasionally (three out of twenty experiments) extended across the dorsal mid line by about 0·3 mm or so in occasional patches but its field never reached the first contralateral orange stripe (Fig. 1*A*; Nicholls & Baylor, 1968; Blackshaw *et al.* 1982). Although the two N cells had fields that reached the ventral mid line, neither crossed it (Fig. 1*A*). Similarly in eight animals (Fig. 2) where one N cell on each side was killed, the fields of the remaining N cells were strictly ipsilateral. Owing to the overlapping receptive fields of N cells on one side of the ganglion, killing a single cell on one side would not be expected to produce full denervation.

In contrast, when three N cells had been killed (one lateral and two medial N cells), a spread of the receptive field of the remaining N cell was clearly evident (Fig. 1*B*). Because the skin had to be cut close to the ganglion, at the ventral mid line, in order to expose it, mapping was carried out in greatest detail over dorsal skin. Fig. 1*B* shows results obtained in an animal in which three N cells had been killed 95 days beforehand. At the time of the experiment, extensive survey of the ganglion indicated that only one lateral N cell remained. In this operated animal, the N cell receptive field covered a considerably larger area than normal, extending across the dorsal mid line to include the entire piece of skin on the contralateral side. In addition clear evidence of spread was seen across the ventral mid line in this and two other animals. At any point in the field the cell responded briskly to gentle squeezing of the skin or to electrical stimulation delivered by a suction electrode. While the threshold for mechanical or electrical stimuli was not obviously different in the normal field or in the expanded area, the latency in the expanded area became progressively longer; for example at two points, one on ipsilateral skin at the orange line and the other 4 mm away at the orange line on the contralateral skin, the delay increased from 80 to 140 msec, corresponding to a conduction velocity of 0·07 m/sec.

In this and all other animals in which three N cells were killed, and the remaining N cell field had expanded, the fields of the touch and pressure cells were also mapped. In no instance was there any spread across the dorsal or ventral mid lines.

The time course over which the spread of N cell receptive fields occurred was slow (Fig. 2). No spread was apparent at 30 days, while the full extent was achieved at about 90 days. Fig. 2 also shows that deleting two N cells, one on each side of the

ganglion, had no effect on receptive field size. Such experiments provided a control indicating that non-specific damage to skin over the ganglion did not play a role in field expansion. Moreover, in one experiment in which both N cells on one side of the ganglion were killed the fields of the two remaining N cells did expand into the denervated territory.

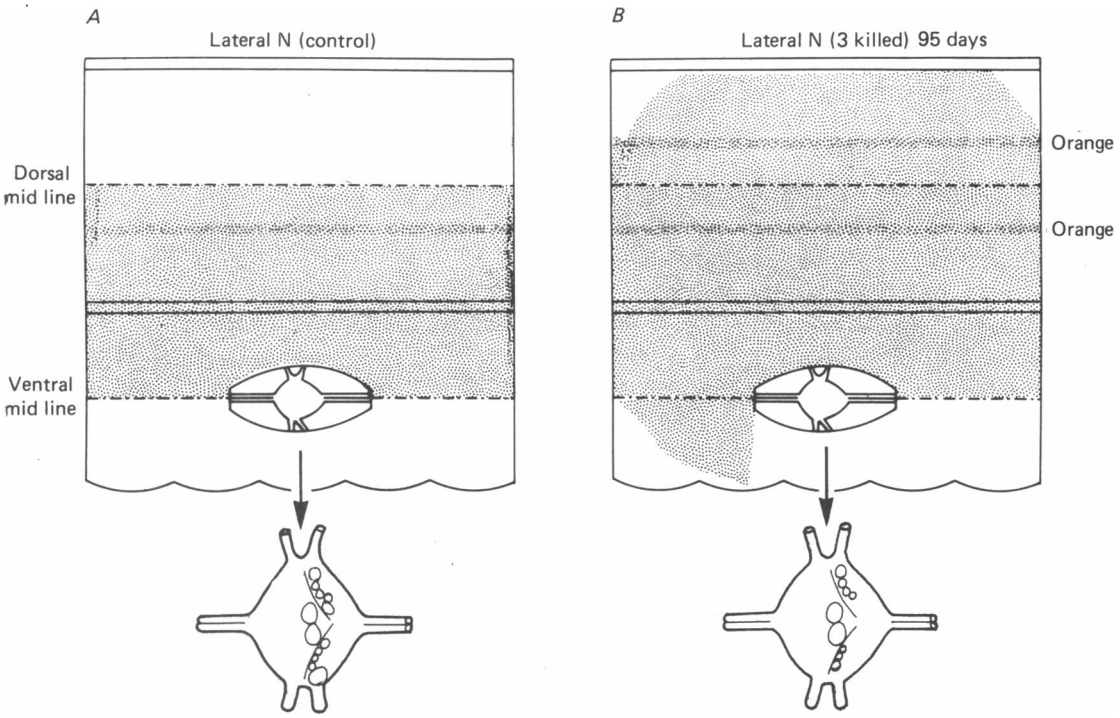

Fig. 1. Normal and expanded receptive fields of N sensory cells. *A*, schematic representation showing that each of the two N cells has a receptive field that extends from the dorsal mid line to the ventral mid line in normal animals. *B*, expanded receptive field of a lateral N cell in a ganglion in which the three other N cells had been killed 95 days beforehand. The cell innervated almost the entire territory including contralateral skin up to the edge, an area it does not normally supply. Some spread occurred across the ventral mid line but this region was inevitably damaged during dissection. Thresholds for electrical and mechanical activation were similar in the normal and expanded fields. The fields of T and P cells in this preparation did not cross the dorsal mid line.

Spread of touch cells

In experiments on T cells, for reference we again used the dorsal mid line to test for expansion of receptive field size. Normally the T cell innervating dorsal skin spreads up to but not beyond the dorsal mid line (Fig. 3*A*). This was shown by careful mapping with a fine stylus driven by a piezo-electric crystal. In seven control experiments, very small shifts of the crystal showed that the field stopped abruptly at the dorsal mid line. In contrast, in five operated animals in which three T cells had

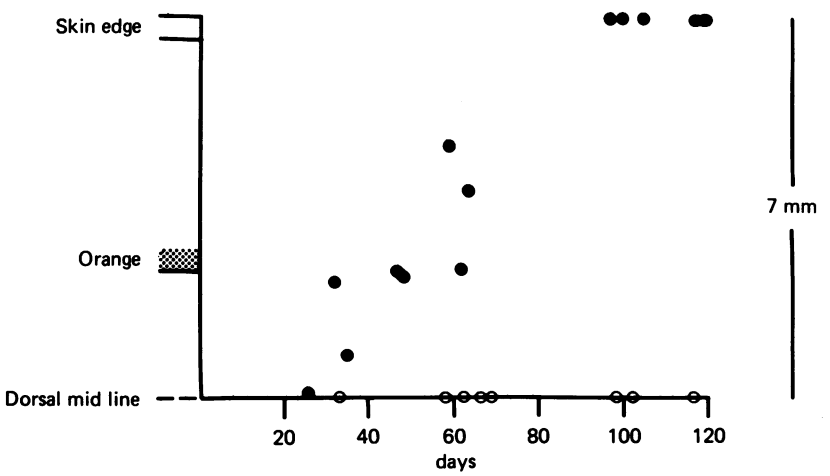

Fig. 2. Time course of spread of N cell receptive fields across dorsal mid line. Abscissa: time in days after killing two (○) or three (●) N cells. Ordinate: extent of spread past dorsal mid line into contralateral skin (see Fig. 1). In experiments in which only two N cells were killed, one was on each side of the ganglion.

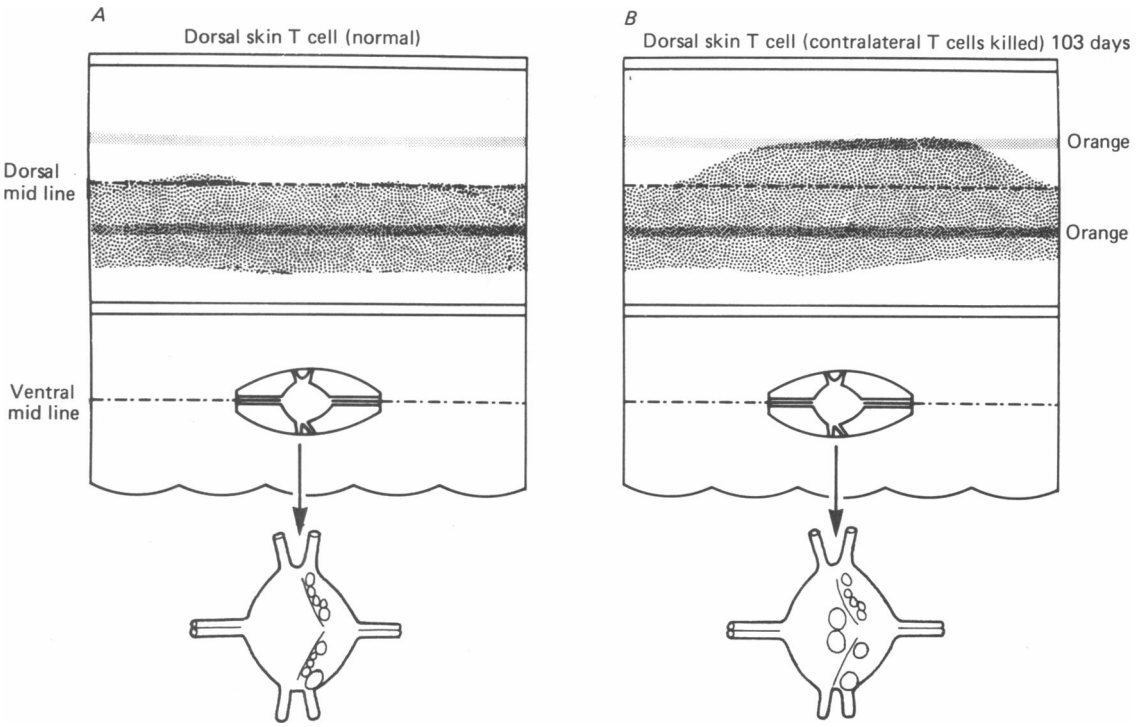

Fig. 3. Expansion of T cell fields following deletion of three contralateral T cells, *A*, normal receptive field of T cell innervating dorsal skin. *B*, 103 days after deletion of contralateral T cells, the field of the dorsal T cell had expanded across the dorsal mid line to reach the contralateral orange stripe. Sensitivity of light touch was similar in normal and expanded territories. No expansion of N or P cell fields occurred.

been killed on one side, the field of the dorsal T cell on the other side clearly expanded to cross the mid line. Two types of expanded fields were seen. Thus in three animals a broad sensitive front was detected between the dorsal mid line and the contralateral orange line (Fig. 3*B*). In two other animals, fingerlike extensions developed; these were long and narrow and could reach almost to the edge of the piece of skin (Fig.

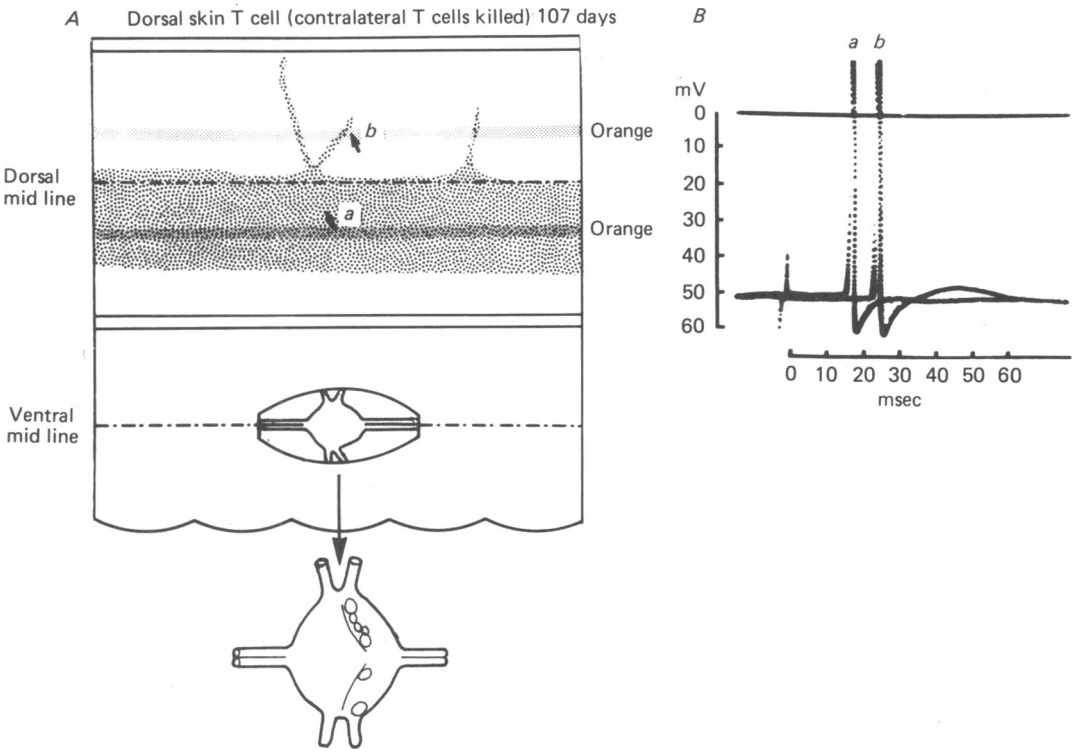

Fig. 4. *A*, expanded receptive field of dorsal T cell showing finger-like extensions beyond the dorsal mid line 107 days after three contralateral T cells had been killed. Light touches applied by a piezo-electric crystal activated the T cell with progressively longer delays. *B*, *a* is the action potential recorded in the soma after touching ipsilateral skin at the orange line; *b* is the action potential initiated by touching the contralateral orange stripe.

4*A*). To detect such fields, careful positioning of the stylus was required, and with small movements to left or right, responses were not obtained. The example given in Fig. 4*A* was obtained 107 days after deleting three T cells on one side.

At greater distances from the mid line there was a corresponding increase in delay of the action potentials recorded in the soma. In Fig. 4*B* the short delay action potential was evoked by stimulation of the ipsilateral orange line (*a*) and the second action potential (*b*) by touching the contralateral orange stripe. The distance between the two points of stimulation was about 4 mm, indicating a conduction velocity of about 0·55 m/sec.

No spread of N or P cell fields was observed in any experiment in which only T cells had been killed.

DISCUSSION

Fett (1978) and others (D. A. Baylor, S. Miyazaki & J. G. Nicholls, unpublished) have in the past tested for enlargement of the fields of sensory cells in response to 'denervation' of an adjacent area produced by cutting nerve roots. At best, the effects were small, probably because the peripheral axons did not degenerate (Van Essen & Jansen, 1977). With pronase, however, which deletes the entire arborization of a cell, the spread of the field of the remaining neurone became apparent and unequivocal. A weakness in the present studies is that because of technical difficulties, we have not yet succeeded in demonstrating anatomically spread of N or T cell terminals. It has proved difficult if not impossible to fill processes at such a distance from the site of injection in the cell body, and the dark pigmentation of the skin makes it difficult to distinguish axons filled with HRP. Hence, while the fields mapped physiologically and by electrical stimulation plainly expanded into new territory, we cannot be sure whether this represents true sprouting or the unmasking of sensory terminals previously 'silent'. The finding of the finger-like processes, which become longer in time, makes the first possibility more attractive. In previous studies on spread of motor fields in the leech (Bowling *et al.* 1978), it was also not certain whether the expansion represented true sprouting.

Of particular interest is the specificity, within a modality, of the mechanism that is responsible for causing the field of a cell to spread. The area of skin innervated by an N cell is also innervated by T and P cells. Yet removal of three N cells led to expansion by only the remaining N cell, and not by T or P neurones. We still have no information about the nature of the stimulus. One possibility is that an area denervated with respect to a particular modality produces a factor that leads to sprouting (Nixon, Jackson, Diamond, Foerster & Diamond, 1980). Alternatively, cells of a given modality might inhibit sprouting by other cells of the same modality.

Another factor that may play a part is damage. Thus, Scott & Muller (1980) observed sprouting within the C.N.S. of the leech by a neurone, the S cell, when a single neurone, the S cell in the adjacent ganglion, was eliminated by pronase. But this occurred only if the surviving S neurone had been damaged at some point on its surface: without such damage its processes could not sprout into 'vacant' territory.

In the present experiments and in those of Bowling *et al.* (1978) inevitably the skin of the leech had to be cut during the initial operation in which pronase was injected. As a result the axons of sensory and motor cells were damaged. Even though the incisions were small, extending for 1 mm or less, some axons of the N cells, whose fields extend from dorsal mid line to ventral mid line, must have been severed. In one series of experiments, however, no overt damage was caused to the neurone under investigation: the T cell that innervates dorsal skin has a field situated dorsolaterally, at a distance from the ventral mid line. And yet this cell showed clear sprouting across the dorsal mid line although its axons had not been cut. Experiments in which connectives were cut (or crushed) in order to produce massive damage did not show faster or larger expansion of fields either for the N cells or for the T cells. These results indicate that whatever factors are involved damage is not a prerequisite for sprouting by sensory neurones innervating leech skin.

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