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Unequal Competition between Axons for Neuronal Targets

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Unequal competition between axons for neuronal targets

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SUMMARY

Precise wiring of the nervous system depends not only on a matching between neurons and their synaptic targets, but also upon competition between neurons for particular targets. Neurons in adult leeches regenerate synaptic connections with their usual neuronal targets in the central nervous system, selecting only those targets with which they connect during embryogenesis. Thus during development axons of nociceptive (N) sensory cells make contacts on the cell bodies of certain neurons in adjacent ganglia but not upon those same types of cells in their own ganglion. After injury the N cell axons accurately regenerate contacts on the appropriate target cells. An abnormal feature observed after injury is that N cell axons sprout and grow to make contacts upon cell bodies within their own ganglion. This is a consequence of the normal innervation of those cells having been removed, thereby eliminating the source of competition. Similar competition during embryogenesis may guide the formation of selective connections.

1. INTRODUCTION

As the nervous system first wires itself together the connections formed are only approximate. This initial coarse wiring appears to depend largely upon an inherent matching between growing neurons and their targets. It is then followed by a fine tuning of that initial wiring, owing in part to a competition between terminals innervating common targets. Thus there is sprouting and retraction of axon terminals to form the final pattern of innervation. Much of our understanding of this process stems from studies of the vertebrate visual and neuromuscular systems (Hubel *et al.* 1977; Landmesser 1986; Rakic 1977, 1981; Fraser & Hunt 1980; Gaze 1970; Landmesser 1980), in which topographic relations and projections between neurons within populations reveal the accuracy with which connections form. Competitive interactions between pre-synaptic motor terminals, for example, appear to be mediated by the post-synaptic target (Grinnell *et al.* 1979), and in the visual system such competition evidently involves post-synaptic transmitter receptors (Constantine-Paton *et al.* 1990; Udin & Scherer 1990). Two important questions have been to what extent do similar mechanisms operate in the adult, and can they play a role in restoring function to the injured nervous system through accurate regeneration.

It has been commonly considered that competition and related 'plastic' phenomena are the exclusive domain of vertebrate nervous systems (Easter *et al.* 1985), but many experiments particularly in the past decade have shown that distinctions between phyla cannot be drawn so easily. Similar processes operate within vertebrates and invertebrates (Murphey 1986). It is in part differences in scale that have produced a different emphasis in studies of invertebrates. The relatively small numbers of neurons in many invertebrate nervous systems permit identification of individual neurons, based on their sizes, shapes, locations within the nervous system, and electrical properties. Consequently, it is known in invertebrates that connections form accurately between individual neurons and even between particular portions of those cells (Jacobs *et al.* 1986; Macagno *et al.* 1973; Kandel 1976; White *et al.* 1976; Muller *et al.* 1981). But are those connections simply 'hardwired'?

To take just one invertebrate as an example, in the leech we know that during embryogenesis the formation of peripheral neuronal projections and, consequently, neuronal connections is influenced by a variety of competitive interactions (Macagno *et al.* 1990). With focal lesions and cell deletions it is possible to alter the normal peripheral connections in a reliable fashion, so that denervated targets become innervated not at random but by homologues of their usual presynaptic inputs. In the adult periphery there is a capacity for accurate regeneration of connections (Van Essen & Jansen 1977) and for sprouting or shifting of those connections (Blackshaw *et al.* 1982*b*; Bowling *et al.* 1978).

It appears that mechanisms similar to those directing peripheral connections underlie the formation of central connections. However, the complex organization of the neuropile in each segmental ganglion of the leech and the large number of possible connections, even in an animal with only 400 neurons per ganglion, have limited analysis of formation and regeneration of

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central synaptic contacts and connectivity. After nerve injury there may develop persistent changes in synaptic transmission, including a shift in the balance of excitation and inhibition (Jansen *et al.* 1974), and the changes may be accompanied by axonal sprouting (Wallace *et al.* 1977). It has been known since the work on leech sensory neurons of Baylor & Nicholls (1971), who were the first to show in any organism that connections could regenerate precisely between two neurons, that qualitative changes in connectivity may occur even when regeneration is successful. A key question has been whether it is neuronal injury *per se* or target denervation which causes sprouting and alters central contacts. This paper addresses this question by analysing a distinctive set of axosomatic contacts of nociceptive (N) sensory neurons in the leech.

2. AXOSOMATIC CONTACTS OF N SENSORY NEURONS IN THE LEECH

The mechanosensory neurons in the leech are among the best studied neurons in the animal (Blackshaw 1981). The N sensory cells resemble other mechanosensory neurons in that they synapse with particular targets in the neuropile of their own ganglion and of adjacent ganglia (Elliott & Muller 1983; Jansen *et al.* 1974; Muller & McMahan 1976; Muller & Nicholls 1974; Nicholls & Purves 1970). Their synapses are made at swellings or varicosities along axonal branches, but without special staining and use of electron microscopy it is difficult to identify particular terminals with particular targets (Macagno *et al.* 1987). In addition to the contacts that the N cells make within the neuropile they wrap processes around the somata of certain neurons in adjacent ganglia to form baskets that are visible with light microscopy (Johansen *et al.* 1984; Muller *et al.* 1978; French & Muller 1986). Electron microscopy has shown that axons and the somata they wrap are in direct apposition, and physiological evidence shows that the wrappings are excitatory (French & Muller 1986). It was previously found that somata of various neurons are sensitive to neurotransmitters (Sargent *et al.* 1977). Axosomatic contacts also exist in other invertebrates, where they can be sites of synapses in the adult (Shkolnik & Schwartz 1980; Zs.-Nagy & Sakharov 1969) and may occur transiently in the embryo (Macagno 1981; Schacher *et al.* 1979; Taghert *et al.* 1982). In the leech the contacts are remarkably specific: N cells will wrap the somata of other N cells, pressure (P) sensory cells, and Leydig cells, but not other somata (figs 1 and 2). The lateral N(*l*N) cells do not wrap somata in their own ganglion, and this additional specificity is investigated in this paper.

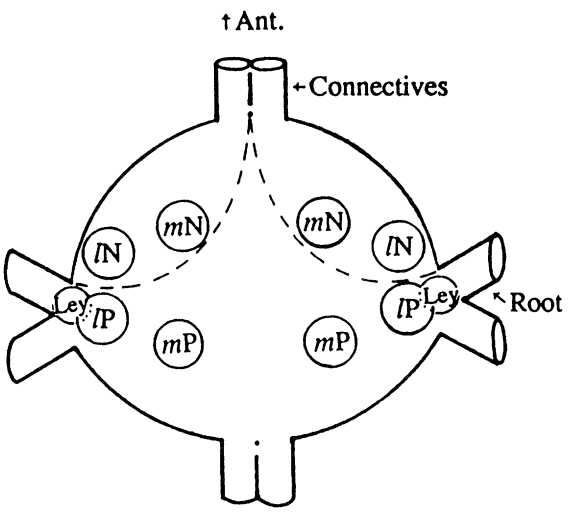

Figure 1. Diagram of ventral aspect of ganglion showing approximate locations of somata ordinarily wrapped by axons of *l*N cell. Wrapped somata are usually situated only in other ganglia, anterior and posterior to the *l*N cell's ganglion. The axosomatic targets are the medial and lateral pressure (*m*P and *l*P) sensory cells, the medial and lateral nociceptive (*m*N and *l*N sensory cells and the Leydig (*Ley*) cells. Ganglia are typically 0.5 mm in diameter, contain 400 neurons, and are linked to one another by 5 mm long connectives. *Ant.*, anterior. (After Gu & Muller 1990.)

3. MATERIALS AND METHODS

(*a*) *Maintenance and operations*

Leeches (*Hirudo medicinalis*) were obtained either directly from suppliers in France and Germany or bred in the laboratory (McGlade-McCulloh & Muller 1989), fed periodically with bovine blood, and maintained at 19 °C in artificial spring water (see Appendix C of Muller *et al.* (1981). When connectives were to be severed, animals were anaesthetized with 8% ethanol in spring water, and small incisions were made in the ventral midbody region superficial to the nerve cord, typically at segmental ganglia 9 (G9) and 13 (G13) when ganglia were to be surgically isolated. Connectives were cut, either singly or in pairs, on both sides of the ganglion after the surrounding blood sinus was opened. For regeneration studies either a single connective was cut or both connectives were crushed through the sinus with fine forceps just posterior to a ganglion. Regeneration of axons anteriorly into the ganglion was studied. For other operations, in which identified cells were to be killed by protease injection, animals were anaesthetized for 25 min in 15 mM chlorobutanol dissolved in artificial spring water, ganglia were exposed in a bath containing leech saline (115 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, and 10 mM Tris maleate, adjusted to pH 7.4 with NaOH), and particular N cells were injected with protease (Sigma, Type VIII, 0.2% in 0.2 M KCl containing 0.4% Fast Green FCF dye) under pressure (Bowling *et al.* 1978). Protease injection destroys the entire neuron without damaging other cells or axons in the vicinity of the killed neuron (Bowling *et al.* 1978; Scott & Muller 1980). Animals were allowed to recover in leech saline 1 or 2 days and then transferred to artificial spring water at 16 °C.

(*b*) *Intracellular labelling*

Regenerated *l*N cells and those in ganglia denervated by severing connectives or by killing cells with protease were examined after periods up to more than one year after the operation, although in most cases the interval

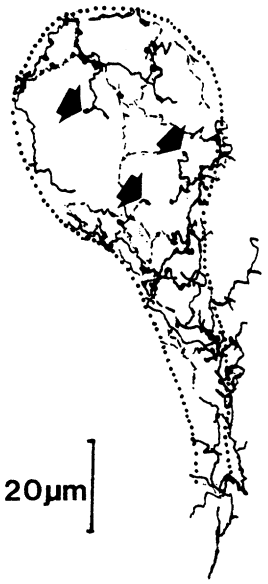

Figure 2. Camera lucida drawing of a P cell (dashed outline) wrapped by axons of an N cell injected with HRP two days earlier in a neighbouring ganglion. Axon branches on the reverse aspect of the cell are drawn dashed. Varicosities are shown with arrows. The N cell axons project along the axon hillock of the P cell to reach the soma.

was from 2 to 6 mos. To determine the extent of typical axonal projections and regenerated projections into adjacent ganglia, N cells were injected with horseradish peroxidase (HRP, Sigma Type VI, 2% in 0.2 M KCl and 0.2% Fast Green FCF) two days and again one day before fixation (Elliott & Muller 1983). To view each of the two *l*N cells in a single ganglion separately and in detail, one cell was usually injected with 0.1 M 5,6-carboxyfluorescein (CF) under pressure and viewed live, before fixation, while the other was injected with HRP and viewed after fixation. For studies of sprouting, HRP was injected one day before fixation to ensure dense staining throughout the cell within the ganglion. Epifluorescence optics (Zeiss filter set 472209 and 10x Neofluor objective, 0.3 N.A.) were used to view briefly the fluorescent axosomatic wrappings. Just before fixation, the pressure (P) cells and medial N cells, the potential targets, were typically marked by intracellular injection of 4% Procion Navy Blue dye after being identified electrophysiologically; this permitted unequivocal identification of P and Leydig cells and of T and N cells in whole mounts of fixed ganglia (French & Muller 1986). Cells from control and experimental leeches were labelled in the same manner.

Preparations were fixed for 30 min at room temperature in 1.6% glutaraldehyde, 0.8% paraformaldehyde, and 5 mM $CaCl_2$ in 0.2 M sodium cacodylate buffer, pH 7.4 (Muller & Carbonetto 1979). Tissue was then processed with 3,3′-diaminobenzidine and H_2O_2 as substrates for the HRP, and mounted whole as previously described (Muller & McMahan 1976). Contrast was enhanced and depth of focus reduced by viewing preparations by using a 40x water immersion objective (0.75 N.A.) with Nomarski interference optics.

4. REGENERATION OF SPECIFIC CONNECTIONS

This section reviews the work of French & Muller, who found that severed N cell axons can regenerate axosomatic wrappings around their usual targets (French & Muller 1986). The experiments therefore showed that the selectivity of contacts is basically preserved in the adult.

The experiments focused on the *l*N cells because it was found that they ordinarily wrap more somata in adjacent ganglia than the medial N sensory neurons (*m*N cells). Furthermore, although the *l*N cells wrap somata on both sides of the adjacent ganglia, ipsilateral wrappings are more prevalent and were exclusively studied for regeneration, although the presence of contralateral wrappings is important in studies of sprouting and specificity (see below). Examination of the incidence of wrapping particular cells indicated that ordinarily wrapping of each target cell is independent of wrapping the others. There was no evidence that this changed during regeneration.

Lesions were made near the target ganglion. Comparison of the somatic contacts made by the anterior regenerated axons in control and experimental groups revealed that the regenerated axons approximated, but did not replicate, the wrappings in intact preparations. Of 72 injured N cells, 23 grew axons into the ganglion anterior to the injured connective. In 14 of these, at least one cell soma was wrapped by the regenerated axons. The fraction of cells that regenerated axosomatic contacts (19%) was almost identical with that observed by using physiological methods to study regeneration of P and N cell contacts with the L motor neuron (Elliott & Muller 1983).

The regenerated wrappings tended to be simpler than normal in two ways. First, fewer cells than usual were wrapped by the regenerated axons. Second, although the amount of regeneration varied, the regenerated wrappings were typically less extensive and less branched than were controls. When leeches that had been maintained for up to a year after injury were compared with those that had been allowed only 6 weeks for recovery, no relationship was seen between complexity of wrapping and time for regeneration. This showed that regeneration of the axosomatic contacts was essentially complete after 6 weeks and neither expanded nor contracted when more time elapsed.

The cell most often wrapped by regenerating axons of the *l*N cell was the medial P cell (*m*P cell), one of the cells most likely to be contacted in normal leeches. However, the N cell somata, which are also usual targets, were contacted less if at all. In addition, a novel cell was frequently wrapped by regenerating N cells. It was a cell of the lateral margin of the posterior cluster of neurons, wrapped in 6 of the 14 preparations in which at least one soma was contacted by a regenerated axon. It may be significant that this cell lies along the pathway of the normal axons and therefore would be expected to be encountered by regenerating axons growing along the normal pathway. In more than 120 ganglia from unoperated

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Table 1. *Axosomatic wrappings by lN cell axons with ganglia*

(Modified from Gu & Muller (1990)

type of preparation	mean no. of wrappings	(%) cells sprouted	$N =$
ganglia fully denervated			
isolated ganglia	2.5	84	62
all 8 N cells killed in anterior and posterior ganglia	2.5	88	16
isolated ganglia, 2 weeks after isolation	1.1	70	10
ganglia half-denervated			
single anterior and posterior connectives cut, one side (injured *l*N/intact *l*N)	1.3/1.0	94/72	22
four N cells killed in anterior or posterior ganglia	0.9	50	24
four *l*N cells killed in anterior and posterior ganglia	0.9	47	15
four *m*N cells killed in anterior and posterior ganglia	0.1	13	8
roots cut	0.5	40	15
controls			
four Retzius cells killed in anterior and posterior ganglia	0.1	14	14
unoperated, anterior/posterior adjacent ganglia	3.1	90/94	62

leeches, this unusual wrapping was seen only once. The regeneration of the lateral N cell's somatic contacts therefore appeared to be highly selective, but the selectivity was somewhat different than in normal leeches.

In many cases multiple axons grew from the cell at the lesion. That the regenerated processes always bypassed several cells before turning and contacting the somata that would be wrapped is additional evidence that a process of selection was operating.

Morphological examination of varicosities along the somatic wrappings did not reveal conventional synapse specializations (Muller & McMahan 1976). However, they resembled, at least superficially, somatic contacts that are evidently sites of synapses in other invertebrates. Moreover, the transmitter sensitivity of somata and the ability of vertebrate neurons to release transmitter at synapses with reduced synaptic specializations (Geffen & Livett 1971) or without vesicles (Schwartz 1982) must be considered when evaluating synapse structure. This morphology, the physiology indicating that the wrappings are normally directly excitatory, and their ability to regenerate suggest that they are not simply vestigial.

A striking feature of the axosomatic wrappings of the *l*N cell is that they are selective for certain types of neurons, and only those in adjacent ganglia and not in the cell's own ganglion. The ability to regenerate indicates that mechanisms that operate during development may persist in the adult. Experiments in the next section examine the selectivity for wrapping somata outside the neuron's own ganglion.

5. TARGET DENERVATION INDUCES SPROUTING

(*a*) *Sprouting in isolated ganglia*

In considering the specificity of N cells contacts, an important question is, are *l*N cells capable of wrapping somata within their own ganglion? If so, are any wrapped somata homologues of their normal targets? In a series of experiments that will be reviewed in this section, we found that *l*N cells can be induced to sprout to wrap in their own ganglion the somata of homologues of their usual targets, and that denervation of the new targets rather than injury to the cell that sprouts is the primary cause of sprouting (Gu & Muller 1990). In this section new work is presented on the speed with which the sprouted axosomatic wrappings form.

It was hypothesized that *l*N cells might sprout axosomatic contacts within their own ganglion if they were prevented from making their usual contacts in other ganglia, or if target homologues in their own ganglion were made more attractive through denervation, or if a combination of the two occurred. Ganglia were therefore isolated from their neighbours by cutting the anterior and posterior connectives. Regeneration did not occur, because the ends of the muscular connectives pulled away from each other. Such operations had previously produced marked changes in the physiology of the isolated ganglion (Jansen *et al.* 1974).

Within two weeks after ganglion isolation, at least 70% of *l*N cells had sprouted axosomatic contacts within their ganglion (table 1), wrapping homologues of their usual axosomatic targets. The proportion making detectable wrappings rose to a plateau level of nearly 85% by 2 months, while the proportion making wrappings in adjacent ganglia was in the range of 90–95% of *l*N cells. Cells made an average of 1.1 wrappings at 2 weeks and 2.5 after 2 months (see also table 1). Eventually, wrapped somata were nearly evenly distributed on both sides of the ganglion, but at two weeks most of the detectable wrappings were ipsilateral. Because contralateral wrappings were farther from the site of dye injection, they might have been more difficult to detect. Only N, P, and Leydig cells were wrapped; it is not known whether *l*N cells wrapped their own somata.

The new and old axosomatic wrappings were similar in appearance. They branched as they coursed over the somata, and swellings or varicosities were present at intervals along those branches (figure 3*a*.) Electron micrographs did not reveal conventional synapses, but

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*l*N cell axons were in direct apposition to the target somata.

In the experiments just described, ganglia were isolated by cutting the connectives close to them, both anteriorly and posteriorly. The neighbouring ganglia, which had only one pair of connectives cut, and those cut at a distance, were also examined for axosomatic wrappings made by axons of their own *l*N cells (Gu & Muller 1990). From 2 to 6 months post-operative, the *l*N cells' axons wrapped somata in only 7% of ganglia ($n = 28$). At longer post-operative intervals, the *l*N cells in 33% of ganglia ($n = 33$) had sprouted axosomatic contacts, but this value was far below the more than 95% of isolated ganglia in which sprouting occurred. In a new set of observations at 2 weeks, when wrappings were observed in 85% of ganglia isolated by cutting close to the ganglion, other ganglia were isolated that had long connectives, cut near the adjacent ganglia. In those preparations, none of 4 *l*N cells examined had sprouted. As discussed below, denervation is the principal factor causing sprouting, and it may be that longer axons either degenerate more slowly or otherwise maintain innervation longer.

(*b*) *Sprouting in ganglia denervated by killing N cells*

Repair of neuronal connections in the leech may be facilitated by long-surviving distal segments of severed axons (Mason & Muller 1983). Such survival of axon segments is seen in various vertebrates and in other invertebrates (Atwood *et al.* 1989; Matsumoto & Scalia 1981; Cook & Wisniewski 1973; Hoy *et al.* 1967). Therefore to ensure that axons degenerate it is not always sufficient that they be cut. An effective approach has been to inject neurons with a protease (Bowling *et al.* 1978), and this was used by us (Gu & Muller 1990) in experiments summarized here.

As a direct test of the hypothesis that target denervation triggers sprouting, N cells in anterior and posterior adjacent ganglia were selectively killed by intracellular injection of a protease. In this way neither the sprouting neuron nor the cells that became wrapped were injured. When the four N cells in both ganglia on either side of a central ganglion were killed, the results were nearly identical with those obtained by isolating the ganglion, as summarized in table 1. Half denervation was approximately half as effective. The denervation was cell specific, in that killing cells other than the *l*N cell, such as the Retzius cells, produced essentially no sprouting.

(*c*) *Cutting only the left anterior and posterior connectives distinguishes effects of injury from denervation*

Although similar levels of axosomatic sprouting occurred following surgical isolation of ganglia or selective killing of *l*N cell inputs, another test was made to determine whether either injury of the sprouting neuron or of the target produced sprouting (Gu & Muller 1990). The basis of the test was the following set of observations. Each *l*N cell usually wraps somata on both sides of the ganglion, its axon projects only along the ipsilateral connective to the adjacent ganglia, and the target neurons also project their axons ipsilaterally. Thus cutting the left connective injures only sensory cells on the left, but denervates cells on both sides, left and right. It was found that both uninjured and injured cells sprouted to wrap somata on both sides. This result was consistent with denervation induced sprouting. As shown in table 1, injured cells sprouted 30% more than uninjured, but it was not clear whether this difference was significant. Moreover, injured cells were closer to more heavily denervated targets, which could account for small differences. Cutting connectives only on one side both anterior and posterior to a central ganglion effectively caused half the denervation of completely isolating the ganglion. Significantly, the level of sprouting was half that seen after complete isolation.

6. SPROUTING DESPITE PERSISTENCE OF AXOSOMATIC WRAPPINGS

Several lines of evidence suggested that sprouting occurs after degeneration of the normally innervating axons. Furthermore, in the present study sprouting was observed within 2 weeks of isolating ganglia. Consequently, a direct test was made for the persistence of axon segments within the isolated ganglia.

The four N cells in one ganglion were injected with HRP within the animal and a week later, after the HRP had diffused throughout the cells, the next posterior ganglion was isolated by severing its connectives. On examination 36 days later, HRP-filled processes were observed wrapping both *l*P somata and one *l*N soma within the isolated ganglion (figure 3*b*). In the same ganglion both *l*N cells were injected with CF and had sprouted wrappings around an *m*N soma and around the *l*P somata that remained wrapped by the axon segments. Sprouts around the *l*N somata would not have been visible, since they were both filled with dye.

The long-term survival is consistent with observations of other leech axon segments, mentioned above. It is not known whether some axons or portions of axon had, in fact, degenerated, but surviving axon segments are reported to remain capable of signalling and even of releasing transmitter at their synaptic terminals. In fact the thin, somewhat smooth appearance of the isolated segments did not look entirely normal. Conceivably it is a reduction in transmission or an interruption in the movement of molecules along the axon rather than its outright degeneration that is crucial to permit sprouting of *l*N cells' axosomatic wrappings within their own ganglion. However, it is possible to conclude that sprouted wrappings can occupy the target somata with the original wrappings. It is not known if the new wrappings follow precisely the old.

7. LIMITED SPROUTING INDUCED BY INJURY

The results of Gu & Muller (1990) indicate that sprouting of *l*N cells within ganglia can be entirely accounted for by denervation of the targets rather than

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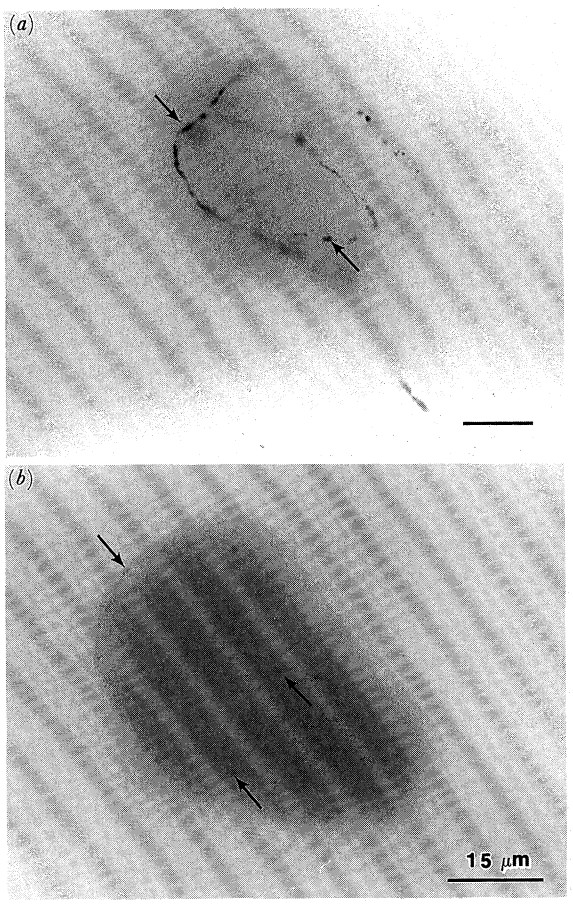

Figure 3. Axosomatic wrapping within an isolated ganglion. (*a*) the *l*N cell axon sprouted to wrap the soma of an *m*P cell in a ganglion isolated $4\frac{1}{2}$ months previously. Sprouted axosomatic wrappings resemble the cell's usual wrappings around N and P somata in neighbouring ganglia. The dark axons of the *l*N cell, injected with HRP, stand out against the blue-stained P cell, a homologue of the usual targets. (*b*) Within isolated ganglia the distal segments of the original axons projecting from adjacent ganglia persist for over a month. The N cells in a neighbouring ganglion were injected intracellularly with HRP a week before their axons were severed near the isolated ganglion and a month later the P and N cells in that ganglion were injected with Procion navy blue, fixed, and the tissue reacted with diaminobenzidine. Persistent axon segments (arrows) retained their HRP and stained. The axons, which are continuous, appear fragmented in (*a*) and (*b*) because of the shallow focal plane.

by injury to the sprouting neurons or to those targets. At first glance this would appear contrary to the conclusion of Blackshaw and colleagues (Bannatyne *et al*. 1989), who reported sprouting of N cells within the ganglion after cutting the ganglion roots rather than connectives. Cutting roots would not be expected to denervate the wrapped somata but would injure targets and the cells that sprouted. Unfortunately, no distinction was made between *m*N cells, which typically wrap somata in their own ganglion, and *l*N cells, which do not. Moreover, the axons of the wrapped targets were evidently also severed. But it also seemed conceivable that with sufficient injury to the *l*N cells, such as severing its largest axons as occurs when the roots are damaged, the cell might be induced to sprout axosomatic baskets within its ganglion.

We have therefore repeated similar experiments, chiefly cutting roots on one side of a series of 12 ganglia in 4 leeches, examining *l*N axons within those ganglia. Of 15 injured *l*N cells, 6 had sprouted to form a total of 7 axosomatic wrappings, an average of 0.5 baskets per cell, compared with 2.5 in isolated or denervated ganglia and 3.1 in unoperated adjacent ganglia. One *m*N, 3 *m*P, 2 *l*P, and one Leydig cell somata were wrapped. Only 40% of injured cells sprouted, compared with 85% of uninjured cells that sprouted during a similar period upon denervation of the ganglion. In the same ganglia 9 *l*N cells whose root axons were not cut did not sprout. Two out of the seven wrapped somata were contralateral and were not injured when the root was cut. Six out of the 15 *l*N cells had had both root axons cut and two of these sprouted, while the remaining four that sprouted had had either their anterior (3) or posterior (1) axon cut, suggesting that no significant distinction could be made between the operations.

Overall, then, injury to the *l*N cell's largest axons appears to induce some sprouting. While minor compared with the sprouting induced by denervation, injury may have accounted for the 30% increased sprouting of injured *l*N cells when connectives were cut on only one side of the connectives (see table 1 and §5).

8. CONCLUSIONS

The axosomatic wrappings *l*N cell axons make in adjacent ganglia can be plainly seen in the light microscope, so that one can readily determine which cells are contacted and which are not. They have thus provided useful information on the specificity with which contacts between neurons regenerate and on triggers of sprouting.

The central sprouting of *l*N cells is apparently similar to sprouting by the same cells in the leech periphery, where denervation rather than injury also appears to be the chief stimulus (Blackshaw *et al*. 1982*a*). Experiments in the leech periphery have relied on functional rather than morphological measures, and therefore complement the present results. In the periphery, however, the regions innervated are those closest to the neuron, in contrast to the curious failure of *l*N cell axons to wrap apparent targets that are closest to them. The reason for this preference remains obscure.

Axonal sprouting in the leech is similar to sprouting reported in various other adult systems, ranging from salamander skin to mammalian brain (Cotman *et al*. 1981; Scott *et al*. 1981; Diamond *et al*. 1976; Yoon, 1972; Raisman, 1969). Of particular interest is sprouting in the red nucleus (Nakamura *et al*. 1974; Tsukahara *et al*. 1975), where inputs were in some cases redistributed on the target cell.

Some support was found in this study for reports that injury might trigger sprouting of N cell wrappings within their ganglia (Bannatyne *et al*. 1989), but the effects of injury in the present study were small compared with effects of denervation. It is interesting that injury triggers sprouting in an interneuron in the leech, the S cell, which will sprout an intact axon when

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that axon is denervated by killing the target, but sprouting occurs only when another axon of the S cell is severed (Muller & Scott 1980; Scott & Muller 1980).

The sprouting of axosomatic contacts to wrap specific targets may also be compared with development of selective connections in vertebrates. The N cells' sprouting new wrappings without retracting the old is in some respects similar to sprouting in the vertebrate visual system during development (Miller & Lund 1975) and regeneration (Yoon 1972), although the mapping between retina and target is not fixed. However, in other systems incorrect target innervation occurs only when the usual targets are missing (Farel & Wray 1989).

Overall the results in leech and in vertebrates might be interpreted in terms of a hierarchy of target suitability. There is a matching between N cell axons and their axosomatic targets, but the final pattern of wrappings results from an exclusion or unequal competition in which axons from N cells in adjacent ganglia triumph.

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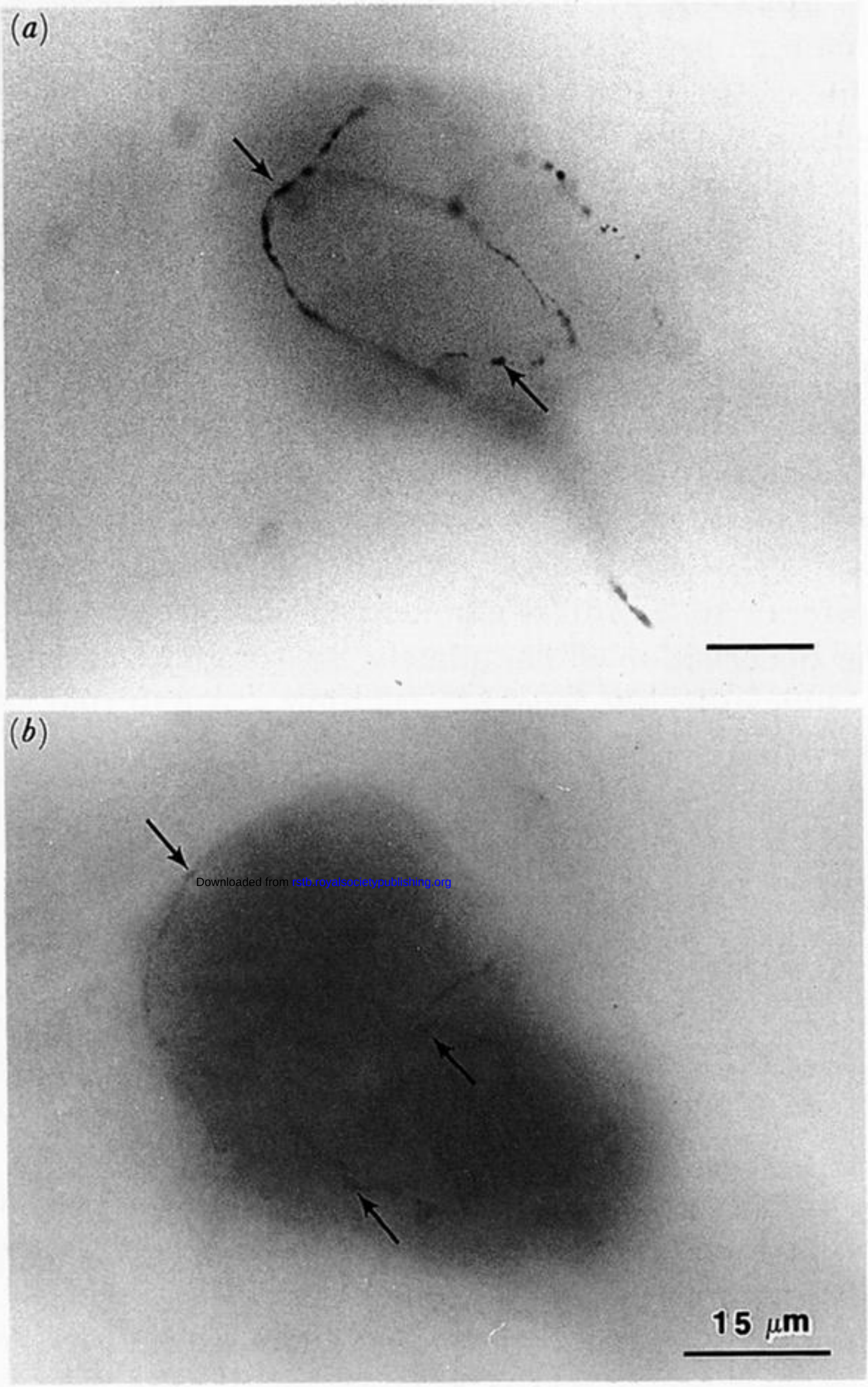
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



igure 3. Axosomatic wrapping within an isolated ganglion.) the *l*N cell axon sprouted to wrap the soma of an *m*P cell a ganglion isolated $4\frac{1}{2}$ months previously. Sprouted xosomatic wrappings resemble the cell's usual wrappings round N and P somata in neighbouring ganglia. The dark xons of the *l*N cell, injected with HRP, stand out against the ue-stained P cell, a homologue of the usual targets. (*b*) Within isolated ganglia the distal segments of the original xons projecting from adjacent ganglia persist for over a onth. The N cells in a neighbouring ganglion were injected ntracellularly with HRP a week before their axons were vered near the isolated ganglion and a month later the P nd N cells in that ganglion were injected with Procion navy ue, fixed, and the tissue reacted with diaminobenzidine. ersistent axon segments (arrows) retained their HRP and ained. The axons, which are continuous, appear fragmented (*a*) and (*b*) because of the shallow focal plane.