# SHORT COMMUNICATION

# STIMULATION OF NEURITIC OUTGROWTH IN AN UNDAMAGED MOLLUSCAN INTERNEURONE

### By P. ALLISON AND P. R. BENJAMIN*

*Medical Research Council Neurophysiology Group, School of Biological Sciences, University of Sussex, Falmer, Brighton, Sussex, BN1 9QG, UK*

Axotomy of neurones from a variety of invertebrate groups induces sprouting and regrowth of neuritic processes (reviewed by Anderson, Edwards & Palka, 1980). However, direct surgical damage is not a necessary prerequisite for inducing sprouting. For instance, Altrup & Kolde (1982) and Bulloch (1984) showed that damage to non-neural tissue could induce sprouting and Pitman & Rand (1981) showed that neural lesions can cause dendritic outgrowth of an undamaged insect neurone. We report here that a similar phenomenon to the latter occurs in the gastropod mollusc *Lymnaea stagnalis*. We have previously shown that lesioning the axon of the giant pedal interneurone (RPeD1) induces sprouting and subsequent recovery of synaptic connectivity (Allison & Benjamin, 1985; Benjamin & Allison, 1985). Here similar techniques were used to lesion the *Lymnaea* CNS, but instead of crushing connectives containing the axon of RPeD1, we lesioned a part of the CNS not occupied by RPeD1 processes. This induced limited, but statistically significant, increases in the number of processes in the posterior three ganglia of the CNS, when normal and lesioned preparations were compared.

The site chosen for the lesion was the left pleural–parietal connective (arrowed in Fig. 1). This part of the brain does not normally contain neuritic or axonal processes of RPeD1 (Fig. 1). Crushing, without completely severing the connective, was carried out in anaesthetized snails (Allison & Benjamin, 1985). In the period from 2 to 59 days after the operation to crush the connective, brains were removed and the anatomy of RPeD1 compared in operated ($N = 26$) and normal ($N = 24$) cells by Lucifer Yellow injection (cf. Allison & Benjamin, 1985). The occurrence and numbers of fibres in peripheral nerves and connectives were examined in whole mount preparations (localization of anatomical regions and morphology of a normal RPeD1 shown in Fig. 1).

The most striking effects of the lesion to the left pleural–parietal connective were seen in the posterior part of the brain, where the percentage of cells extending into particular nerve trunks and connectives of the visceral and parietal ganglia (Fig. 1)
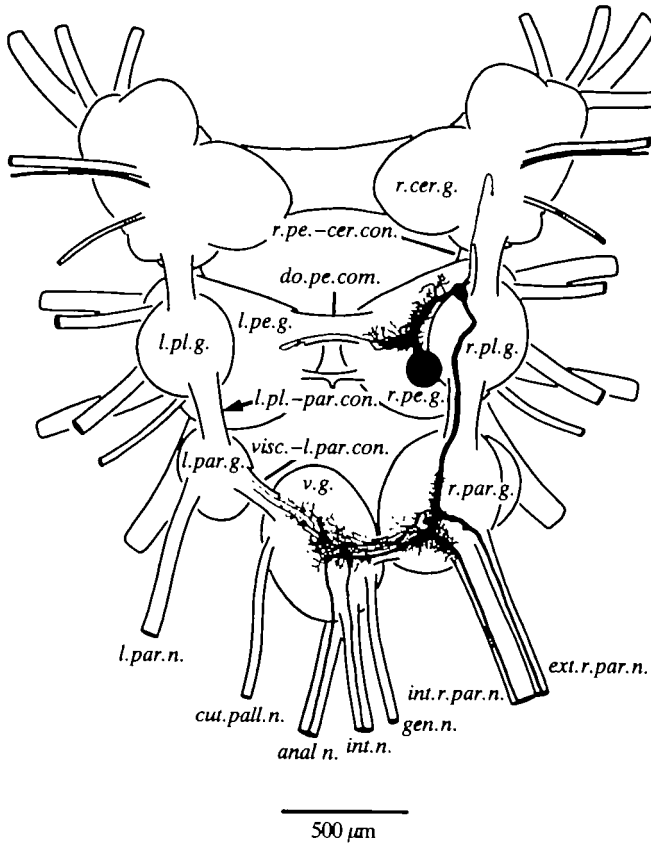
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500 μm

Fig. 1. Drawing of Lucifer Yellow-filled giant pedal interneurone, RPeD1, from the dorsal view showing the main anatomical features of the normal cell in the *Lymnaea* CNS. An arrow indicates the left pleural–parietal connective (*l.pl.–par.con.*) which was crushed in the experimental animals. Abbreviations: *anal n.*, anal nerve; *cut.pall.n.*, cutaneous pallial nerve; *do.pe.com.*, dorsal pedal commissure; *ext.r.par.n.*, external right parietal nerve; *gen.n.*, genital nerve; *int.r.par.n.*, internal right parietal nerve; *int.n.*, intestinal nerve; *l.par.g.*, left parietal ganglion; *l.par.n.*, left parietal nerve; *l.pe.g.*, left pedal ganglion; *l.pl.g.*, left pleural ganglion; *r.cer.g.*, right cerebral ganglion; *r.par.g.*, right parietal ganglion; *r.pe.–cer.con.*, right pedal–cerebral connective; *r.pl.g.*, right pleural ganglion; *v.g.*, visceral ganglion; *visc.–l.par.con.*, visceral left parietal connective; *r.pe.g.*, right pedal ganglion.

were significantly greater in damaged brains compared with normal ($\chi^2$ test). These new sprouts were fine neuritic processes originating from neuritic arbors already present in normal cells. In the visceral ganglion, for example, RPeD1 processes were present in the anal, intestinal and genital nerves in every damaged brain whereas in normal brains the interneurone projected consistently only to the anal nerve (Fig. 2) (the significance of this variation in peripheral projections in the normal cell is unknown but it may be that RPeD1 has access to peripheral targets *via* alternative nerves). Similarly, extension of RPeD1 neurites across the visceral–left parietal connective and into the left parietal ganglion was observed in all the lesioned brains but in only 58 % of normal brains (Fig. 2). In a small number of damaged brains

(19 %) neurites had grown through the repaired lesion site of the left pleural–parietal connective compared with 8 % in normal brains. The stimulation of sprouting also showed itself in an increase in the *number* of fibres entering a particular nerve or connective (Fig. 3). For instance, the numbers of fibres entering the anal, intestinal and genital nerves were significantly greater in lesioned compared with normal snails. Similar increases occurred in nerves with roots in the right parietal ganglion but here only the internal right parietal nerves showed significant increases. Within the connectives of the posterior ganglia, increases in the number of fibres occurred in the visceral–left parietal connective (Fig. 2). In the anterior regions of the CNS, the pattern and degree of branching was indistinguishable from normal (Figs 2, 3). For instance, there were no differences between normal and lesioned cells in the proportion of cells sending fibres from the right pedal arbor of RPeD1 into either the dorsal pedal commissure or the right pedal–cerebral connective.

Our previous regeneration study, which examined the effects of direct lesions of RPeD1, demonstrated that there was a preference for particular nerve trunks in both normal and regenerated cells (Allison & Benjamin, 1985). Thus the anal nerve almost always contained an axon whereas the intestinal genital and cutaneous pallial nerves showed more variability in both normal and damaged cells (an example of a normal cell with a 'variable' branch in the intestinal nerve is shown in Fig. 1). This
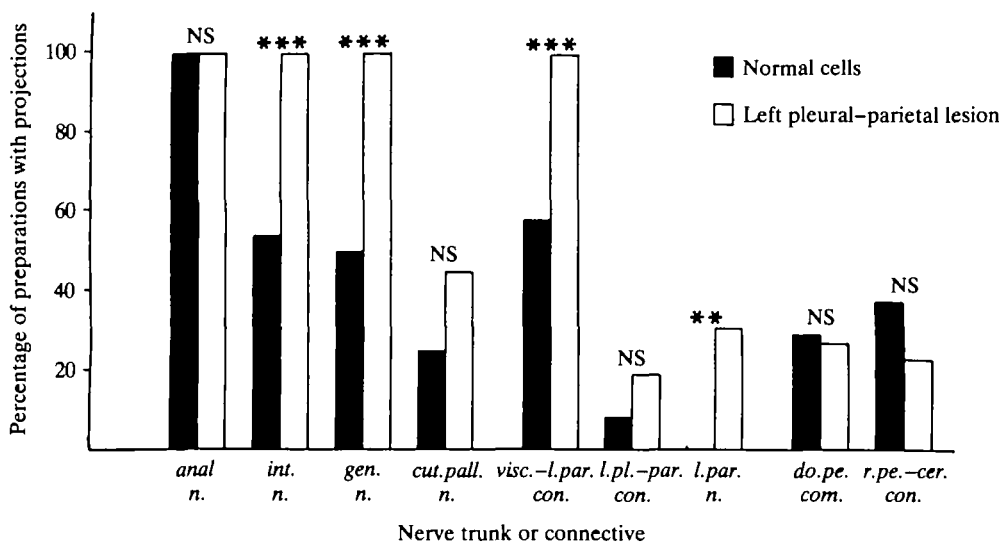


Fig. 2. Histogram comparing the percentage of preparations with giant pedal interneurone (RPeD1) fibres in nerves and connectives in normal and lesioned snails. Lesions increased the percentage of preparations receiving projections in several nerves and connectives in the posterior regions of the CNS (e.g. the intestinal nerve, *int.n.*; anal nerve, *anal n.*, and visceral–left parietal connective, *visc.–l.par.con.*) but not in more anterior regions close to the cell body (e.g. dorsal pedal commissure, *do.pe.com.*). Abbreviations: *cut.pall.n.*, cutaneous pallial nerve; *gen.n.*, genital nerve; *l.par.n.*, left parietal nerve; *l.pl.–par.con.*, left pleural–parietal connective; *r.pe.–cer.con.*, right pedal–cerebral connective. ** Significant at the 1 % level; *** significant at the 0·1 % level; NS, no significant difference.
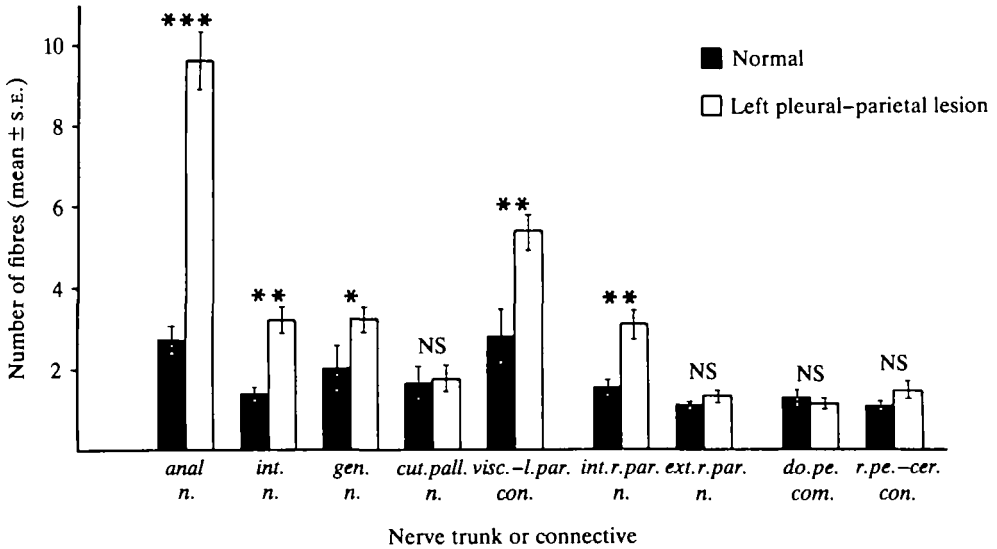
Fig. 3. Histogram comparing the numbers of RPeD1 fibres present in nerve and connectives in normal and lesioned snails. There were significantly more fibres in five out of the seven posterior nerves examined and this was particularly striking in the anal nerve (*anal n.*). No differences were seen in the more anterior CNS locations (dorsal pedal commissure, *do.ped.com.*; right pedal–cerebral connective, *r.pe.–cer.con.*). Abbreviations: *cut.pall.n.*, cutaneous pallial nerve; *ext.r.par.n.*, external right parietal nerve; *gen.n.*, genital nerve; *int.n.*, intestinal nerve; *visc.-l.par.con.*, visceral–left parietal connective; *int.r.par.n.*, internal right parietal nerve. * Significant at the 5 % level; ** significant at the 1 % level; *** significant at the 0·1 % level; NS, no significant difference.

same selectivity of nerve trunks was also found in the present experiments where the number of nerve fibres was again greater in the anal nerve compared with the other visceral nerve trunks, even though all four nerve trunks of this ganglion contained at least one fibre in the operated animals (Fig. 3).

The stimuli which initiate the development of new sprouts in RPeD1 are unknown. One possibility is that substances are released by injured neurones at the lesion site (Pitman & Rand, 1981). The observation that new sprouts were associated particularly with the RPeD1 arbor in the visceral ganglion may result from the close proximity of this region to the site of the injury compared with more distal arbors in the right pedal ganglia. The loss of pre- or postsynaptic connections of RPeD1 may also play an important role in stimulating development of new neurites, and it is significant that the crush site used in the present experiments contains the axon of the visceral white interneurone (VWI) which is presynaptic to RPeD1 (Benjamin, 1984). A final possibility is that cells damaged by the lesion were less able to compete with RPeD1 (because they were axotomized) and this allowed RPeD1 to sprout. Detailed evidence for this phenomenon has been obtained in the vertebrate CNS (e.g. Raisman & Field, 1973).

These experiments have clearly established that the undamaged RPeD1 inter-neurone can respond to damage within the CNS by growth of new processes from

pre-existing dendritic regions. Damage to non-neural tissue can also lead to sprouting in molluscs (glia, Altrup & Kolde, 1982; body wall incisions, Bulloch, 1984) and so a variety of factors other than direct damage can induce this phenomenon. It should be noted, however, that the extent of the sprouting in RPeD1 is considerably less than that following direct damage to the cell, particularly if the lesions occur in the more proximal axon regions (e.g. the right pleural–right parietal connective, Allison & Benjamin, 1985). Axotomy led to extensive proliferation of neurites into pedal nerves and more anterior CNS regions, as well as posteriorly into the right parietal and visceral ganglia and their nerves. We assume that axotomizing the axon induces specific changes in RPeD1 adding to the more generalized responses to damaged tissue produced by the present experiments.

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