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MECHANISMS OF FUNCTIONAL RECOVERY AND REGENERATION AFTER SPINAL CORD TRANSECTION IN LARVAL SEA LAMPREY

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SUMMARY

1. Large sea lamprey larvae, close to metamorphosis, regained swimming co-ordination after several weeks following complete spinal cord transection. Recovery was much faster when animals were kept at 23 than at 12 °C.

2. The behavioural recovery involved a regenerative mechanism in the spinal cord, since stimulation of the head resulted in tail curling, even when all tissue other than spinal cord and notocord was stripped away for several cm above and below the transection.

3. Following complete behavioural recovery, stimulation of the rostral cord evoked electrical signals recorded from the cord dorsum for only 10 mm below the transection.

4. Dorsal cells and giant interneurones, which normally project to the brain, could not be antidromically activated across the transection zone. However, giant interneurones could be activated polysynaptically by descending volleys.

5. Twelve of eighteen large reticulospinal axons followed in serial sections regenerated across the glial-ependymal scar, but branched abnormally and migrated away from their customary locations. They became smaller, and were finally lost within 4 mm of the centre of the transection zone.

6. These data suggest that behavioural recovery does not involve long axon tract regeneration. An alternate hypothesis, that short distance sprouting of axons across the transection zone may result in synapse formation with propriospinal interneurones which relay the necessary information, is discussed.

INTRODUCTION

Studies of recovery following transection of optic nerves in fish and Amphibia have suggested a considerable degree of specificity in the regeneration of retinotectal connexions (Sperry, 1943, 1944, 1948; Gaze, 1959; Gaze & Jacobson, 1963; Jacobson & Gaze, 1965). In the peripheral nervous system, experiments with competitive reinnervation of muscles in fish and Amphibia also suggest specificity in the re-establishment of neuromuscular connexions (Mark, 1969; Marotte & Mark, 1970; Cass, Sutton & Mark, 1973). However, in the spinal cord (and elsewhere in the vertebrate nervous system) recovery from transection is usually incomplete or absent (for reviews see Windle, 1955, 1956; Clemente, 1964; Pettegrew & Windle, 1976).

According to one hypothesis this is because regeneration of axons is inhibited by contact with many deafferented neurones before the original target neurones can be reached (Bernstein & Bernstein, 1967). Thus when functional recovery does occur, following lesions in most parts of the nervous system, it may involve mechanisms other than direct point-to-point axonal regeneration.

The present study was undertaken because the sea lamprey larva, a primitive vertebrate, has been reported to recover swimming movements following spinal cord transection (Marón, 1959; Hibbard, 1963; Rovainen, 1976; Selzer, 1976), and has several advantages for the study of spinal cord regeneration. The cord can be studied *in vitro*. By transillumination it is possible to identify many large neurones and axons (Pl. 1). They can then be impaled with micro-electrodes and, their responses to remote spinal cord stimulation can be tested (Rovainen, 1967*a*, *b*). Several 'giant' reticulospinal axons (Mauthner's and Müller's fibres), can be followed in serial sections with light microscopy (Rovainen, Johnson, Roach & Mankovsky, 1973).

The previous reports of recovery following spinal cord transection in lamprey larvae included histological descriptions which differed with regard to the extent of axonal regeneration. In the present study, histological and electrophysiological observations suggest that point-to-point axon tract regeneration does not occur. On the other hand, functional recovery might occur by axon sprouting across the transection and new synapse formation with propriospinal interneurones in the area of transection.

MATERIALS AND METHODS

Ten larval sea lampreys (*Petramyzon marinus*) 11–15 cm long were anaesthetized in tricaine methanesulfonate (MS 222 Finquel, Ayerst Laboratories; 1:4000). The spinal cords were exposed and completely transected through a dorsal incision about 1·5 cm caudal to the last gill. The cut ends were allowed to re-appose and the wounds sutured. The animals were returned to fresh water aquaria, where they were maintained at 12 °C, and their movements observed and recorded on Super 8 film. Two additional animals were maintained in the dark at 23 °C and fed finely ground fish food (Hibbard, 1963). Animals were re-anaesthetized for physiological and histological experiments at 3, 5, 7, 9, 10, 11, 13, 15, and 17 weeks after transection. Five additional animals were transected, kept at 23 °C, and observed behaviourally, but have not yet been studied physiologically or histologically.

Behavioural observations

Animals were placed in a shallow glass pan and their spontaneous swimming movements observed. Once the animal was still, the tail was stimulated either by pinching with forceps or by a train of 110 V 1 msec shocks at 10 Hz with a bipolar platinum electrode. The normal response to these manoeuvres is a lashing escape movement of the tail, followed immediately by rapid swimming, in which a sinusoidal wave of movement is propagated from the head to the tail. Next, the animal was stimulated on the face, and the responses of the head and tail once again observed. The normal response may be the same as for tail pinch, or a local withdrawal of the head may precede swimming. Animals were also observed crawling on a dry surface. The usual pattern is a coarse, sinusoidal movement. In spinal transected animals the portion of the body caudal to the transection lies still, while the rostral portion engages in slow writhing or searching movements. A more detailed description of several types of lamprey movements has been given by Rovainen (1976). In the present study the effects of mechanical and electrical stimulation were always the same. This acted as control for the possible effects of electrical stimulus spread along the body surface on the one hand, and on the other, secondary cues due to passive movement of the animal through the water as the result of mechanical stimulation. The effects of the latter alone were also controlled by pinning the animals to the bottom of a dissecting tray and observing the responses to mechanical stimulation.

Electrophysiology

In animals 7 weeks or more post-transection (i.e. mostly those in which nearly normal swimming movements had been re-established), electrophysiological observations were made before fixation of the spinal cord for histology. The anaesthetized animals were eviscerated through a ventral incision. The ventral surface of the notocord was exposed and split longitudinally to improve visibility of the transilluminated spinal cord. The animals were then pinned in the dissecting tray and the entire central nervous system exposed dorsally. The skin and muscle were completely removed for several cm caudal and 2 cm rostral to the transection. This allowed testing of the intactness of the spinal cord pathways by observing the response of the most caudal segments of the tail to mechanical stimulation of the head in the absence of any possible extraspinal pathways. It also reduced muscle twitching and movement artifacts during electrophysiological recording. The dissection and the electrophysiological experiments were performed at 12 °C in a physiological solution of the following composition (Rovainen, 1967*a*): 111 mM-NaCl, 2·1 mM-KCl, 2·6 mM-$CaCl_2$, 1·8 mM-$MgCl_2$, 4 mM-glucose, 10 mM-Tris buffer adjusted to a final pH of 7·4. The dissected preparation was transferred to a transilluminated Plexiglass chamber lined on the bottom with Sylgard (Dow Chemical). The physiological solution was equilibrated in room air and re-circulated at 5 ml per minute. A chlorided silver wire connected the bath to ground. Two bipolar silver–silver chloride electrodes were positioned on the dorsal surface of the spinal cord for stimulation and recording. Intracellular recordings were made with 2·0 M-K acetate-filled glass micro-electrodes with resistances of 30–60 MΩ. Micro-electrode signals were recorded with a high input impedance preamplifier and photographed from an oscilloscope screen. A modified bridge circuit allowed simultaneous intracellular stimulation and recording.

Histology

Lampreys with exposed central nervous systems were pinned to Sylgard strips and fixed overnight at 4 °C in 3 % glutaraldehyde in 100 mM-phosphate buffer adjusted to pH 7·4. They were post-fixed in 1 % osmium tetroxide for 1·5 h. Following dehydration in ethanol series and infiltration with propylene oxide, the animals were cut into 5 mm segments and embedded in Epon. The hardened blocks were cut at 4 and 10 μm alternate sections with glass knives, and stained with toluidine blue. In this way serial sections could be studied from each of about thirty 5 mm segments along the length of the neuraxis. Several of the Müller's and Mauthner's axons were assigned arbitrary numbers and traced through the transection zone in approximately 1100 serial sections with the aid of a Nikon drawing attachment.

RESULTS

Behavioural studies

Following transection, the recovery of the animals kept at 12 °C could be divided into four stages. *Stage 1.* During the first 4–5 weeks there was no evidence of functional connexions between rostral and caudal portions of the spinal cord. Neither mechanical nor electrical stimulation of the head produced movement in the tail. However, both did produce an abnormally high frequency oscillation of the head and body rostral to the transection. Spontaneous swimming also took this form, the tail hanging limp. Stimulation of the tail resulted in a rapid reflex lashing of the tail, propelling the animal forward, but did not elicit movements of the head. A few animals moved their heads in response to mechanical or electrical tail stimulation within a day or so of transection. This may have been due to impulses carried in untransected lateral line nerves, as suggested by Rovainen (1976). *Stage 2.* Between 5 and 7 weeks post-transection, the animals' behaviour suggested a dissociation between recovery of ascending sensory function and continued absence of functional descending connexions. Stimulation of the tail resulted in head movement, but face stimulation

did not elicit tail movement. *Stage 3.* Between 7 and 9 weeks animals began to develop rostrally controlled swimming movements. Face stimulation now elicited tail movements, although often with a delay of up to 2–3 sec. Swimming was still awkward and lacked the normal sinusoidal quality. It was characterized by the same high frequency oscillations of the head described for stages 1 and 2, but also included active tail movements. Co-ordinated crawling movements began to appear at this stage. *Stage 4.* Between 10 and 13 weeks post-transection, swimming movements became smoother and, in most animals, grossly indistinguishable from normal. Stimulation of the body on either side of the transection resulted in prompt escape movements of the tail and co-ordinated swimming. Seven animals kept at 23 °C showed patterns of recovery identical to those kept at 12 °C but the time course was approximately half as long. Thus, head movements in response to tail stimulation appeared after 2–3 weeks, and swimming movements appeared normal by 7 weeks.

Effects of body wall removal

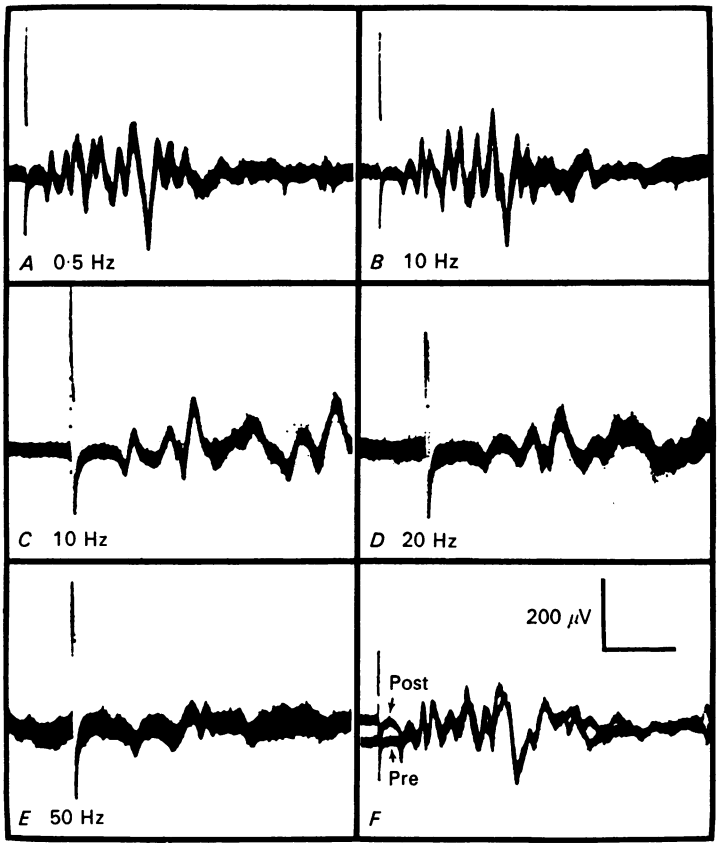
Five animals which had recovered normal swimming movements were studied following removal of the body wall from 2 cm rostral to at least 4 cm caudal to the transection site. In all of these animals, despite the effects of anaesthetic, evisceration and extensive body wall removal, either mechanical or electrical stimulation of the face resulted in curling movements of the remaining tail. Since these animals were pinned to the floor of the dissecting chamber, secondary cues resulting from body movements through water were also eliminated. When electrical stimuli were used, raising the stimulating electrodes a fraction of a mm off the face eliminated the response in the tail. Therefore, the tail responses were not produced by volume conduction of the stimulus across the transection. Thus at least part of the behavioural recovery was shown to be mediated through the spinal cord.

Electrophysiology

In the untransected spinal cord, electrical stimulation produced a complex electrical signal which could be recorded at a distance caudal to the stimulating electrode (Text-fig. 1*A*). Most of the components of the signal persisted at stimulus frequencies of 10 Hz or more (Text-fig. 1*B–E*), although some peaks showed amplitude depression. (Surface potentials showed little or no post-tetanic potentiation (Text-fig. 1*F*).) Thus, most of the electrical potentials recorded from the cord dorsum probably represented the synchronous firing of directly stimulated axons and the synaptic currents of monosynaptically activated neurones. Most polysynaptically activated action currents and synaptic currents are probably not synchronous enough to generate signals recordable from the cord dorsum.

In transected spinal cords, electrical activity could always be recorded on the same side of the transection as the stimulating electrodes (Text-fig. 2*A* and *D*). During the first 7 weeks, however, no electrical activity could be recorded from the cord surface anywhere caudal to the transection, on stimulation of the spinal cord rostral to the transection. By the tenth week small electrical potentials could be recorded from the region between 2 and 10 mm caudal to the transection (Text-fig. 2*B*), but not further than this (Text-fig. 2*C*). Raising the recording electrode a fraction of a mm off the cord surface, or placing it on muscle or notocord a fraction of a mm lateral to the

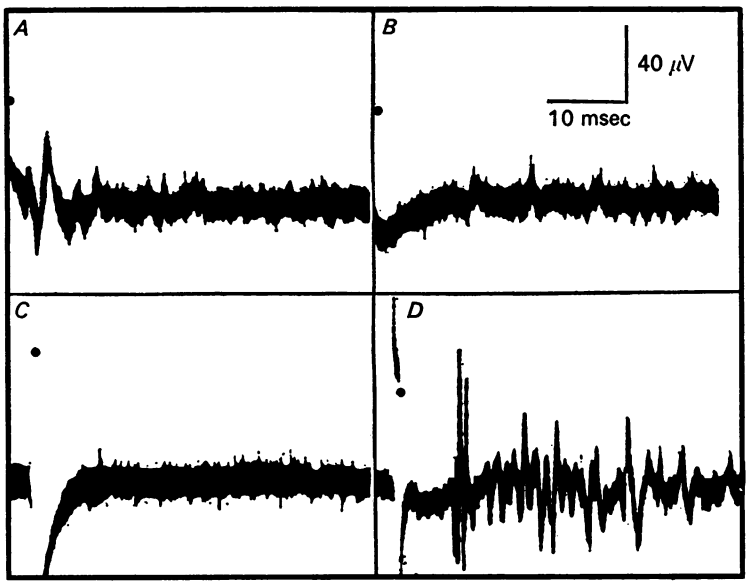
cord, completely eliminated the recorded signal. Therefore, the electrical activity recorded within 10 mm across the transection was generated by neurones and/or axons in the spinal cord subjacent to the recording electrode, and could not have been electrotonically conducted across the transection. This situation persisted until 17 weeks post-transection.



Text-fig. 1. Spinal cord surface potentials in response to high frequency supramaximal cord stimulation in an untransected control animal; stimulated 15 mm caudal to last gill, recorded 23 mm caudal to last gill. *A–E* consist of about ten superimposed traces each. *A*, stimulus frequency 0·5 Hz; note constancy of most components of the response. *B*, 10 Hz. *C*, same as *B* but with expanded time scale. *D*, 20 Hz. *E*, 50 Hz. *F*, two superimposed sweeps, before and 3 sec after a 5 sec 50 Hz tetanus; note lack of potentiation in most components of the response. Time calibrations: *A*, *B*, and *F*, 10 msec.; *C*, *D*, and *E*, 4 msec.

Two types of spinal neurones, dorsal cells and giant interneurones, are known to project rostrally for long distances (probably to the brain stem) in the spinal cord (Rovainen, 1967*b*). Dorsal cells are primary sensory neurones (Martin & Wickelgren, 1971) whose rostral projections travel in the ipsilateral dorsal columns. They receive no synaptic input from descending axons, but can be antidromically activated by stimulating the rostral cord in about 75 % of cases. They are easily seen and impaled in the transilluminated spinal cord. Giant interneurones are also easily seen in the transilluminated cord. Their axons project rostrally in the contralateral lateral

columns. Normally, giant interneurones can be activated antidromically by rostral cord stimulation in about 80 % of cases, and show characteristic large excitatory post-synaptic potentials (e.p.s.p.s on rostral cord) stimulation (Text-fig. 4*D*). Five dorsal cells and three giant interneurones caudal to the transections were recorded from in animals 10–17 weeks after transection. The experimental arrangement is shown in Text-fig. 3. None could be activated antidromically by cord stimulation rostral to the transection (Text-figs. 4 and 5). However, giant interneurones showed small, long latency depolarizing potentials which showed considerable latency jitter and did not follow stimulation at frequencies higher than 1–2 Hz, suggesting that they were not monosynaptic. Occasionally these potentials gave rise to action potentials (Text-figs. 4*C*, 5*C* and *D*). While the nature of these potentials was not established, they were not



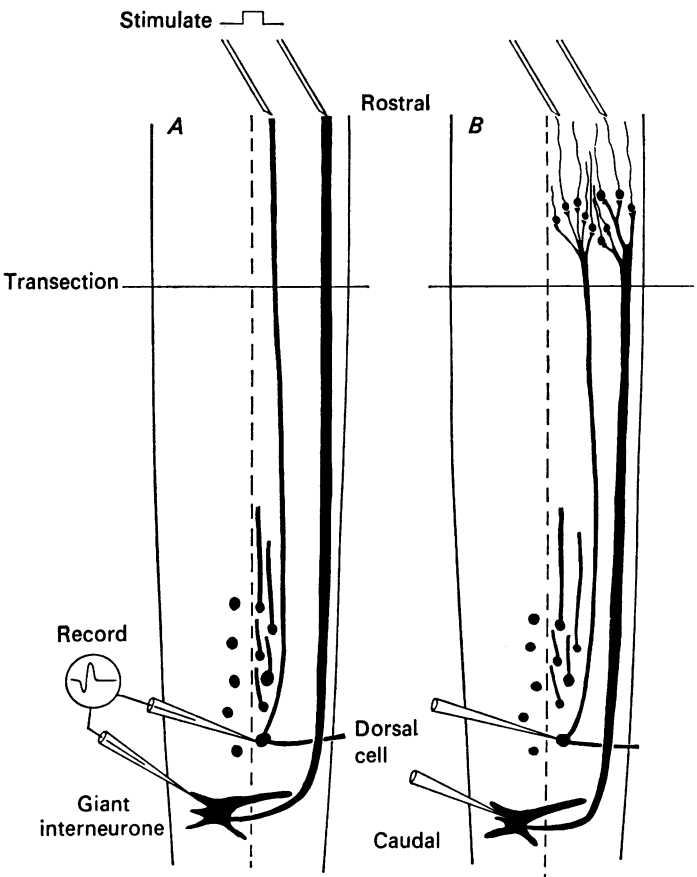
Text-fig. 2. Spinal cord surface potentials in response to supramaximal cord stimulation (kept 10 weeks post-transection at 23 °C). *A*, *B*, and *C* consist of five superimposed tracings each. *D* is a single response. *A*, stimulating electrode was 14 mm rostral to transection. *B*, stimulated 9 mm rostral, recorded 6 mm caudal to transection; note small but constant response. *C*, stimulated 9 mm rostral, recorded 12 mm caudal to transection; no electrical response seen. *D*, stimulated 7 mm caudal, recorded 30 mm caudal to transection; stimulus artifact is indicated by a dot.

present in the extracellular field recordings following removal of the micro-electrode from the giant interneurones (Text-fig. 5*E*). Thus they may be polysynaptic e.p.s.p.s. Their small amplitudes and long latencies are in marked contrast to the synaptic responses of giant interneurones of non-transected animals.

These results suggest that axons of dorsal cells and giant interneurones did not regenerate for long distances rostral to the transection at times when the animals had regained normal swimming movements and responses of the head to stimulation of the tail. Moreover, while direct synaptic contact between descending axonal systems and giant interneurones was not re-established, indirect (i.e. polysynaptic) connexions were formed.

Histology

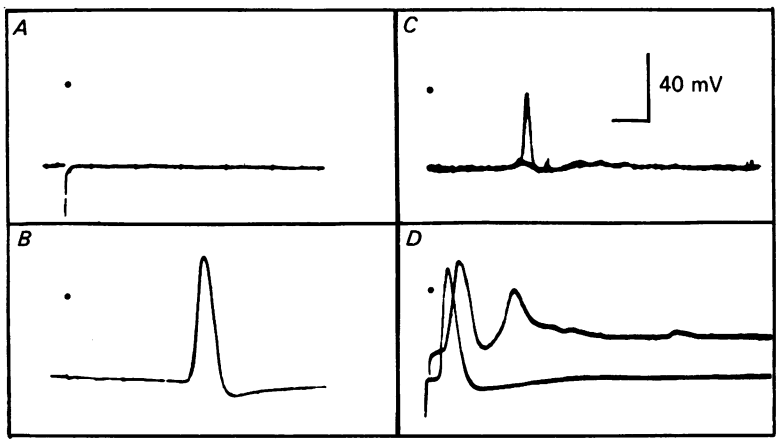
The appearance of the normal spinal cord in cross section is shown in Pl. 2*A*. A central canal lined by ependymal cells is located half way between a dorsal column of small axons and a ventral column which contains several very large reticulospinal axons (Müller's axons), which descend, mostly unbranched, almost the full length of the spinal cord (Rovainen *et al.* 1973). Mauthner's fibre runs in the lateral column



Text-fig. 3. Two hypotheses to explain recovery of ascending sensory function following spinal cord transection. *A*, long axon tract regeneration. *B*, local axon sprouting. The Figure includes design of an experiment to test hypothesis *A*. Failure to activate dorsal cells and giant interneurones antidromically would argue against *A*, but would not argue against *B*. Other hypotheses discussed in text.

on each side. One is seen in Pl. 2*B* on the left. Following transection, Müller's and Mauthner's fibres degenerate over a 3–10 week period. Thus, examination of isolated sections caudal to the transection during the first 10 weeks could give the erroneous impression of regeneration of these large fibres. Despite the behavioural recovery once they have degenerated these axons do not reappear in their customary locations. Pl. 2*B* demonstrates that rostral to the transection site these large axons persist,

while the dorsal columns are degenerated, resulting in dorsal retraction of the central canal. A typical dorsal cell is present in this section. In this same animal, caudal to the transection, the ventral columns no longer contain the giant axons, and are therefore much reduced in size, resulting in downward retraction of the central canal. This is shown in Pl. 2*C*, which also contains two giant interneurones. Early in the regeneration process the transection zone is filled in by cellular elements which are derived, at least in part, from ependymal cells. Glial cells and extraspinal connective tissue cells also seem to be involved (Pl. 3*A*). By the 13th week (Pl. 3*B*) the transection zone is well delineated with a spinal cord surrounded by a connective tissue

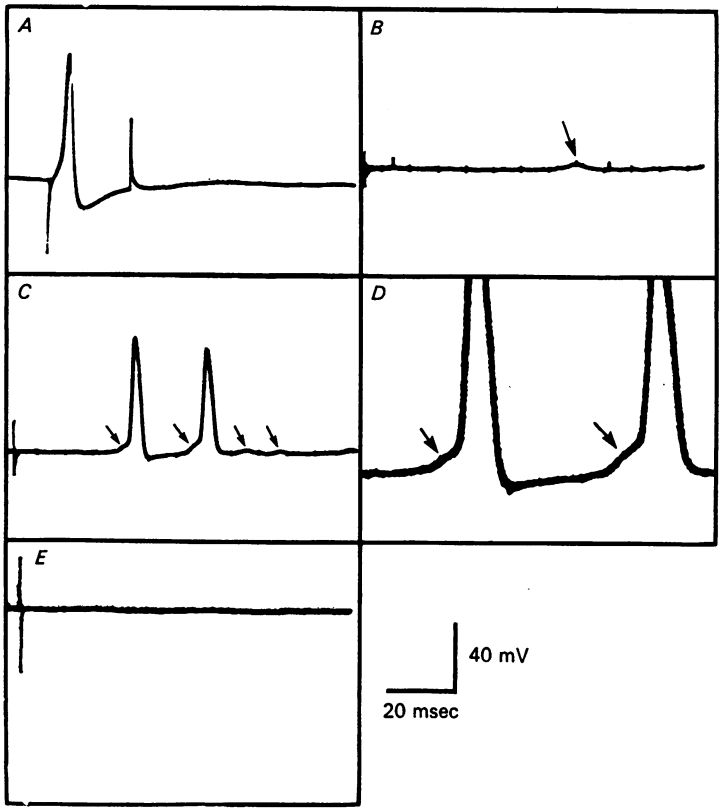


Text-fig. 4. Results of experiment illustrated in Fig. 3. *A*, *B*, and *C* refer to experiment done on an animal kept 15 weeks post-transection at 23 °C. *A*, failure to activate dorsal cell located 21 mm caudal to the transection zone on supramaximal stimulation of the cord 12 mm rostral to the transection. *B*, stimulating electrode moved to 9 mm caudal to the transection: dorsal cell is now activated antidromically. *C*, two superimposed sweeps of response of giant interneurone to supramaximal stimulation of the cord 9 mm rostral to the transection spike failure in one sweep reveals a subthreshold depolarizing potential; second sweep shows a spike arising from the crest of the depolarizing potential. *D*, response of a giant interneurone (upper trace) and dorsal cell (lower trace) to cord stimulation in an untransected control animal; stimulated 9 mm rostral to giant interneurone; note the early antidromic spike in the giant interneurone: a delayed orthodromic spike is attenuated by current shunting caused by a very large complex e.p.s.p. Time calibration: *A*, *B*, and *D*, 5 msec; *C*, 20 msec. Spikes retouched.

capsule (the meninx primitiva). The central canal is re-formed, but is much larger than normal, and irregular in shape. In the central 100 μm of the transection zone there are no neurone somata, but medium sized axon profiles can be seen. Often the axons are orientated transversely to the plane of section, and can even be seen branching. Such axon profiles are not seen normally. By the 17th week the central canal is more regular in shape. Large axon profiles can be seen. Many axons form large dilatations as they are followed caudally (Pl. 4). These may be analogous to terminal enlargements in regenerating peripheral nerves (Weiss, 1944).

The loss of giant axons from the ventral columns supports the theory that these axons do not regenerate for long distances beyond the transection zone. However, some medium sized axons can be seen in the lateral and dorsal columns. These are

most likely ascending axons of giant inteneurones and possibly other rostrally projecting neurones whose axons may have enlarged in response to the degeneration of descending axons in the spinal cord. However, they might also be regenerating reticulospinal axons which have migrated away from their customary locations. The latter possibility was shown to be unlikely by following seventeen large Müller's fibres and one Mauthner's fibre of the 17th week post-transection cord in serial sections, from 5 mm rostral to the transection to their disappearance more caudally.



Text-fig. 5. Responses of a giant interneurone located 30 mm caudal to transection in animal kept 10 weeks post-transection at 23 °C. Stimulating electrode 12 mm rostral to transection. *A*, activation by intracellular current injection. *B*, subthreshold stimulation of rostral cord; note small long latency depolarizing potential. *C*, response to supramaximal cord stimulation; note no antidromic action potentials, but two action potentials arising from small depolarizing potentials (arrows). Spikes did not follow 1/sec stimulation. *D*, enlarged detail of *C*. *E*, extracellular field. Calibrations apply to all but *D*.

The centre of the transection zone was identified as the region of maximal morphological distortion and central canal enlargement. Twelve of the fibres regenerated beyond this point. The level at which each of the axons was lost relative to the centre of the transection zone is listed in Table 1, together with their original and final locations. Most of the axons were noted to branch several times, and branches which were large enough were also followed in subsequent sections. None of the axons

could be followed for more than 3·6 mm beyond the level of the transection. The region of most prolific branching was in the first 300 μm caudal to the centre of the transection zone. Those axons which regenerated across the transection often migrated considerably away from their customary positions and even to the contralateral side of the spinal cord. Neurone somata were absent only from the 30 μm rostral to 90 μm caudal to the centre of the transection zone.

TABLE 1. Fates of seventeen regenerating Müller's axons and one Mauthner's axon (Mt) followed caudally in serial sections, 17 weeks post-transection. Levels at which axons were lost are listed in μm rostral to (−) or caudal to (+) the centre of the transection zone. LVC = left ventral column. LVLC = left ventrolateral column, LDC = left dorsal column, LDLC = left dorsolateral column, RVC = right ventral column, etc. Levels lost and final locations are listed for each large branch of several axons

Axon No.	Original location	Level lost (μm)	Final location
1	LVC	−68	LVC
—	—	+38	LVC
2	LVC	−845	LVC
3	LVC	−38	LVC
4	LVC	−142	LVC
—	—	−68	LVC
—	—	+223	RVLC
—	—	+238	RDC
5	LVC	−4455	LVC
6	LVC	−1285	LVC
—	—	−620	LVC
7	LVC	−460	LVC
—	—	−180	LVC
8	LVC	+3638	RDC → RVC
9	RVC	+75	RVC
—	—	+1908	RDC
10	RVC	−895	RVC
11	RVC	+75	RVC (turns rostrally)
12	RVC	+1572	RDC
13	RVC	+23	RVLC
14	RVC	+223	RVC
15	RVC	+23	RVCL
16	LVLC	+223	LVC
—	—	+432	LVC
—	—	+440	LVC
—	—	+600	LVC
—	—	+818	LVC
17	RVC	+238	RDC
Mt	LDLC	+238	LVLC → LDLC

DISCUSSION

The behavioural studies reported above confirm that lamprey larvae can recover functionally from complete spinal transections. (Marón, 1959; Hibbard, 1963; Rovainen, 1976). The shorter recovery times and longer distances of axon regeneration observed by Rovainen may reflect the fact that his larvae were younger than those used in the present study and were kept at room temperature, rather than 12° C. However, the behavioural and histological results of these two studies are

qualitatively similar. Behavioural recovery following spinal transection has also been reported in fishes (Koppanyi, 1955) and some amphibians (Piatt, 1955*a*, *b*).

Possible mechanisms of spinal cord regeneration include: (1) fusion of proximal and distal cut ends of axons, (2) regeneration of axons for long distances back to their original target neurones and (3) sprouting of axons for short distances across the transection and synapse formation with propriospinal interneurones which relay the necessary information to more distant spinal or brain levels.

Fusion of the cut ends of transected axons has been seen in crustaceans (Hoy, Bittner & Kennedy, 1967; Bittner & Johnson, 1974), the leech (Frank, Jansen & Rinvik, 1975) and the earthworm (Birse & Bittner, 1976). However, the loss of giant fibres from the ventral and lateral columns caudal to the transection in the present study shows that Mauthner's and Müller's axons in the lamprey resemble axons of higher vertebrates in degenerating distal to a transection.

The second and third mechanisms (long axon tract regeneration and local sprouting) are illustrated in Text-fig. 3. The failure of cord stimulation to elicit recordable surface potentials beyond 10 mm across a transection in behaviourally recovered larvae suggests that direct axonal conduction and monosynaptic contacts by regenerating axons do not extend beyond this distance. This argues against the hypothesis of long axon tract regeneration, but does not rule out the local sprouting hypothesis, since polysynaptic neuronal activity generated beyond the transection zone might not be synchronous enough to produce recordable surface potentials. The tracings of giant fibres in serial sections across the transection zone, where they branch abnormally and disappear within a few mm, strengthens the plausibility of the local sprouting hypothesis. However, further experiments will be necessary to demonstrate whether this process does in fact occur. For example, these results, as well as the surface potential recordings, could also be explained if only small axons regenerated for long distances and/or large axons regenerated only after breaking up into fibres too small to be followed in serial section. Such fibres might conduct so slowly and generate such small currents that their activity on cord stimulation could not be recorded from the cord surface. The rostrally projecting axons of giant interneurones are smaller than most of the Müller's fibres, and dorsal cell axons are much smaller. Therefore, the inability to activate these neurones antidromically by stimulation 9 mm rostral to the transection in behaviourally recovered larvae suggests that even small axons do not regenerate for long distances.

The activation of giant interneurones by long latency (presumably polysynaptic) orthrodomic pathways confirms the *a priori* conclusion that indirect neuronal pathways must be re-established between rostral and caudal parts of the spinal cord. It is interesting that re-establishment of synaptic contacts has been demonstrated in leech ganglia following transection of connective nerves (Baylor & Nicholls, 1971; Jansen & Nicholls, 1972), although it is not clear whether those contacts are monosynaptic. The considerable number of identified monosynaptic connexions among spinal neurones (Rovainen, 1974*a*) and between reticular neurones and spinal neurones (Rovainen, 1974*b*) in the lamprey offers an opportunity to test the specificity of new synaptic connexions in regenerating spinal cord.

In the regenerating lamprey spinal cord, the transection is filled in by a glial-ependymal scar before any axons can be observed to cross the transection. Subsequent

axon regeneration would seem to occur through an already forming scar, suggesting that the scar does not prevent regeneration. These findings support the conclusion of Bernstein & Bernstein (1967) in goldfish, that the glial scar is not the limiting factor in axonal regeneration. In higher vertebrates, a more dense scar may contribute to the prevention of functional recovery, since recent reports suggest that inhibition of scar formation by administration of proteases resulted in some functional recovery of spinalized rats (see Pettegrew & Windle, 1976).

Conclusions

The present study demonstrates that large larval sea lampreys can recover their co-ordinated swimming movements following spinal cord transection. This process is temperature sensitive and involves a regenerative process in the spinal cord. Despite the formation of a glial-ependymal scar, many axons traverse the transection zone. However, tracings of giant axons in serial sections, studies of electrical activity recorded from the cord surface, and intracellular recordings from rostrally projecting neurones caudal to the transection, suggest that regeneration of axons does not progress beyond a few mm at recovery times sufficient for animals to regain normal appearing swimming movements. Although still unproven, a local sprouting mechanism of regeneration seems more likely. The use by sea lamprey spinal cords of such a mechanism, rather than long axon tract regeneration or fusion of proximal and distal cut ends of axons, would make them a realistic model for the regenerative potential of higher vertebrates.

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EXPLANATION OF PLATES

PLATE 1

A, appearance of living transilluminated spinal cord. Note translucent cellular profiles, including round, medially located dorsal cells (dc), and longitudinally orientated Müller's fibres (mf); cc, central canal. *B*, for comparison with *A*, a toluidine blue stained whole mount of spinal cord showing the above features and a giant interneurone (gi).

PLATE 2

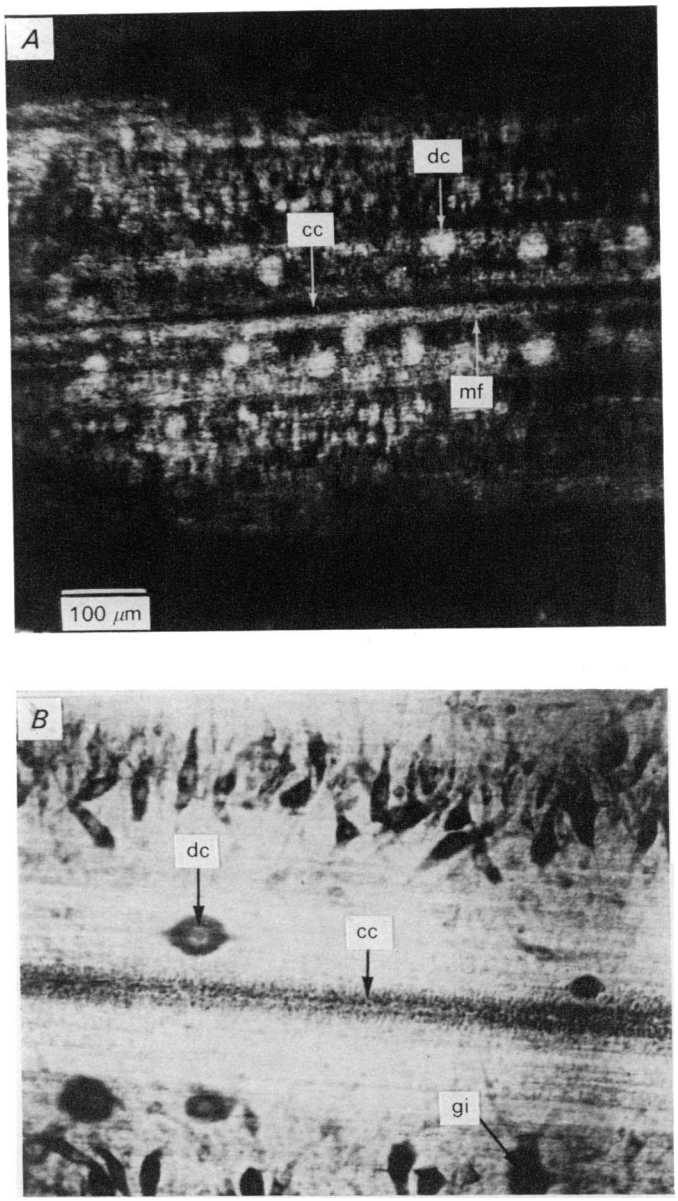
Lamprey spinal cord in cross-section. Epon embedded 10 μm section stained with toluidine blue. *A*, untransected control, gill region. Note dorsal cell (dc) and Müller's fibres (mf). Central canal is located midway between dorsal and ventral surfaces. *B*, at 17 weeks post-transection, 2 mm rostral to the transection. Note the presence of Müller's fibres in ventral columns and the loss of ascending fibres from dorsal columns. The central canal is closer to dorsal surface. *C*, same animal, 40 mm caudal to the transection. Note the presence of two giant interneurones (gi) in the cell body layer. Note also the loss of Müller's fibres in the ventral columns and the retraction of the central canal ventrally.

PLATE 3

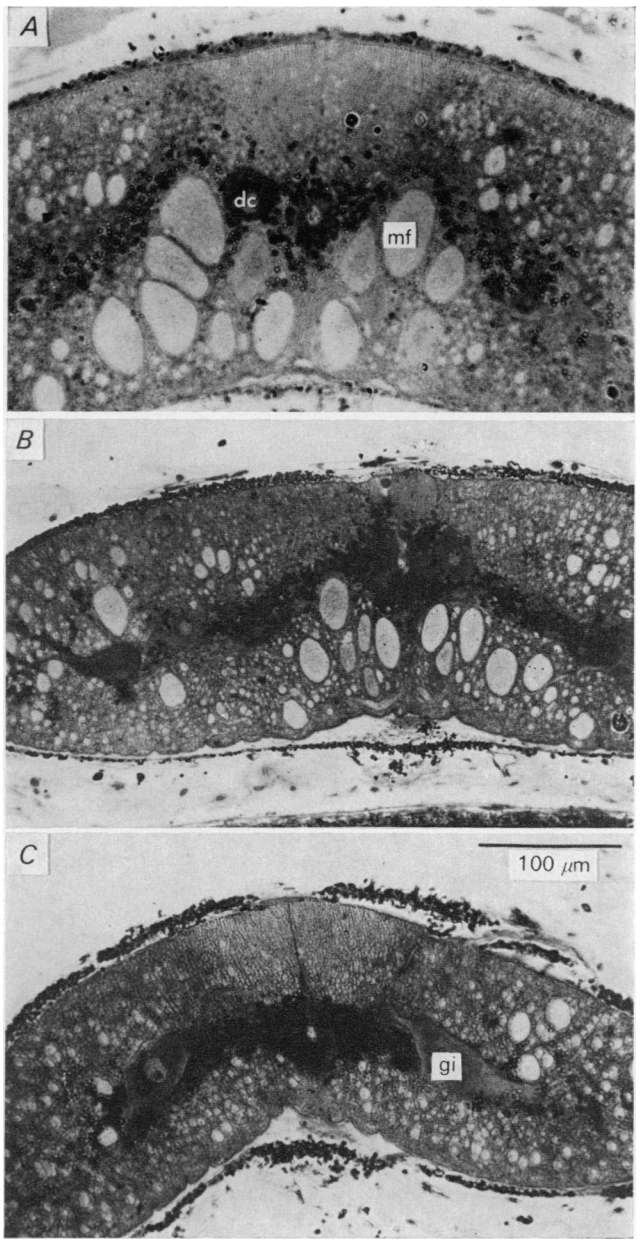
Appearance of the centre of the transection zone in cross-section. *A*, 7 weeks post-transection. Connective tissue elements fill in the transection zone. The central canal is poorly formed. Sheets of extraspinal connective tissue cells surround the cord. Arrow points to a small regenerating axon. Only a few axons are seen at this stage. *B*, at 13 weeks, the central canal (cc) is reformed, surrounded by ependymal cells (e), which are greatly proliferated and appear to migrate away from the central canal, contributing to the glial scar. Medium sized axons are seen. *C*, at 17 weeks the cord is a regular oval shape. The central canal is also regular in shape. Large axon profiles are seen. Arrow points to an axon branching transversely to the plane of section.

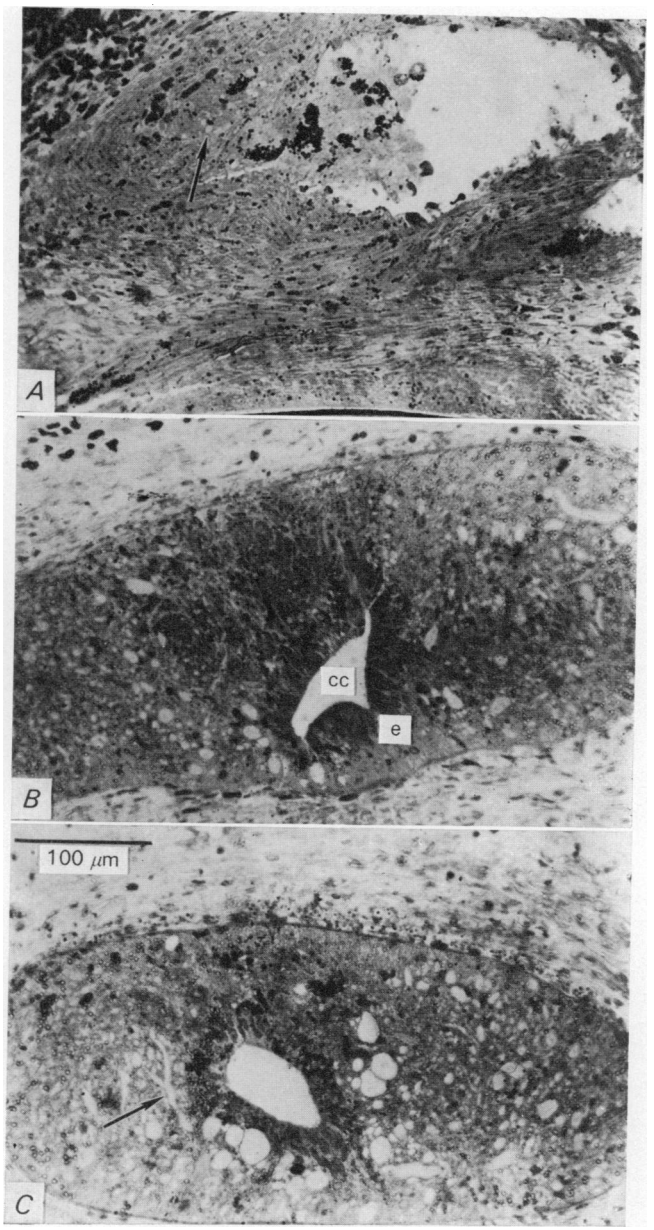
PLATE 4

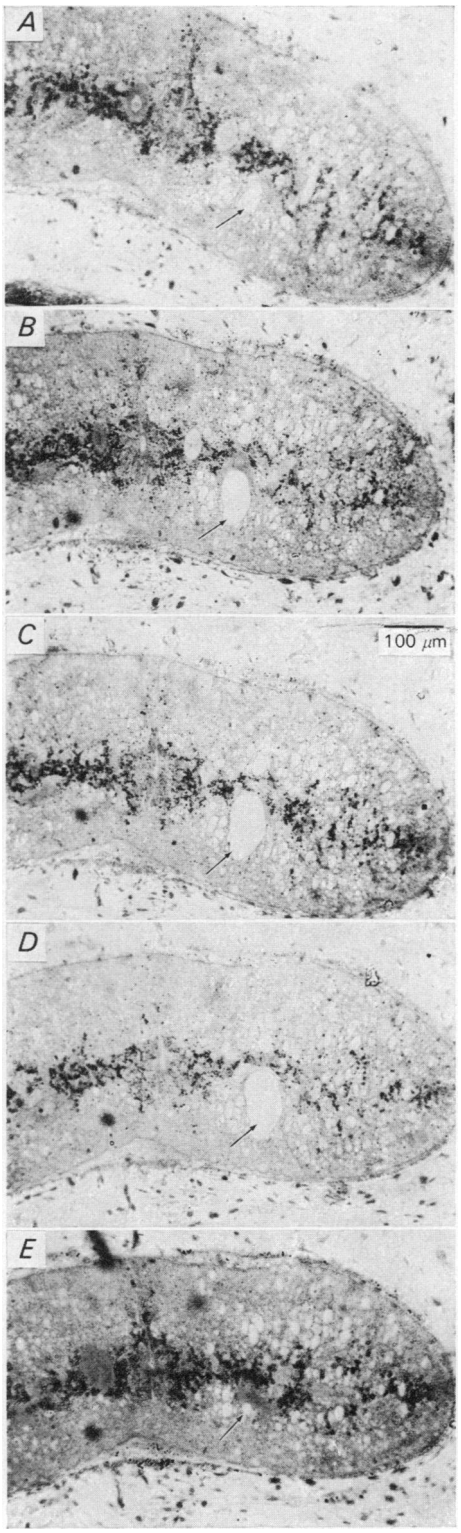
Terminal enlargement (arrow) of regeneration axon 2 mm caudal to the transection zone at 17 weeks post-transection. *A*–*E* are 5 μm sections taken every 15 μm progressing caudally.



(*Facing p.* 408)


A
dc
mf
B
C
100 μm
gi


A
cc
e
B
100 μm
C


A
B
C
100 μm
D
E