J. Physiol. (1980), **303**, *pp.* 243–263
With 7 text-figures
Printed in Great Britain

LONG-TERM EFFECTS OF AXOTOMY ON NEURAL ACTIVITY DURING CAT LOCOMOTION

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(*Received 15 May 1979*)

SUMMARY

1. Neural activity was recorded from cats during locomotion on a treadmill using electrodes in Silastic cuffs placed around the sciatic nerve and the lateral gastrocnemius-soleus, medial gastrocnemius, common peroneal and tibial nerve branches. Each branch gave characteristic patterns of activity which were studied before and after it was cut distal to the recording cuffs. Sensory and motor components were separated and measured using cross-correlation techniques. The amplitude of the cross-correlation peaks was compared with the amplitude of compound action potentials evoked by electrical stimulation and recorded from the same sites in the anaesthetized animal.

2. Sensory activity declined rapidly following axotomy and did not recover unless reinnervation occurred. Sensory activity even 5 months after nerve section and resuture had recovered to only a fraction of the control values. This reduction is attributed to a decline in the evoked compound potentials and to many fibres being unsuccessful in regenerating to appropriate sensory organs.

3. Motor activity declined more than could be accounted for by a decline in evoked potentials over the first month after axotomy. The extra reduction represents a decline in the number of impulses generated by α-motoneurones after axotomy. If regeneration was permitted, motor activity recovered to higher levels than did the evoked potentials for the whole nerve. Even if regeneration was prevented by nerve ligation, motoneurones continued to generate activity at a stable level over a period of months during which whole nerve compound potentials continued to decline.

4. The modulation of motor activity in ligated nerves during the step cycle was still appropriate to the required movement. Thus, activity recorded from severed nerves in human amputees may be useful in controlling powered artificial limbs. The persistence of motor activity may be responsible for the lesser degree of atrophy found in motor fibres than in sensory fibres following ligation (Hoffer, Stein & Gordon, 1979*b*).

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0022-3751/80/8330-0211 $07.50

INTRODUCTION

Histological and electrophysiological studies have shown that peripheral fibres can survive and conduct action potentials even years after a nerve is cut and ligated (Ranson, 1906; Cragg & Thomas, 1961). Axons shrink in diameter and recover slowly if connexions are reformed in the periphery (Gutmann & Sanders, 1943; Berry & Hinsey, 1946; Davis, Gordon, Hoffer, Jhamandas & Stein, 1978); atrophy is more severe in sensory fibres than in motor fibres of the same nerve (Hoffer, Gordon & Stein, 1979*a*). However, the level and pattern of activity in cut nerves are relatively unknown. The generation of impulses during locomotion has been shown to persist in motor nerve fibres following dorsal rhizotomy (Taub & Berman, 1968) and after acute de-efferentation (Viala & Buser, 1969; Grillner & Zangger, 1974), but there are few chronic studies of motoneuronal discharge after nerve section. De Luca & Gilmore (1976) indicated that the discharge from the cut common peroneal nerve in rabbits fell but stabilized within 3 weeks. Acheson, Lee & Morison (1942) found that phrenic nerve activity declined sharply following axotomy but began to recover after about 20 days and could return to control levels. No procedures were undertaken to prevent regeneration, so presumably the phrenic nerves reinnervated muscles. Acheson *et al.* (1942) suggested several possible explanations for the early decline, including a reduction in synaptic transmission onto the injured motoneurones.

This suggestion has been experimentally verified and the synaptic depression after axon injury has been correlated with the loss of synaptic contacts from injured neurones in the hypoglossal nucleus (Hamberger, Hansson & Sjöstrand, 1971), the facial nucleus of the rat and the mouse (Blinzinger & Kreutzberg, 1968) and spinal motoneurones in cats (Mendell, Munson & Scott, 1976). In the sympathetic ganglion, synaptic connexions between pre- and post-ganglionic neurones were so depressed that some post-ganglionic neurones could not be activated even by maximal preganglionic stimulation (Purves, 1975). Similarly, in cat lumbar motoneurones which supply hind limb musculature, progressive changes in post-synaptic excitatory potentials culminated in loss of detectable connectivity of many 1a spindle afferent fibres onto homonymous motoneurones (Mendell *et al.* 1976). Suprasegmental inputs onto motoneurones may also be affected (Shapovalov & Grantyn, 1968). Changes in connectivity and in synaptic efficacy should alter the activity of motoneurones after axotomy. The electrical properties of the motoneurones also change, e.g. the dendrites of axotomized motoneurones develop the ability to generate action potentials and the safety factor for antidromic invasion of the initial segment is altered (Eccles, Libet & Young, 1958; Kuno & Llínas, 1970*a*, *b*).

The functional implications of these changes can only be assessed by recording the motor output in nerves after axotomy. Do motoneurones remain active after nerve section and generate a similar pattern of impulses during behaviour? If so, the activity in motoneurones may be available in human amputees for controlling powered artificial limbs. Furthermore, if the activity of the motoneurones can be enhanced by reconnecting the nerves to convenient muscles, this may be a useful surgical procedure for human amputees.

We have recorded the activity generated during locomotion for over 200 days in cat nerves which were either ligated to prevent regeneration, sutured to a nearby

muscle, or resutured to their distal stumps (Davis *et al.* 1978). Our results indicate that cut motoneurones continue to be activated reflexly (Jhamandas, 1976) and during appropriate phases of locomotion. Activity levels remain low in nerves which are prevented from reinnervating muscle, but recover substantially if regeneration is successful. Brief accounts of some results have been presented elsewhere (Gordon, Hoffer & Stein, 1977; Hoffer, Gordon & Stein, 1977; Stein, Hoffer, Gordon, Davis & Charles, 1979).

METHODS

Silastic cuffs with platinum–iridium electrodes were implanted in cat hind limbs around the sciatic nerve and one or more of its branches using methods which have previously been described in detail (Stein, Charles, Davis, Jhamandas, Mannard & Nichols, 1975; Stein, Nichols, Jhamandas, Davis & Charles, 1977; Davis *et al.* 1978). In a second surgical procedure some time after implantation, the nerve branches were cut 10–20 mm distal to their cuffs and treated in one of three ways. (1) Proximal and distal ends were individually *ligated* with a 4-0 Mersilene suture after removing several millimetres of nerve between them. This largely prevented regeneration, although a few fibres eventually managed to reinnervate end-organs in some preparations. (2) A nerve was cut and the proximal end was sutured directly to a nearby muscle, which was in turn denervated (*nerve–muscle suture*). (3) A nerve was cut cleanly with a scalpel, realigned and the perineurium of the proximal stump was connected to the perineurium of the distal stump with a number of fine sutures (7-0 silk) (*nerve–nerve suture*).

TABLE 1. Cat hind-limbs nerves were studied before and after the operative procedures indicated. The procedures were done distal to the recording electrodes and are described fully in the text.

Procedure	Tibial	CP	LGS	MG	Total
Ligation	1	3	0	0	4
Nerve–muscle suture	1	1	3	1	6
Nerve–nerve suture	1	1	0	1	3
Total	3	5	3	2	13

Four nerves were used: the tibial over its long unbranched length above the ankle, the common peroneal, the lateral gastrocnemius-soleus and the medial gastrocnemius. Altogether, thirteen nerves in thirteen cats provided data for this study, and the numbers of successful procedures carried out on each nerve are given in Table 1. Another four nerves had a cap placed over the distal end of the recording cuff containing the cut and tied nerve. Data from these experiments have not been considered here since the blood supply was seriously compromised by this procedure and the tied nerves without caps were more viable (Davis *et al.* 1978). In addition, nerves died back a short distance from the capped end which affected records from distal electrodes.

Recording

Compound action potentials were elicited electrically and recorded in the anaesthetized cat, as described previously by Davis *et al.* (1978). For recording neural and e.m.g. activity during conscious locomotion, a flexible cable was connected to a 12-pin socket embedded in a percutaneous connector in the animal. The cable was attached to a harness to prevent undue stress on the percutaneous connector during walking and led to a switching box. Each nerve was recorded using a tripolar configuration and coupled by a step-up transformer (Hammond 585D) to a low noise preamplifier (Grass P-15) with a band-pass of 300–10,000 Hz. The transformer provided isolation, some filtering and a valuable improvement in the signal-to-noise ratio (Stein *et al.* 1975), although some shunting of neural signals occurred as a result of the low impedance of the primary coil of the transformer. Thus, the 'effective gain' varied somewhat for different electrodes and frequency components, and was not known exactly. Based on detailed measurements for a

few electrodes (Stein *et al.* 1975), all data shown in this paper were divided by a nominal transformer gain of 20. Any uncertainty in absolute gain will not affect the relative values over time presented here, since the electrode impedances remained steady for months after an initial increase associated with connective tissue growth (Stein, Charles, Gordon, Hoffer & Jhamandas, 1978).

Signals were monitored on an oscilloscope and audio amplifier, displayed on a pen recorder and recorded on FM tape for later analysis. Before being displayed on the pen recorder, the signals were rectified and filtered using a third-order low-pass Paynter filter (Gottlieb & Agarwal, 1970) with a frequency cut of 30 Hz. The envelope or pattern of neural and electromyographic activity was faithfully reproduced during walking (frequency components up to 10 Hz) but contamination from power lines (60 Hz) or components from individual nerve impulses (300–10,000 Hz) were rejected. The signals from implanted bipolar e.m.g. probes sutured to the muscle surface (Hoffer, 1975; Hoffer, Milner & Stein, 1976) were treated identically to the nerve signals except that no transformer was used and the pass band of the preamplifier was 30–3000 Hz. In a few animals a length gauge was also implanted in the experimental leg to correlate neural and e.m.g. activity with phases of the step cycle. Length gauges were constructed similarly to those described by Loeb, Walmsley & Duysens (1979). They consisted of two Teflon-coated platinum-10% iridium wires fixed in the ends of distensible Silastic tubes which were filled with hypertonic saline and sealed. The proximal end was attached with heavy sutures through a hole drilled near the antero-proximal edge of the tibia and the distal end was sutured to the calcaneum for monitoring the length changes of soleus muscle. Increasing muscle length increased the resistance of the saline path between the two wires, which was monitored using a 25 kHz signal from a carrier amplifier.

Cross-correlation and spectral analysis

To determine the cross-correlated neural activity recorded at two sets of electrodes on the tibial nerve (Fig. 1) or at electrodes on the sciatic and one of its branches, FM tapes were played back at 1/16th speed into the analog-to-digital converters of a general purpose computer. Using computer programs which have been previously described (French, 1973), the power spectrum of each signal and the cross-spectrum of the two signals were computed. The inverse Fourier transform of the cross-spectrum gives the cross-correlation function (Bendat & Piersol, 1971). The cross-correlation function measures the amount of correlated activity between these two recordings. Motor fibre impulses travelling with velocity v between two electrodes separated by a distance l will reach the distal electrode at time $\Delta t = l/v$ after they are recorded at the proximal electrode. If the signals recorded by the proximal electrode are delayed an amount Δt and multiplied by the signals recorded at the distal electrode, a positive output will result for each motor spike travelling at velocity v. Sensory spikes will contribute a positive output if the signals at the distal electrode are in turn delayed, since they conduct in the opposite direction (this is equivalent to delaying the proximal record by a negative amount). For other values of Δt, positive or negative products may result, but these will average out to a small value over time in the absence of correlated activity. The use of spectral analysis facilitates calculation of these average products for many values of Δt (see Fig. 5 of Results).

The amplitude of each peak in the cross-correlogram is related to the total number of impulses recorded from all fibres conducting at a given velocity and to the average unitary spike amplitude. Peak-to-peak measurements from cross-correlations (such as Table 2 and Fig. 7) were subject to two kinds of errors. First, particularly when signal levels were small following axotomy, values were subject to substantial random errors due to noise. Secondly, there is generally some bias toward over-estimating the value of a peak. In particular, with no signal at all a non-zero peak due to noise is still measured, as explained below.

We carried out two types of studies to determine the magnitude of these errors. First, from data stored on magnetic tape, we took a stretch with a high signal-to-noise ratio and hence a fairly accurate measurement of the peaks and added to it various amounts of noise. Noise amplitude and spectral characteristics were varied to cover the entire range of frequencies (white noise) or just the range of frequencies at which e.m.g. pickup tended to contaminate our measurements (filtered noise). From these measurements, we verified that peak values were biased upward, particularly when the signal-to-noise ratio was low. In the absence of any signal, the measured peak-to-peak amplitude was still about 4 times the noise level, since there is a

5% chance of a point being more than two standard deviation units from the mean of a Gaussian distribution. Positive and negative peaks were added to obtain the peak-to-peak measurement.

Secondly, we added a known cross-correlation signal lasting 32 msec to cross-correlations which had already been computed. Sensory and motor peaks were contained in the first millisecond of data on either side of zero (Figs. 2 and 5). Therefore, we could add the known signal to each of thirty other records, measure the peak-to-peak signal and compute not only the mean but also the standard deviation. The extrapolated value (for zero signal added) occurred again at

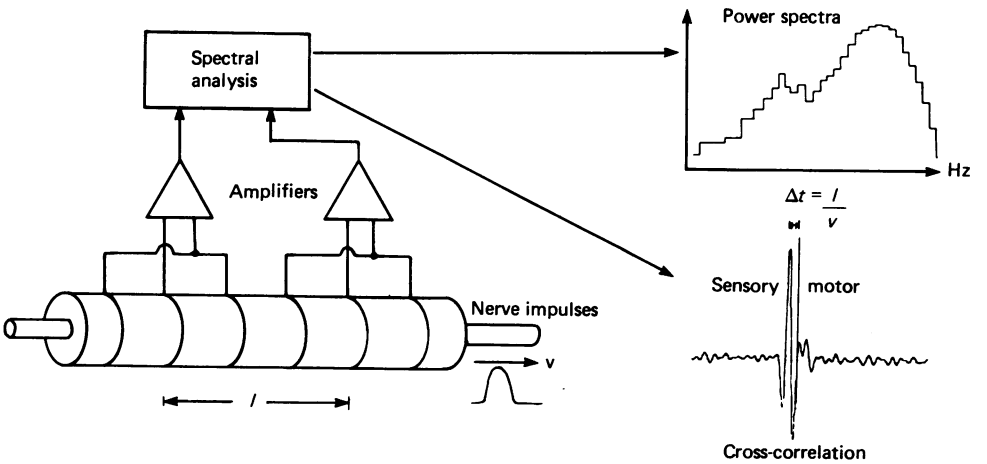


Fig. 1. Method for determining the cross-correlated neural activity recorded at two sets of electrodes on a tibial nerve. The power spectrum was computed for the signals at each site and the cross-correlation function obtained from the inverse Fourier transform of the cross-spectrum. Nerve impulses travelling with velocity v between the two electrodes separated by a distance l will reach the second electrode at time $\Delta t = l/v$ after they are recorded at the first electrode. If the signals recorded by the proximal electrodes are delayed an amount Δt and multiplied by the signals recorded at the distal electrodes, a positive output will result for each motor spike travelling at velocity v. The cross-correlation in this way measures the amount of correlated activity between the two recording sites as a function of time delay Δt between the two signals. Motor impulses occur with a positive latency at the distal electrodes while sensory impulses have a negative latency, since they travel in the opposite direction.

about 4. The bias was decreased to about half with a measured signal-to-noise ratio of 6 (an actual value of 4), and the standard deviation bars were about twice the noise level in each direction. This behaviour is expressed by

$$y = x + \frac{4}{1 + 0{\cdot}25x},$$

In which x represents the actual signal-to-noise ratio, y represents the measured signal-to-noise ratio, and the extent of the bias is given by the rectangular hyperbolic term on the right. Although the correction given by this equation was tested on much of our data, the results shown in Results are uncorrected for reasons that will be discussed in that section.

RESULTS

Patterns of whole nerve and muscle activity

Fig. 2*A* shows the neural activity recorded from the tibial nerve of a cat walking on a treadmill. Also shown is e.m.g. recorded from the long extrinsic muscles of the

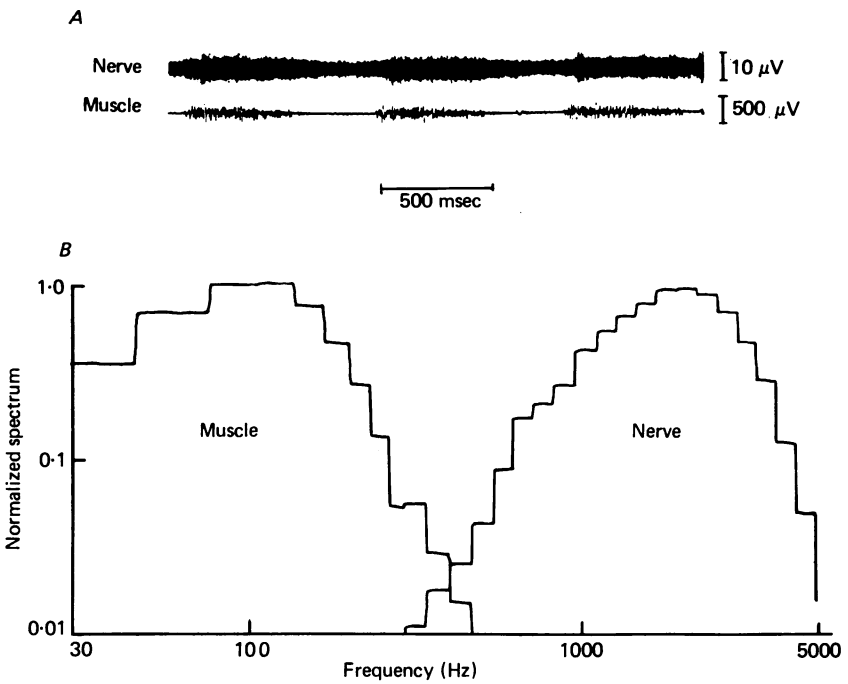


Fig. 2. *A*, neural and e.m.g. signals recorded in a cat walking on a treadmill. Neural signals were recorded from the tibial nerve and e.m.g. from the long extrinsic muscles of the toes and the ankle extensor muscles. The tibial nerve shows a diffuse burst in phase with the extensor e.m.g. activity. Although the amplitude of the neural signals is smaller than the e.m.g. signals, neural signals are clearly distinguishable from the noise level. Tibial nerve spikes are much more numerous than e.m.g. spikes in synergistic muscles due to the large number of afferent fibres that are also being recorded. Modulation of activity appears as a change in the envelope of signals recorded. Nerve action potentials are much briefer and therefore contain higher frequency components than those recorded from the muscles, as can be seen from the power spectra computed in *B* for the same steps as in *A*.

B, spectra computed from the neural and e.m.g. signals in *A* and normalized with respect to the highest peak. The units of each spectrum before normalization were $\mu V^2/Hz$. The e.m.g. peak occurs at 100–200 Hz, whereas the neural peak is well above 1000 Hz. Because of the tenfold separation in frequency, further filtering could be used to reduce e.m.g. pick-up by the neural recording cuffs while preserving the neural components. Both co-ordinates in *B* are logarithmic.

toes, such as flexor hallucis longus and flexor digitorum longus, as well as the ankle extensor muscles recorded by electrodes on the outside of the neural cuff.

Two differences between neural and e.m.g. activity are apparent. (1) Neural spikes are much smaller than muscle signals. However, the smaller neural signals were still clearly distinguishable from the noise level of the recording system, even though

recordings were made in an unscreened room with the treadmill motor nearby. (2) Action potentials recorded from nerves are much briefer and therefore contain higher frequency components than those recorded from muscles (see the power spectra in Fig. 2*B* computed for the neural and e.m.g. activity recorded over several steps). The peak of the spectrum from the tibial nerve occurs well above 1000 Hz, whereas the peak of the e.m.g. spectrum is at 100–200 Hz. Neural records were contaminated

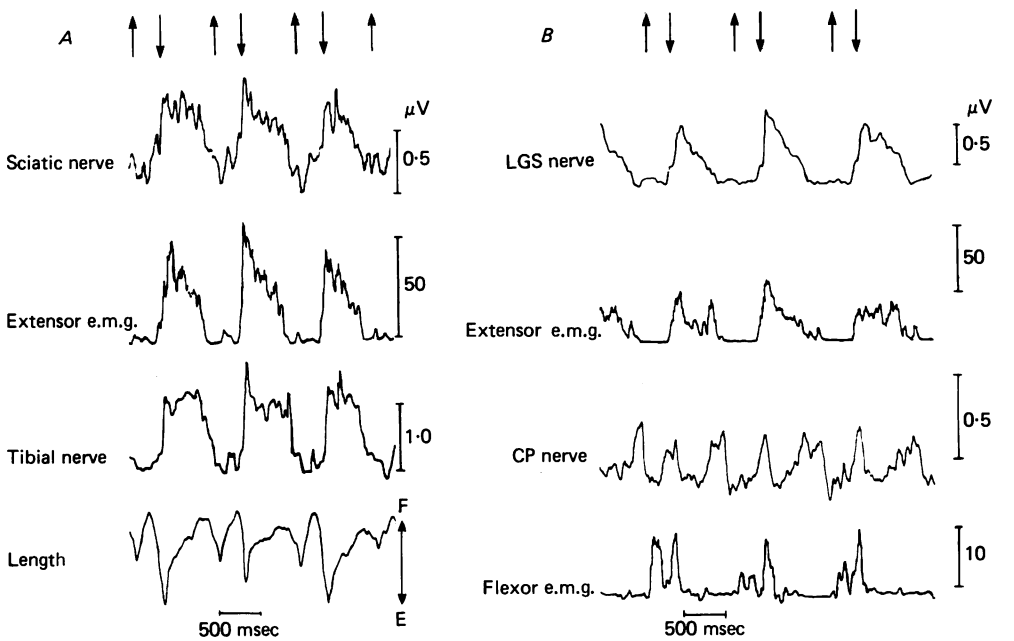


Fig. 3. Modulation of activity in *A*, the tibial nerve and *B*, the lateral gastrocnemius (LGS) and common peroneal nerves. Modulation of activity has been correlated in time with (1) the activity in the whole sciatic nerve, (2) the corresponding e.m.g. ankle extensor e.m.g. for the tibial and LGS, which are physiological extensor nerves, and the ankle flexor e.m.g. for the common peroneal, a physiological flexor nerve, and (3) length changes across the ankle joint. In *A* an increase in length corresponds to ankle flexion (stretching of the ankle extensor muscles). Arrows at the top indicate the time of foot lift at the beginning of the flexion phase of the step cycle and foot fall during the extension phase. After foot fall, the ankle 'gives' or flexes under the weight of the body for some time, despite the continued extensor activity.

with variable amounts of e.m.g. pickup from nearby muscles, but most of the power occurred at frequencies near the neural peak. The neural spectrum was obtained routinely in computing the cross-correlation function (Fig. 1) for each nerve and proved to be a useful way of assessing the e.m.g. contamination of the neural signal. If necessary, the signals on the neural recording cuffs were further filtered to reduce the low-frequency components due to e.m.g. with a sharp high-pass filter (80 db/decade; Krohn-Hite model 3700). The filter was adjusted to the frequency of the trough between the neural and e.m.g. peaks (400–700 Hz, Fig. 1). The neural spectrum shown in Fig. 2*B* was computed after filtering the signals at 600 Hz.

The electrodes in cuffs record impulses from all large myelinated sensory and motor

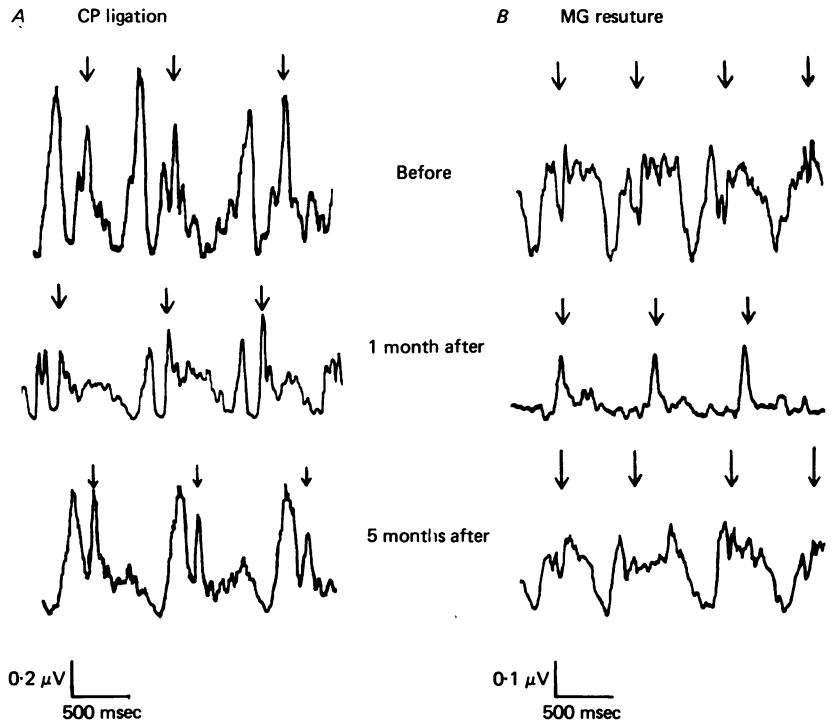
fibres without too much attenuation (Stein & Oğuztöreli, 1978), whereas e.m.g. electrodes are mainly sensitive to potentials from superficial muscle fibres close to the electrodes. Neural spikes are more numerous than muscle potentials, so neural recordings often have a smoother envelope despite their small amplitude, as shown in Fig. 3. All traces in Fig. 3 were amplified, full-wave rectified and filtered with a third-order low-pass filter at 30 Hz (see Methods). The frequency components of the neural and e.m.g. signals occur almost exclusively above 30 Hz (Fig. 2*B*), but the modulation in activity during walking is largely below this frequency.

Fig. 3*A* shows the modulation in activity of the tibial nerve and extensor e.m.g. which was shown in Fig. 2*A*, together with modulation on the whole sciatic nerve. The phases of the step cycle (Phillipson, 1905) were identified by the length changes in the ankle extensor group of muscles recorded with an implanted length gauge (Prochazka, Westerman & Ziccone, 1976, 1977; Loeb & Duysens, 1979). Activity was recorded from the tibial nerve well below the knee, where it contains fibres to the intrinsic muscles of the foot and cutaneous branches to the sole of the foot. The nerve shows a burst of activity during the extension phase of the step cycle with a characteristic peak when the foot hits the ground (indicated by the arrow). Activity continues after the cessation of extensor e.m.g. to the end of the stance phase (E3 in Phillipson's notation). The nerves supplying extensors of the ankle, lateral gastrocnemius-soleus in Fig. 3*B*, medial gastrocnemius and the whole sciatic nerve show a single main burst of activity in phase with extensor e.m.g. Most of the activity in the sciatic nerve occurs during extension, although a smaller burst of activity can sometimes be distinguished during flexion.

Although the extensor muscle group dominates the fibre representation in the sciatic nerve at the level of recording (midway between knee and hip), the sciatic also contains nerve fibres which supply the ankle flexor muscles via the common peroneal nerve. The modulation of activity in the common peroneal nerve and the flexor e.m.g. recorded from the anterior tibialis show characteristic double burst patterns (Fig. 3*B*). The two bursts in the common peroneal and the flexor e.m.g. do not correspond exactly because the activity in the nerve is dominated by sensory impulses (see next section of Results). Activity in the common peroneal peaks toward the end of extension, declines during the flexion phase of swing and then rises to a second peak at the end of swing. The flexor e.m.g. bursts are closer together, mainly because the first burst is delayed, as required to lift the foot from the ground. The second burst of flexor e.m.g. activity overlaps the beginning of the extensor burst. The double burst pattern of the ankle flexors has been observed in other flexor muscles (Engberg & Lundberg, 1969; Peret & Cabelguen, 1975) and has also been described for activity in other flexor nerves (Hoffer, 1975; Hoffer & Marks, 1976).

After nerve section which disconnects fibres from their end-organs, the common peroneal nerve still shows a double burst pattern (Fig. 4). However, the pattern now resembles that of the flexor e.m.g. with two bursts of activity occurring closer together. Note that the second burst of activity still occurs at the time of footfall, so the first burst must be delayed. The peaks are separated by 150–250 msec (20–30 % of the step cycle duration) compared to 250–400 msec (30–50 % of the cycle duration) in the intact nerve. The characteristic double burst pattern of the common peroneal nerve could still be seen 150 days after section and ligation, and actually increased in

amplitude in this animal. Cats with cut common peroneal nerves generally modified their gait by swinging the limb more vigorously from the hip to compensate for the inability to flex the ankle. Despite continued ligation, a few nerve fibres managed to reinnervate muscle in the example shown in Fig. 4.



Fig, 4. Modulation of activity in proximal stump of the cut common peroneal and medial gastrocnemius nerves. *A*, the double burst pattern of activity of the intact common peroneal nerve was maintained after section. Although much smaller and more irregular, the pattern became more like that of the flexor e.m.g. (see Fig. 3) with two bursts of activity occurring closer together. The double burst pattern of the common peroneal was actually larger and clearer 5 months after nerve section and ligation (see Discussion). *B*, one month after nerve section, the medial gastrocnemius nerve showed a single burst of activity although the level was not maintained during the burst. In addition, the onset of activity was delayed until just before foot fall (arrows). The activity during the burst recovered after the first month and returned to the original pattern once reinnervation occurred.

After axotomy, the modulation of activity in nerves supplying extensors (tibial, lateral gastrocnemius soleus and medial gastrocnemius) showed the single broad burst of activity characteristic of the intact nerves. However, the onset was delayed after axotomy (Fig. 4*B*) so that the burst coincided with the extensor e.m.g. A yield of the ankle accompanied section of the lateral gastrocnemius nerve. Gait changes were barely noticeable with section of either medial gastrocnemius or tibial nerves. The gait improved and the original pattern returned after reinnervation had taken place.

Motor and sensory components of neural activity

Fig. 5 shows cross-correlation functions for a tibial nerve obtained before and at three different times after a nerve-nerve suture. Correlated activity from two sites was measured over several steps and plotted as a function of the difference in latency Δt between activity recorded at the two sites. The vertical line represents $\Delta t = 0$;

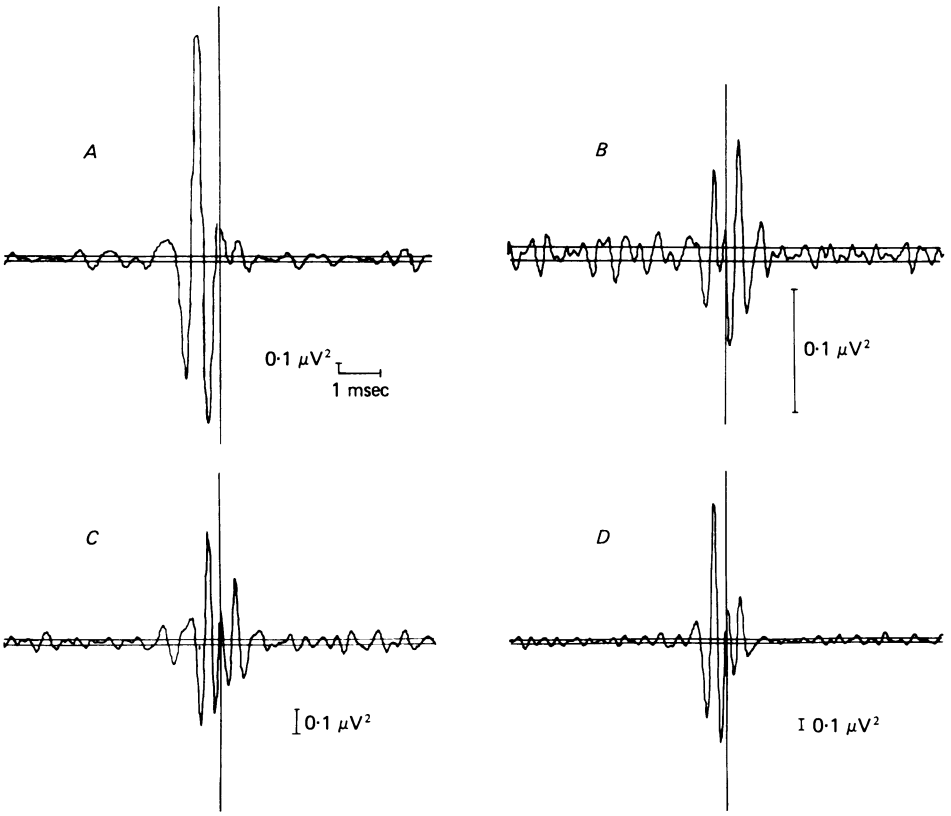


Fig. 5. Motor and sensory components of neural activity obtained from the cross-correlation functions of the tibial nerve, (*A*) before, (*B*) 42 days, (*C*) 78 days, and (*D*) 185 days after nerve section and nerve–nerve suture. Correlated activity from two sites on the nerve was computed as a product in μV^2 over several steps and plotted as a function of the difference in latency between activity recorded at the two sites. The vertical line represents zero difference in latency and the sensory activity is displayed to the left of the line and the motor activity to the right. These peaks represent the mean level of neural activity during three to four steps and the two horizontal lines represent 1 S.D. unit of the scatter from the base line. The first signs of motor reinnervation were seen at 43 days after suture.

sensory activity is displayed to the left of this line and motor activity to the right (sensory impulses occur at the distal recording site first and motor impulses occur proximally first). There is some scatter at long delays and the two horizontal lines represent plus and minus one standard deviation unit of the scatter from the baseline.

This 'neural noise' was lowest for the tibial nerve, where a long unbranched length

was available. Correlated activity was determined from two sites on the same nerve, and all spikes occurring at one site had corresponding spikes in the second record. Cross-correlation peaks were large compared to the noise level due to uncorrelated activity, in the tibial and to a lesser extent in the other normal nerves.

The cross-correlation of triphasic potentials is complex and contains a number of peaks which are dispersed in time. Therefore, we have not been able to distinguish different peaks corresponding to Group I and Group II fibres. The main peaks for each nerve in Fig. 5 correspond to the expected conduction latency for α-motoneurons

TABLE 2. Peak-to-peak amplitude of sensory and motor components of the cross-correlograms obtained from four hind-limb nerves for three to four step cycles during locomotion. The square root of the ratio of the sensory to motor peak amplitudes (S/M) gives a measure of the relative activity of sensory and motor fibres during stepping. This ratio is larger than the relative contributions of sensory and motor fibres to the nerve compound evoked potentials, as shown by the charge ratio at the spinal roots (Hoffer *et al.* 1979*b*). Cross-correlation values shown in this Table are geometric means for two to five intact nerves.

	Cross-correlation p–p amplitude (μV^2)				
	Sensory	Motor	S/M	$\sqrt{S/M}$	Charge ratio
Tib	7·78	0·81	9·60	3·10	1·1
CP	9·33	2·62	3·56	1·89	1·2
LGS	1·13	0·72	1 57	1·25	0·38
MG	1·04	0·33	3·15	1·77	0·40

and for the largest sensory fibres. The distance between the electrodes in the tripolar configuration at each recording site and the distance between the two sets of electrodes determines the shape of the function and whether the positive or negative peak is greater. For each preparation, the shape of the function was reproducible from day to day and peak-to-peak amplitudes were used to measure sensory and motor activity.

In intact nerves, the peak-to-peak amplitude of the sensory peak in the cross-correlogram was always larger than that of the motor peak, as illustrated for the intact tibial nerve in Fig. 5*A* and shown for four nerves in Table 2. The ratio of sensory to motor peak amplitudes was greatest for the common peroneal and tibial nerves, which contain large numbers of cutaneous afferents in addition to muscle afferents. The ratio is enhanced by the multiplication operation inherent in a cross-correlation, which makes the units of output μV^2. The square root of the ratio indicates the relative contribution of sensory and motor fibres to the modulation of activity during locomotion. Table 2 compares the relative contribution of sensory and motor fibres with the compound action potentials for each nerve obtained previously by measuring the total charge delivered to the dorsal and ventral roots by the peripheral nerve (Hoffer *et al.* 1979*b*). The sensory contribution to the modulation of activity during locomotion was two to four times greater than expected from its contribution to the compound action potentials in each nerve. This greater contribution is due to the higher mean firing rates of sensory fibres and by the recruitment of relatively more sensory than motor fibres during the step cycle.

Following nerve section, the sensory peak declined several fold. Interestingly,

levels of sensory fibre discharge did not decline to zero immediately. It is likely that spontaneous discharge occurred near the cut end (Govrin-Lippmann & Devor, 1978). Alternatively, cut sensory fibres may have discharged in response to mechanical stimulation during walking. Although the amplitude of the motor peak also declined, the motor peak became a relatively more prominent peak in the cross-correlogram (Fig. 5*B*). If reinnervation was permitted by either nerve–nerve suture or nerve–muscle suture, sensory and motor peaks recovered. However, the large sensory to motor ratio was not restored, since the motor peak recovered toward its former amplitude while the sensory peak reached only a fraction of its pre-operative value, even 200 days after nerve–nerve suture (Fig. 5*D*).

Time course of changes following axotomy

Neural recordings were made at similar walking rates both preceding and following axotomy. The cross-correlation functions were obtained for the same number of steps on each day. Data were obtained from individual nerves in single animals for 200 days after various procedures (Table 1). Because the amplitude of evoked action potentials decreases after axotomy (Davis *et al.* 1978), we compared the cross-correllation functions (measured in μV^2) with the product of the compound evoked potentials recorded at the same recording sites (in mV^2). The results are summarized in Fig. 6, which shows the geometric means of potentials recorded for three different procedures.

Evoked activity in all nerves declined after axotomy, as shown previously (Davis *et al.* 1978). In that study, the amplitudes for nerves which were ligated to prevent them from regrowing and making peripheral connections, declined to a quarter of the initial values. The evoked potentials plotted in Fig. 6*A* are for the squared values, being the product of the evoked compound action potentials recorded at the proximal and distal sites. The corresponding decline is to about $(0{\cdot}25)^2 = 0{\cdot}06$ of initial values.

In the sutured nerves, evoked signals recovered after reinnervation. The first signs of reinnervation (weak but measurable contractions in the reinnervated muscle evoked by nerve stimulation) were seen 30–40 days after nerve–nerve sutures and 40–50 days after nerve–muscle sutures. Evoked signals began to recover a few weeks later and the product reached 23 and 13% of initial values 200 days after nerve–nerve and nerve–muscle sutures respectively. The recovery of the evoked signals of resutured nerves is, however, under-estimated due to the slow ongoing decline of axons that have not made connections (Davis *et al.* 1978). The recovery of fibres that have successfully remade connections is better indicated by deviation of the data for the sutured nerves from the simple decay curve for the tied nerves. The compound potentials were larger, compared to the ligated nerves, by a factor of roughly four for nerve–nerve sutures and by a factor of 2 for nerve–muscle sutures. Although these results suggest that reinnervation occurs earlier and is more successful for nerve–nerve sutures than for nerve–muscle sutures, the samples are too small to detect significant differences between the recovery after the two procedures.

The modulation of activity in ligated nerves shown in Fig. 4*A* indicated that some motor activity continued to be generated during locomotion, but bursts were reduced in amplitude and duration. Fig 6 shows the change in amplitude of the motor and sensory peak components of the cross-correlogram as a function of time after axo-

tomy for tied and sutured nerves. Initially the amplitude of the motor potentials decreased, but did not decay to zero even in the tied nerves (Fig. 6*B*). The motor signals in the cut and tied nerves stabilized after a month and then remained at between 20 and 25% of their initial values during the 200 days of study. If axons were permitted to grow and remake connexions, motor signals returned toward pre-operative values, reaching 80% for the nerve–nerve sutures and 52% for the

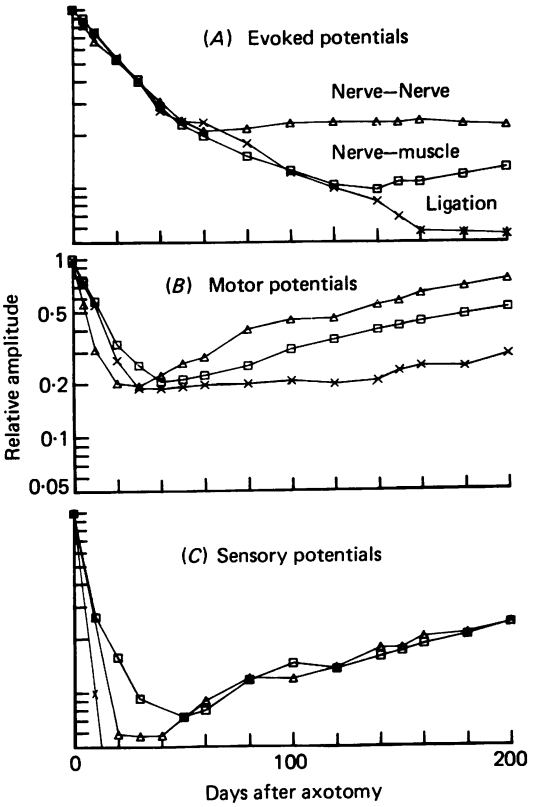


Fig. 6. Change in the evoked, motor and sensory activity following axotomy. Each point is the geometric mean for four nerves which were ligated (×), three that were cut and resutured to the distal nerve stump (△), or six that were cut and sutured directly into muscle (□). The evoked potentials are the product of the compound signals recorded at the two sites in mV^2 while the motor and sensory cross-correlation peaks have units of μV^2. However, in Figs. 6 and 7 the data have been normalized with respect to the pre-operative values to allow direct comparison of the curves.

nerve–muscle sutures by 200 days. Values for nerve–nerve and nerve–muscle sutures at 200 days are 3·8 and 2·5 times greater than the minimum values for the same nerves measured 30–40 days after axotomy, which compares well with the relative recovery of evoked signals discussed above.

Unlike the motor activity, which continued even months after the nerves were tied, sensory signals eventually disappeared below the noise level of the recording system unless reinnervation of the periphery was permitted by suturing the nerves. Fig. 6*C* shows the recovery of sensory activity in sutured nerves from minimal levels

recorded a month after axotomy. Sensory activity began to recover soon after peripheral connexions were made but recovered to only 24% of control values even 5 months after suture of nerves. The extent of the recovery would be even slightly less if a correction for possible bias in the measurement of small peaks was applied (see Methods).

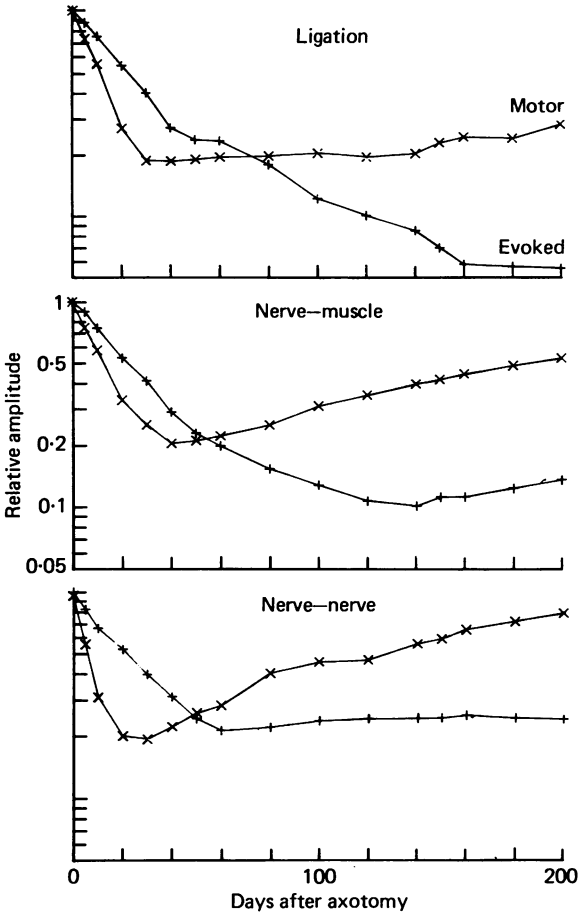


Fig. 7. Comparison of the motor activity and evoked potentials recorded for ligated and resutured nerves. The data are the same as in Fig. 6, but have been rearranged to show the greater decline in motor signals in the first month and the greater recovery of motor signals relative to evoked potentials at 150–200 days. By pooling data from all three procedures (thirteen nerves), the differences were significant ($P < 0{\cdot}05$) at 30 and 150 days.

Recovery of motor and sensory signals after reinnervation generally paralleled the recovery of evoked signals for fibres that were successful in regenerating to appropriate end-organs. However, quantitative differences were noted in the time course of changes in evoked potentials and of motor activity generated during locomotion, as shown in Fig. 7. The early decline in motor activity for all procedures was greater than the corresponding decline in evoked signals. The motor activity fell to about 20% of initial values in the first month after axotomy, at a time when the

evoked signals had fallen to 40%. Thus, the fall in motor cross-correlation peak amplitudes could not be accounted for by the decline in amplitude of the evoked signals alone. Applying the correction for bias described under Methods would only accentuate this difference.

In contrast, recovery of motor activity exceeded the recovery of evoked signals after reinnervation. Even in tied nerves where little or no regeneration occurred, motor activity stabilized after the initial decline and was maintained over a period of months while evoked signals continued to decline. The decay curves of motor and evoked potentials cross at about 75 days and diverge. A similar crossing of the data for motor and evoked potentials is observed for sutured nerves, although the separation is not as great at 150–200 days.

DISCUSSION

The use of chronic neural recording techniques has enabled us to describe patterns of activity in four hindlimb nerves of the cat. With cross-correlation techniques, quantitative measures of the motor and sensory components of the activity were obtained which were followed in single animals for long periods of time before and after cutting the nerves distal to the cuffs. Sensory activity tended to dominate in intact nerves during locomotion, particularly for mixed musculocutaneous nerves (common peroneal and tibial). Following axotomy, a motor pattern was observed for the nerve similar to that which had previously been recorded from the e.m.g. For example, a common peroneal nerve normally has two bursts of activity during each step: one at the end of extension, when the flexor muscles are maximally stretched, and the second at the beginning of extension close to the time of footfall. Following axotomy, the nerve still showed a double burst pattern, but the first burst was shifted in time into the flexion phase of the step, while the second burst still occurred at a time when extensors are active.

After axotomy, total motor activity during walking declined to a new and stable level, as was previously shown for the amplitude of the evoked potentials recorded from cut and tied nerves (Davis *et al.* 1978). However, changes in motor activity could not be fully accounted for by the corresponding changes in the compound potentials evoked from the whole nerve. Motor activity declined more steeply than evoked compound potentials in the first month, but then stabilized while evoked potentials continued to decline (Fig. 7). These two results will now be discussed in turn.

Early decline in motor activity following axotomy

Evoked potentials recorded from the whole peripheral nerve do not represent the actual atrophy in motor axons unless both sensory and motor fibres are equally affected by axotomy. Recently we showed that sensory fibres are more severely affected by axotomy than motor fibres, although this differential atrophy does not become apparent until after the first 45 days (Gordon, Hoffer & Stein, 1978; Hoffer *et al.* 1979*a*, *b*). Therefore, the decline in motor fibre diameter may be assessed in the first month from the decline in evoked signals from the whole nerve. This decline can only account for part of the reduction in motor signals during voluntary activity.

The extra reduction must therefore be due to a reduction in the numbers of nerve impulses generated by motoneurones.

The first obvious change after severing a muscle nerve is the interruption of the normal traffic in homonymous afferent fibres. Therefore, phasic afferent volleys during locomotion which may play a role in reflexly facilitating the recruitment of α-motoneurones (Severin, Orlovski & Shik, 1967) are lost immediately. In these experimental animals, however, nerve section did not cause a large decline in motor output for the first few days. Thus, the larger than expected decline in motor output which developed over the first month presumably comes from other sources. Furthermore, these data suggest that homonymous afferent input is not essential in determining the over-all level of motor activity during locomotion.

Dramatic electrophysiological changes take place in α-motoneurones within days of nerve section which reach a maximum 2–3 weeks later (Eccles *et al.* 1958; Kuno & Llínas, 1970*a*, *b*; Kuno, Miyata & Muñoz-Martinez, 1974*a*), concurrent with the series of morphological and biochemical changes that are collectively referred to as chromatolysis or the axon reaction (Lieberman, 1971; Watson, 1976).

One likely explanation for a reduction in numbers of motor impulses in the first month after axotomy is less efficient synaptic transmission onto motoneurones. Excitatory post-synaptic potentials (e.p.s.p.s) are smaller in amplitude, longer in duration and much more variable in axotomized than normal motoneurones (Eccles *et al.* 1958; Kuno & Llínas, 1970*a*). These changes in synaptic efficacy are associated with morphological changes, including swelling of the soma and retraction of dendrites (Cerf & Chacko, 1958). Eventually the post-synaptic thickenings are lost and boutons are displaced from the soma and proximal dendrites (Sumner & Sutherland, 1973; Cull, 1974; Sumner, 1975). Electrophysiological measurement of monosynaptic connections onto normal and axotomized motoneurones indicates that although the e.p.s.p.s are smaller and longer in the first month after axotomy, 1a afferent connectivity is normal until 1–2 months after axotomy, when connections are lost (Mendell *et al.* 1976). Motor output in the first month may therefore be reduced by diminished efficacy rather than loss of connectivity.

Stabilization of motor activity in ligated nerves

Following the first month after axotomy, motor activity remained at about 20% of initial values, despite a continuing decline in the evoked signals for motor fibres. Many structural and biochemical changes associated with chromatolysis subside after 1 month (reviewed by Lieberman, 1971; Watson, 1976). Some electrophysiological properties of motoneurones also stabilize after the first 20–30 days following axotomy, such as input resistance, duration of the hyperpolarization and amplitude of the overshoot of motoneuronal impulses recorded intracellularly (Kuno, Miyata, Muñoz-Martinez, 1974*b*). The diameter of motor fibres continues to decline, although the later decline of evoked action potentials in whole nerves is dominated by the relatively greater atrophy of sensory than motor fibres (Gordon *et al.* 1978; Hoffer *et al.* 1979*a*, *b*). Changes in synaptic transmission also continue past the first month and often culminate in loss of connectivity of many 1a synapses to axotomized motoneurones during the second and third months (Mendell *et al.* 1976). From another series of acute experiments, Hoffer *et al.* (1979*b*) calculated separate curves for the decay of electrical

charge measured on the ventral and dorsal roots following nerve ligation. These curves indicate that the charge due to motor fibres decayed to 0·42 of its initial value. For comparison with Fig. 7, the decay would be $(0{\cdot}42)^2 = 0{\cdot}18$ or 18 % at 200 days. Thus, much of the difference between the decay of the evoked potentials (to 6 % in Fig. 7) and the motor cross-correlation peaks (to 24 % in Fig. 7) was probably due to less atrophy of motor fibres than of sensory fibres. However, the value of 18 % is probably an over-estimate because it represents the total charge generated by all fibres when recorded monophasically from the ventral roots. In contrast, the peaks of the triphasic evoked potentials recorded from the nerves can be considerably reduced by dispersion and some cancellation of positive and negative potentials (Davis *et al.* 1978) Thus, part of the discrepancy between motor cross-correlation peaks and compound evoked potentials may arise from other sources.

Stabilization of the level of motor output while the diameters and hence the size of signals from motor axons is declining suggests that the motoneurones may be generating more nerve impulses. Partial recovery due to reinnervation in a few fibres could not be completely discounted. However, increased numbers of motor impulses could arise from a relative increase in synaptic drive on to motoneurones, which would compensate for the loss of synaptic connexions. For example, shortly after the common peroneal nerve was cut, the animals showed 'foot drop' similar to that observed in human patients. Because the ankle cannot be flexed actively, the foot hangs limply down during the flexion phase of the step cycle and drags along the ground. After several months, these symptoms were not observed during walking at normal speed because the animal swung his leg more vigorously from the hip, which caused some passive flexion of the ankle. In the more vigorous flexion of the limb from the hip, there must be a stronger descending drive to the hip flexors and some of this increased drive may extend to motoneurones in the common peroneal nerve as well. No striking qualitative changes were seen in the patterns of motor activity generated during walking which were still appropriate for the intended movements. A similar type of compensation was noticed in the ankle extensor muscles. The e.m.g. generated in the medial gastrocnemius muscle was increased after cutting the lateral gastrocnemius-soleus nerve and vice versa. Thus, in compensating for the loss of power resulting from ligation of a muscle nerve, the animal may increase synaptic drive onto all synergistic motoneurones, including the axotomized cells. This increased synaptic drive could offset to some extent the loss of synapses on to the cells and the reduction in the evoked signals we recorded due to declining fibre diameter.

Effects of activity on fibre diameter

A decline in axon diameter is often associated with and may be caused by a decline in motor and sensory activity. Cell bodies of sensory nerves show chromatolytic changes only when the peripheral axons but not axons central to the ganglion are cut (Marinesco, 1896; Ranson, 1914; reviewed by Cragg, 1970). Similarly, conduction velocity of sensory fibres is slowed only after peripheral axotomy (Czéh, Kudo & Kuno, 1977). Marinesco in 1896 attributed the difference in the chromatolytic effect of the two lesions to the silencing effect of peripheral axotomy on impulse traffic in sensory fibres. Many atrophic changes in denervated muscles have been attributed to and produced by disuse, so that activity imposed upon muscle may account for

some of the trophic influence of nerve on muscle (reviewed by Vrbová, Gordon & Jones, 1978). Similarly, nerve fibre atrophy after axotomy may be partly due to reduction in impulse traffic. This is also suggested by the evidence that nerve fibres atrophy after tenotomy (Aitken, Sharman & Young, 1947) and immobilization by skeletal fixation (Eisen, Carpenter, Karpati & Ballavance, 1973). Hypertrophy of motoneurones supplying medial gastrocnemius has also been demonstrated following removal of the remaining ankle extensor muscles (Walsh, Burke, Rymer & Tsairis, 1978).

Continued activity in motor fibres described here may be partly responsible for sustaining the motor fibres relatively better than the silent sensory fibres several months after axotomy (Hoffer *et al.* 1979*a*, *b*). In the first month after section, when many sensory fibres still conduct impulses generated spontaneously from the neuroma (Govrin-Lippmann & Devor, 1978), sensory and motor fibres atrophy similarly. This correlation between the amount of activity in nerves and their atrophy following axotomy supports the hypothesis that impulse traffic plays a role in the maintenance of nerve fibres.

Recovery following reinnervation

Many changes due to axotomy are reversed when connections are remade in the periphery. The electrophysiological properties of motoneurones return toward normal (Kuno *et al.* 1974*b*), conduction velocity increases (Cragg & Thomas, 1961; Davis *et al.* 1978), cells regain their former dimensions (Lieberman, 1971; Sumner & Watson, 1971) and synaptic connections are remade (Cull, 1974; Mendell & Scott, 1975; Sumner, 1975). As soon as the first signs of reinnervation were seen, motor activity began to recover toward preoperative values. Sensory activity recovered, but this recovery was not as complete as that of motor activity within the period studied. However, recovery of sensory activity depends on reformation of peripheral connections while motor activity continues in both isolated and reinnervated motor fibres. The motor signals from regenerating motor nerves also increased to higher levels than expected from the evoked potentials and for nerve–nerve sutures, control levels were almost regained. A compensation by increased synaptic drive, such as was discussed for ligated nerves, may also be operating for regenerating nerves, although this compensation would be needed less as the force output returns toward control values (Davis *et al.* 1978).

Clinical applications of these results should also be considered. The continued generation of motor impulses in ligated nerves may offer additional ways for amputees to control powered artificial limbs. Perhaps the signals in cut nerves of human amputees can be enhanced by suturing the nerves to nearby muscles, since this procedure appears to be almost as successful as resuturing nerves to their distal stumps in promoting recovery of motor activity in cut nerves. The generation of e.m.g. signals near the skin surface from nerve–muscle grafts would also eliminate the necessity for surgical implantation of cuffs around nerves and the associated problems of recovering these small signals from within the body. Thus, the use of chronic recording methods should be useful in studying both the basic interaction between nerve and muscle and clinical conditions where these interactions are modified.

We thank Mr Dean Charles for constructing many of the cuffs which were used in this study. Dr Lyle A. Davis did much of the surgery and was a constant source of encouragement and useful suggestions during the course of this work. The research was supported in part by grants to R.B.S. from the Medical Research Council of Canada and the Muscular Dystrophy Association of Canada, and to R.B.S. and L.A.D. from the Medical Research Fund of the University of Alberta. J.A.H. was a postdoctoral fellow of the Muscular Dystrophy Association of Canada.

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