*J. Physiol.* (1980), **305**, *pp.* 151–169
*With* 10 *text-figures*
*Printed in Great Britain*



# EPHAPTIC TRANSMISSION BETWEEN SINGLE NERVE FIBRES IN THE SPINAL NERVE ROOTS OF DYSTROPHIC MICE

BY MICHAEL RASMINSKY

*From the Division of Neurology, Montreal General Hospital, 1650 Cedar Avenue, Montreal, Canada H3G 1A4 and Department of Neurology and Neurosurgery, McGill University, Montreal, Canada*

(*Received 11 July 1979*)

SUMMARY

1. Ephaptic transmission was observed between spontaneously active single nerve fibres in the spinal nerve roots of dystrophic mice.

2. In the five ephaptically interacting pairs of fibres studied in detail, the conduction velocities in the exciting fibres were < 1 m/sec and the conduction velocities in the excited fibres were 2–10 m/sec in the immediate vicinity of the ephapses at 26–28 °C.

3. Membrane current analysis suggested that conduction was continuous in the exciting fibres. In some cases conduction away from the ephapse in the excited fibre was saltatory in at least one and possibly in both directions of transmission.

4. It is concluded that in at least some cases the direction of ephaptic transmission is from bare axon to myelinated axon.

5. Transmission time across the ephapses, measured as the interval between peaks of inward membrane current in exciting and excited fibres, was $\leqslant$ 100 $\mu$sec–240 $\mu$sec.

6. Ephaptic transmission is not necessarily contingent upon the direction of propagation of the impulse in the exciting fibre.

7. Ephaptic transmission between two fibres can remain stable at frequencies of at least 70 Hz.

8. There may be multiple sites of spontaneous ectopic excitation in single dystrophic mouse spinal root axons. An impulse traversing a site of ectopic excitation may incite a subsequent burst of impulses to arise from that site following a delay of more than 100 msec.

## INTRODUCTION

In the lumbosacral spinal roots of the mutant mouse *dystrophia muscularis* most axons are either thinly myelinated or devoid of any Schwann cell investment (Bradley & Jenkison, 1973; Bray & Aguayo, 1975; Stirling, 1975). Conduction velocity of nerve impulses is reduced in these spinal root fibres (Huizar, Kuno & Miyata, 1975; Rasminsky, Kearney, Aguayo & Bray, 1978); the finding that the mode of conduction in some of these fibres is continuous implies that at least some of the bare axons are electrically excitable (Rasminsky *et al.* 1978).

Spinal root axons of dystrophic mice act as ectopic generators of nerve impulses (Rasminsky, 1978*a*). Some of this ectopic activity is due to cross talk or ephaptic

0022-3751/80/1100-0357 $07.50 

transmission between contiguous fibres within the spinal roots (Huizar *et al.* 1975; Rasminsky, 1978*a*). Ephaptic transmission between mammalian axons also occurs as a transient phenomenon following acute injury of normal peripheral nerves (Granit, Leksell & Skoglund, 1944; Granit & Skoglund, 1945) and as a more stable phenomenon in experimental neuromas beginning about a month after nerve injury (Seltzer & Devor, 1979). In all previous experiments on ephaptic transmission in mammalian fibres, recordings were made from the exciting and excited fibres at a greater or lesser distance from the site of ephaptic interaction and no conclusions could be drawn concerning the conduction properties of the interacting fibres at or near the ephapse. The present experiments were designed to elucidate the conduction properties of the exciting and excited fibres in ephaptically interacting pairs of fibres within dystrophic mouse spinal roots. Brief preliminary accounts of these experiments have previously been published (Rasminsky, 1978*b*, 1979).

## METHODS

Experiments were performed on adult (8–45 weeks) 129B6F1J dy/dy dystrophic mice weighing 16–23 g. The mice were obtained from Jackson Laboratories, Bar Harbor, Maine, U.S.A.

*Preparation of animals.* Anaesthesia was induced with sodium pentobarbitone 50 mg/kg body wt. I.P. and was maintained throughout the experiment with inhalation of fluothane in oxygen. A lumbosacral laminectomy was performed; the paraffin pool made over the laminectomy site usually remained at ambient temperature of 26–28 °C.

*Recording system.* The recording arrangement which is similar to that used in previous experiments (Rasminsky *et al.* 1978; Rasminsky, 1978*a*) is illustrated in Fig. 1. A dorsal or ventral root in continuity was gently lifted on to two pairs of 125 $\mu$m silver wire hook electrodes each with interelectrode spacing of about 1 mm. The proximal pair of electrodes supported the root near the spinal cord, the distal pair near the exit from the spinal canal. One of these two pairs of electrodes was used as the reference pair of electrodes. A third pair of parallel, horizontal 25 $\mu$m platinum-iridium electrodes separated by about 150 $\mu$m (Rasminsky *et al.* 1978) was gently applied to the root between the pairs of reference electrodes and will be referred to as the mobile electrodes.

Each pair of recording electrodes was led to a high input impedance differential amplifier (Neurolog NL 100) near the preparation and further amplification was accomplished by a Neurolog NL 103 amplifier and Neurolog NL 105 AC amplifier (band pass 10 Hz–10 kHz). Permanent records of spontaneous activity were made on magnetic tape using a Racal Store 4 tape recorder. For most experiments activity on the mobile electrodes and one pair of reference electrodes was recorded on FM channels with band pass 0–10 kHz. For experiments in which activity on both pairs of reference electrodes was recorded, activity on the second pair was recorded on a direct channel with band pass 100 Hz–75 kHz. This led to some distortion of the lower frequency components of the recorded signals on playback which was not critical since there was no change in the polarity of the initial deflection of the recorded nerve impulses (see below).

The amplitudes of the differentially recorded potentials generated from single fibres were as large as 50–100 $\mu$V for the mobile electrodes and somewhat larger for the reference electrodes, the noise level being generally about 10 $\mu$V or less. No attempt was made to introduce a current calibration as has been accomplished by Bostock & Sears (1978) in a similar recording situation and for this reason no amplitudes are indicated for the records shown in the illustrations.

The potentials recorded differentially between two electrodes applied to the root reflect the longitudinal currents flowing between the electrodes external to nerve fibres along which impulses are propagating. The initial direction of external longitudinal current is opposite to the direction of propagation and thus defines the direction of propagation for each impulse. In all external longitudinal current records, an upward deflexion reflects current flowing away from the spinal

cord towards the periphery; thus impulses giving rise to an initial upward deflexion are propagating towards the spinal cord and impulses giving rise to an initial downward deflexion are propagating away from the spinal cord.

*Recognition of cross-talk.* One of the reference electrodes was positioned so that the spikes from a single spontaneously active fibre (recognized by invariant shape and amplitude) could be used to trigger the sweep of the oscilloscope. The output pulse from the trigger circuit was in turn used to trigger a delay line in a Nicolet 1070 averaging computer and events occurring on any of the

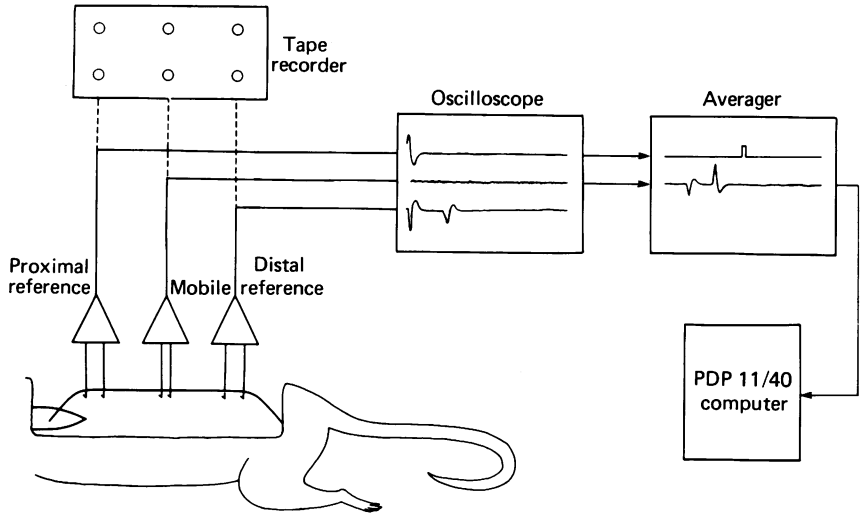


Fig. 1. Schematic diagram of the recording system.

two or three sets of electrodes during the 5 msec before and after the trigger pulse were averaged at a digitization rate of 20 $\mu$sec/bin. Ephaptic transmission was recognized when the trigger impulse from one fibre was consistently associated with an impulse in a second fibre either following or preceding the trigger impulse. The delay line was only required to recognize ephaptic transmission if the associated impulse preceded the trigger impulse.

In the diagrammatic example shown in Fig. 1, the trigger impulse is a centripetally propagated impulse (upward deflexion) recorded at the proximal reference electrodes. No activity is seen on the oscilloscope screen at the mobile electrodes, but by use of the delay line it is apparent that at the site of the mobile electrodes a centrifugally propagated impulse (downward spike) precedes a centripetally propagated impulse (upward spike), indicating that the site of ephaptic interaction is distal to the mobile electrodes. At the distal reference electrodes, two centrifugally propagated impulses (downgoing spikes) are apparent on the oscilloscope screen without use of the delay line, and the site of ephaptic interaction is thus localized between the mobile electrodes and the distal reference electrodes.

When a pair of cross-talking fibres was identified, the position of the reference electrodes was kept unchanged and the mobile electrodes were moved along the root by a micromanipulator in steps of 100 or 200 $\mu$m until recordings had been made over an excursion of one to three or more mm on either side of the site of cross talk. At each recording site 32–128 sweeps were usually averaged although it was occasionally useful to examine single sweeps. After each averaging procedure the memory contents of the Nicolet 1070 computer were transferred to the memory of a PDP 11/40 computer and stored on disk for subsequent analysis. Most experiments were done off line; the acquisition of sufficient iterations of the ephaptic interaction was monitored with the Nicolet 1070 averaging computer but entry of data into the PDP 11/40 computer was usually done on rerunning the experiment from the tape.

The major disadvantage of this method of examining ephaptic transmission was its total dependence on the persistence of spontaneous activity in appropriate single fibres. Unsuccessful attempts were made to isolate ephaptically interacting pairs of fibres by means of our previous

technique for stimulating single fibres in the periphery (Rasminsky *et al.* 1978). In preliminary experiments, the single fibres excited at low stimulus intensity (presumably the larger diameter fibres) did not ephaptically excite other fibres and at higher stimulus intensities it was impossible to recognize possible ephaptic interactions between single fibres within the compound action potential evoked on the spinal roots.

## RESULTS

Fig. 2 illustrates ephaptic transmission between two nerve fibres in a ventral root of a dystrophic mouse. The initial downward deflexion of the uppermost record of Fig. 2*A* represents an impulse propagating away from the spinal cord. This impulse gives rise to a similar downward deflexion at a progressively greater latency at successive positions along the root over the almost 4 mm illustrated (dashed arrow). A second impulse arises in midroot and is propagated towards the spinal cord (upward deflexions in records 8–1) and away from the spinal cord (downward deflexions in records 10–20) (open arrows). At record 9 the recording electrodes straddle the site of origin of this impulse and no clear-cut initial upward or downward deflexion is seen. As indicated diagramatically in Fig. 2*B* this experiment thus defines (to within about 200 $\mu$m) the site of ephaptic interaction between two fibres within a spinal root.

In Fig. 2*C* the latencies of the impulses in the two fibres are plotted against distance along the root. For the exciting fibre, latencies are plotted to the downgoing peak; for the excited fibre latencies are plotted to the upgoing peak for the portion of the fibre proximal to the ephapse in which conduction is towards the spinal cord and to the downgoing peak for the portion of the fibre distal to the ephapse in which conduction is towards the periphery.

For the exciting fibre the increase in latency with distance is uniform in the immediate vicinity of the ephapse, and the conduction velocity is the reciprocal of the slope of the distance *vs.* latency plot. For the exciting fibre illustrated in Fig. 2 the conduction velocity in the vicinity of the ephapse is 0·9 m/sec. Conduction velocity in the excited fibre is less easily specified since in neither direction of propagation is the impulse conducted away from the ephapse with a uniform increase in latency at successive recording sites. An estimate of the conduction velocity in the excited fibre in the immediate vicinity of the ephapse can be obtained by measuring the latency from the site of the ephapse to sites arbitrarily close to the ephapse. For this purpose the time of origin of the excited impulse at the ephapse is taken as the average of the peaks of external longitudinal current generated by the impulse in the excited fibre at the recording sites closest to the ephapse on either side of the ephapse. These external longitudinal currents flow in opposite directions towards the ephapse where inward membrane current is generated in the excited fibre on initiation of the excited impulse.

For the fibre illustrated in Fig. 2 latencies from the site of the ephapse to 0·5 mm distal and proximal to the ephapse were 70 and 90 $\mu$sec respectively corresponding to conduction velocities of 7 and 6 m/sec. The corresponding conduction velocities calculated for distances 1·0 mm away from the ephapse were 4 and 8 m/sec and for distances 1·5 mm away from the ephapse 5 and 6 m/sec. The absolute values of the conduction velocities are of less importance than the fact that no matter how

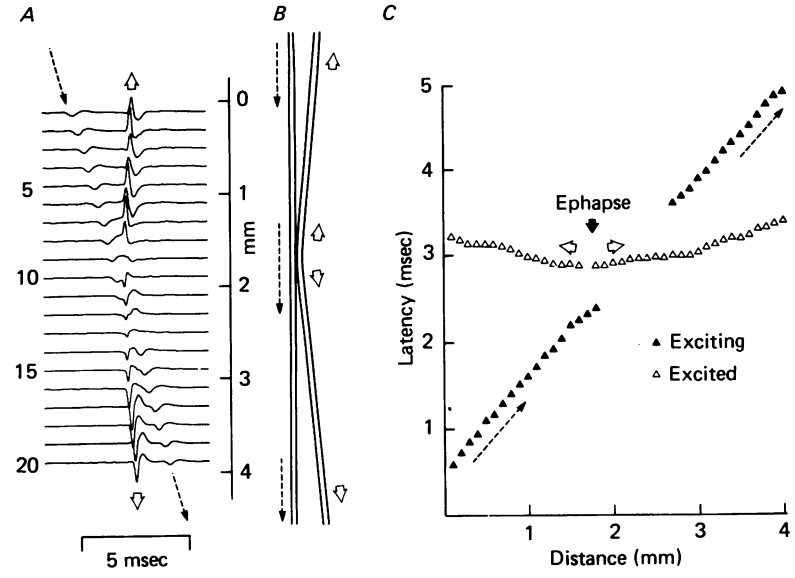


Fig. 2. Ephaptic transmission between two nerve fibres in a dystrophic mouse ventral root. *A*, external longitudinal current records at intervals of 200 $\mu$m from proximal (top) to distal (bottom). The directions of propagation in the exciting and excited fibres are indicated by the dashed and open arrows respectively. Zero on the distance scale is arbitrary. *B*, diagrammatic indication of the site of ephaptic interaction near record 9. *C*, latency to the peak of external longitudinal current vs distance along the root for the exciting (▲) and excited (△) fibres. The distance scale corresponds to *A*; zero time is arbitrary.

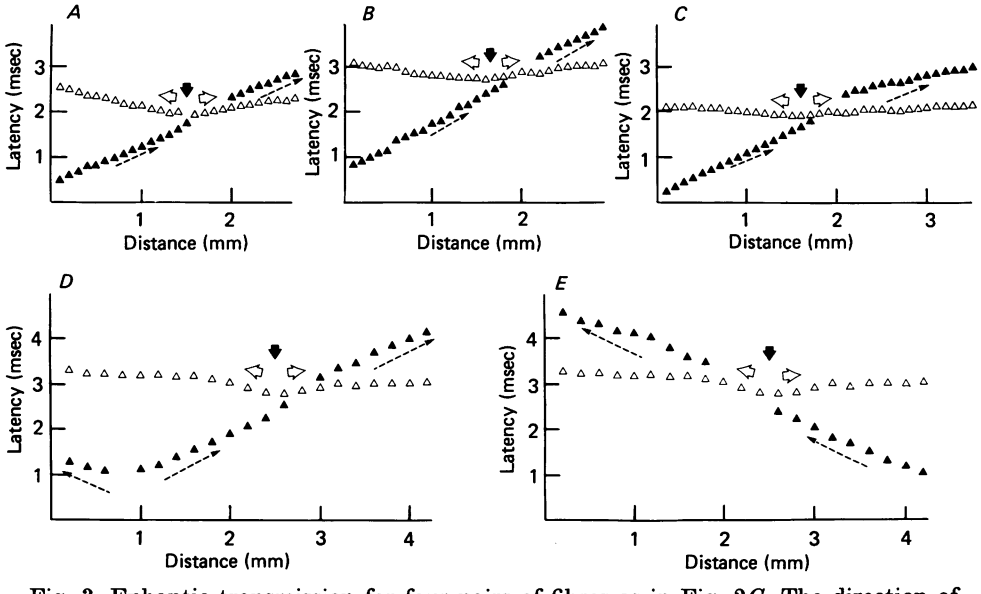


Fig. 3. Ephaptic transmission for four pairs of fibres as in Fig. 2*C*. The direction of propagation in the exciting fibre (▲) is indicated by the dashed arrow, in the excited fibre (△) by the open arrows. The sites of the ephapses are indicated by the vertical arrows. *D* and *E* represent one pair of fibres for which external longitudinal current records are shown in Fig. 6*A* and *B*. In all cases distance increases from proximal to distal. Zero distances and latencies are arbitrary.

measured, the conduction velocity of the impulse in the excited fibre is clearly several times greater in both directions than the conduction velocity of the impulse in the exciting fibre.

Fig. 3 illustrates similar plots of latency *versus* distance for four other pairs of fibres; the data are summarized in Table 1. In all cases, conduction in the exciting

TABLE 1. Conduction properties of the exciting and excited fibres in the immediate vicinity of ephapses. The paired values for latencies and conduction velocities in the excited fibres are for propagation in the centrifugal and centripetal directions. All experiments at 26–28 °C

| Conduction velocity of exciting fibre (m/sec) | Latency from ephapse for impulse in excited fibre ($\mu$sec) | | Conduction velocity away from ephapse of impulse in excited fibre (m/sec) | | Latency across ephapse ($\mu$sec) |
|---|---|---|---|---|---|
| | Over first 500 $\mu$m | Over first 1000 $\mu$m | Over first 500 $\mu$m | Over first 1000 $\mu$m | |
| 0·9 | 130 | 290 | 3·9 | 3·5 | $\leqslant$ 100 |
| | 190 | 390 | 2·6 | 2·6 | |
| 0·9 | 70 | 230 | 7·1 | 4·4 | 240 |
| | 90 | 130 | 5·6 | 7·7 | |
| 0·8 | 120 | 260 | 4·1 | 3·9 | $\leqslant$ 120 |
| | 50 | 200 | 10 | 5·0 | |
| 0·9 | 50 | 90 | 10 | 11 | 160 |
| | 70 | 130 | 7·1 | 7·7 | |
| 0·8* | 150 | 210 | 3·3 | 4·8 | $\leqslant$ 140 |
| 0·9† | 240 | 360 | 2·1 | 2·8 | $\leqslant$ 200 |

* For propagation centrifugally. † For propagation centripetally

fibre proceeds at a slow ( < 1 m/sec) uniform velocity in the immediate vicinity of the ephapse and conduction velocity in the excited fibre is greater in both directions of propagation away from the ephapse.

Table 1 represents the relatively few pairs of fibres in which spontaneous activity in the exciting fibre and the ephaptic interaction persisted for a long enough period to permit complete definition of conduction velocities on either side of and across the ephapse. In some of these pairs the interaction persisted for more than one hour and repeated recording runs over the site of the ephaptic interaction demonstrated no change in conduction velocity of the exciting or excited fibres. Ephaptic transmission was recognized between at least ten other pairs of fibres in which spontaneous activity persisted for too brief a period (i.e. less than about ten minutes) to permit complete definition of conduction velocities on both sides of the ephapse.

In a few experiments the temperature of the pool was raised to 37 °C by a thermostatically controlled heater. It was not possible to hold any ephaptic pair long enough at this temperature to define conduction velocities but one pair of fibres was found to interact at both 28 and 37 °C and a second pair of fibres interacted at 37 but not 28 °C. Although the temperature dependence of ephaptic transmission has not been studied in any detail, it is clearly not a phenomenon which occurs only at unphysiologically low temperatures.

*Membrane current analysis for ephaptically interacting fibres*

The mode of conduction in spinal root fibres can be determined by subtracting adjacent records of external longitudinal current to obtain membrane current and then by identifying sites of excitable membrane as sites at which inward membrane current is generated (Rasminsky & Sears, 1972; Bostock & Sears, 1978; Rasminsky *et al.* 1978). For fibres in which conduction is saltatory, sites of excitable membrane

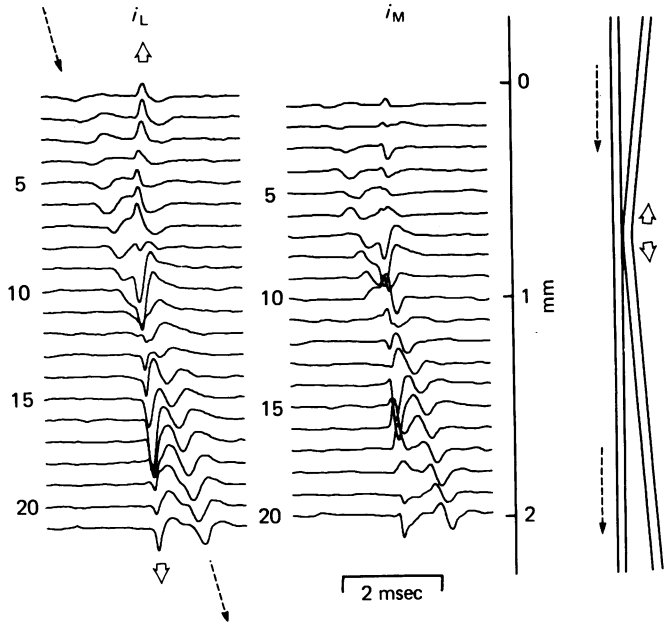


Fig. 4. Ephaptic transmission. External longitudinal current records ($i_L$) at intervals of 100 $\mu$m from proximal (above) to distal (below). The directions of propagation in the exciting and excited fibres are indicated by the dashed and open arrows respectively. Membrane current records ($i_M$) are obtained by differencing adjacent longitudinal current records. Inward membrane current is downwards. The diagram on the right indicates the site of ephaptic interaction as defined by the longitudinal current records.

are spatially separated (Huxley & Stämpfli, 1949; Rasminsky & Sears, 1972; Bostock & Sears, 1978; Rasminsky *et al.* 1978); for portions of fibres in which conduction is continuous such as some axons demyelinated with diphtheria toxin and bare axons in the spinal roots of dystrophic mice, sites of inward membrane current flow appear to be distributed along the axon with no intervening passive membrane (Bostock & Sears, 1976, 1978; Rasminsky *et al.* 1978).

Fig. 4. illustrates the interaction between another pair of fibres in a dystrophic mouse spinal root in which records of membrane current were obtained by subtraction of adjacent records of longitudinal current. As in Fig. 2 the site of the ephapse is identified from the longitudinal current records as the site at which the initial polarity of the external longitudinal current in the excited fibre reverses in direction. For the exciting fibre, the mode of conduction past the ephapse appears to be continuous; at each position along the root an initial phase of outward mem-

brane current is followed by a phase of inward membrane current which is displaced continuously in time at each successive recording site. For the excited fibre the mode of conduction is less immediately obvious. At the site of the ephapse a large inward membrane current is generated in the excited fibre (second downward deflexion in membrane current records 7 and 8 corresponding to the initial excitation of this fibre). On the proximal side of the ephapse no significant inward membrane current is generated by the excited fibre at sites 6 and 5 but the initial phase of outward


Fig. 5. Membrane currents at the ephapse for three pairs of interacting fibres. Inward membrane current downwards.

membrane current is followed by a relatively large inward membrane current at sites 4 and 3. This suggests that the mode of conduction in the proximal direction of transmission in the excited fiber is saltatory from the site of origin of the impulse at the ephapse across an internode between 200 and 300 $\mu$m in length.

Over the first few hundred $\mu$m distal to the ephapse before the impulse in the excited fibre has outdistanced the impulse in the exciting fibre, the membrane currents generated by the two fibres are superimposed (membrane current records 9–11), and it is not possible to attribute inward or outward membrane current definitively to one fibre or the other. There is clearly inward membrane current generated in the excited fibre in membrane current record 12 and there is further saltation to sites defined by recordings 14 and 15 and recordings 19 and 20.

In this example, it thus appears that cross talk occurs between an axon conducting continuously, presumably a bare axon, and an axon in which conduction is saltatory away from the site of interaction in at least one and possibly in both directions of transmission. For the other pairs of fibres conduction invariably appeared to be continuous in the exciting fibre but as in the example illustrated in Fig. 4 there was ambiguity in the interpretation of the mode of conduction in at least one direction in the excited fibre.

Transmission time across the ephapse could be estimated from the membrane current records as the latency between the peaks of inward membrane current in the two fibres at the site closest to the ephapse (e.g. membrane current record 7 in Fig. 4). Fig. 5 shows membrane current records straddling an ephapse for three other pairs of fibres. Four distinct phases of membrane current are apparent: (1) an initial upward deflexion corresponding to outward membrane current in the exciting fibre as this fibre begins to be depolarized by the advancing impulse, (2) an initial downward deflexion corresponding to inward membrane current generated in the exciting fibre as the impulse passes, (3) a sharper downward deflexion corresponding to inward membrane current generated in the excited fibre as it is ephaptically excited,

(4) a final inconstant upward deflexion corresponding to outward membrane current drawn from both fibres in the vicinity of the ephapse as these fibres now act as current sources for sinks at nearby sites of depolarization. In some cases, as for the third example in Fig. 5, the inward membrane current generated by the excited fibre overlaps the preceding peak of inward membrane current generated by the exciting

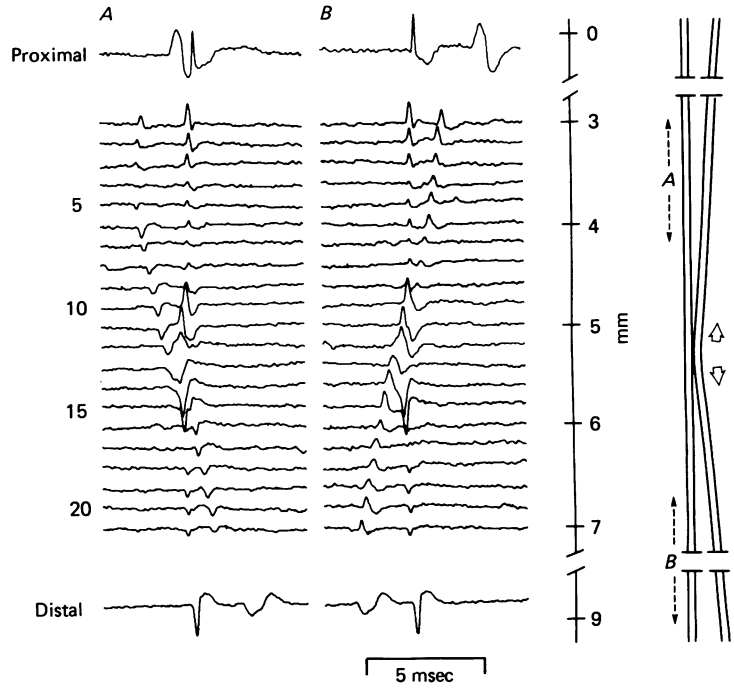


Fig. 6. Ephaptic transmission between two fibres for two directions of propagation in the exciting fibre. Simultaneous recordings were made from proximal and distal reference electrodes which remained fixed throughout and from mobile electrodes (records 1–21) which were moved in steps of 200 $\mu$m. The trigger spike for all records was the large amplitude downward spike at the distal electrodes. Single sweeps from the proximal and distal electrodes were monitored on a storage oscilloscope. Sequence *A* was generated for single sweeps in which the large amplitude downward spike at the distal electrodes was followed by a smaller amplitude downward spike. Sequence *B* was generated for single sweeps in which the large amplitude downward spike at the distal electrode was not followed by a smaller spike but the large amplitude upgoing spike at the proximal electrodes was followed by a smaller wider upgoing spike. The positions of the ephapse and sites of origin of the exciting impulses are indicated in the diagram on the right, dashed and open arrows indicating the directions of propagation in the exciting and excited fibres respectively. The proximal channel was recorded on a direct channel of the tape recorder. See text for further details.

fibre and the interval between peaks is indeterminate but less than or equal to the time between the inflexion on the superimposed inward membrane currents and the peak for the excited fibre. Transmission times across the ephapse estimated in this manner ranged from $< 100$ $\mu$sec to 240 $\mu$sec (Table 1).

*Direction of propagation in the exciting fibre*

The experiment illustrated in Fig. 6 demonstrates that ephaptic transmission is not necessarily contingent upon the direction of propagation of the impulse in the exciting fibre. In sequence *A* an impulse arises at recording site 4 and is propagated both towards the spinal cord (upgoing impulses with progressively greater latencies in records 3–1 and Proximal) and towards the periphery (downgoing impulses with

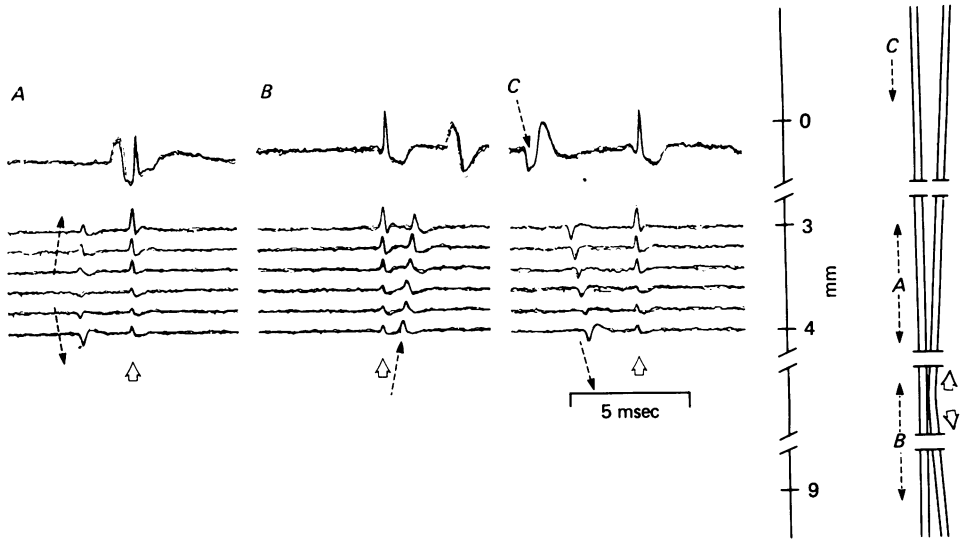


Fig. 7. Three sites of origin of impulses in the exciting fibre depicted in Fig. 6. Each sequence is three superimposed single sweeps from the proximal site and sites 1–6 as in Fig. 6. *A* and *B* correspond to Fig. 6*A* and *B*. In *C* the impulse in the exciting fibre originates proximal to the proximal recording site.

progressively greater latencies in records 5–21 and Distal). Between recording sites 12 and 13 an ephaptic interaction occurs and an impulse in a second fibre is propagated both towards the spinal cord (upgoing impulses with progressively greater latencies in records 12–1 and Proximal) and towards the periphery (downgoing impulses with progressively greater latencies in records 13–21 and Distal). This interaction is similar to that illustrated in Figs. 2 and 4 except that in this case the exciting impulse arises at a defined site in midroot. In sequence *B* an impulse arises between recording site 21 and the distal recording site and is propagated towards the spinal cord (upgoing impulse with progressively greater latencies in records 21–1 and Proximal) and towards the periphery (downgoing impulse at Distal). Between recording sites 12 and 13 an ephaptic interaction occurs, and as in sequence *A*, an impulse in the second fibre is propagated towards both the spinal cord and the periphery.

A number of criteria establish that sequences *A* and *B* do indeed represent interaction between the same pair of fibres. At each recording site there is a striking similarity in the shape and relative latency of the external longitudinal current

recorded from the excited fibre, whether the excitation resulted from a centrifugal impulse in the exciting fibre as in *A* or a centripetal impulse in the exciting fibre as in *B*. This comparison cannot be made record for record for the exciting fibre since the direction of propagation is different in comparable records except for records 1–3 and the records at the proximal and distal recording sites. The external longitudinal currents recorded from the exciting fibre do appear similar in sequences *A* and *B* at the proximal and distal recording sites. When the impulse in the exciting fibre is

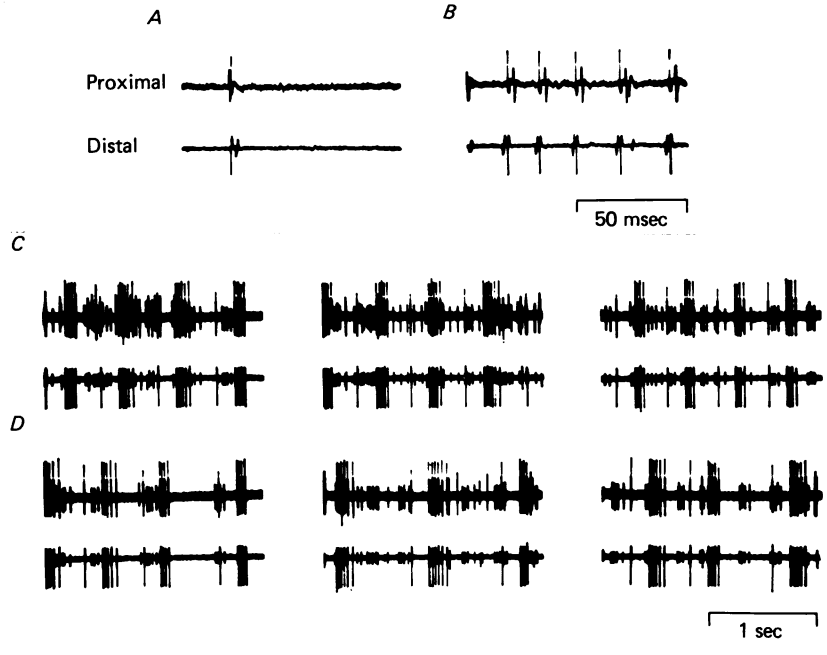


Fig. 8. Patterns of spontaneous activity in the pair of fibres illustrated in Fig. 6. Upper trace proximal reference electrodes, lower trace distal reference electrodes. *A*, exciting impulse originates at the proximal ectopic site as in Fig. 6*A*. This complex invariably appeared singly. *B*, part of a burst of complexes in which the exciting impulse originates at the distal ectopic site as in Fig. 6*B*. *C*, single complexes as in *A* are followed by bursts as in *B*. *D*, the burst originating at the distal ectopic site does not occur unless preceded by a single impulse from the proximal ectopic site. 50 msec time scale for *A* and *B*; 1 sec time scale for *C* and *D*.

propagated past site 4 as in sequence *B*, sites distal to 4 act as current sinks for external longitudinal currents flowing outside the portion of the fibre proximal to site 4. This is not the case when the exciting impulse originates near site 4 as in sequence *A*; thus the external longitudinal currents near the site of origin of this impulse (i.e. at sites 1–3) are somewhat smaller in amplitude for sequence *A* than for sequence *B*.

Very infrequently, a third site of origin of the impulse in the exciting fibre was observed proximal to the proximal recording site (Fig. 7). This could have represented either occasional spontaneous firing of the cell body of this fibre or spontaneous ectopic activity arising at a site near or within the spinal cord.

*Stability of ephaptic interaction*

Ephapses are able to transmit bursts of impulses (Fig. 8*B–D*). For the pair of fibres illustrated the instantaneous frequency at the beginning of the burst was > 70 Hz and every impulse in the exciting fibre was followed by an impulse in the excited fibre. Ephaptic pairs for which the bursting frequency of the exciting fibre was greater than this were not observed; the maximum possible frequency of

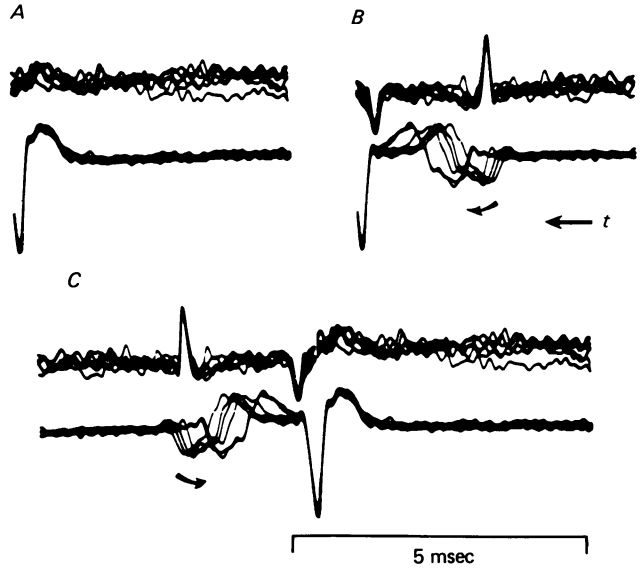


Fig. 9. Traces showing erratic propagation away from the site of origin of the exciting impulse and secure transmission across the ephapse. Upper trace from recording site 21 and lower trace from distal reference electrodes as in Fig. 6*B*. The exciting impulse arises between the two recording sites; the site of ephaptic interaction is proximal to both recording sites. Each panel represents an entire burst of 6 impulses. *A*, events following the trigger spike; *B*, events preceding the trigger spike recorded by reversed playback of the tape recorder; *C*, *A* and *B* superimposed. The curved arrow beneath the lower trace in *B* and *C* indicates the order of successive single sweeps. Note the constant latency between the impulses in the exciting (upward deflexion) and excited (downward deflexion) impulses on the upper trace indicating constant latency across the ephapse.

transmission across an ephapse is not known. For this pair of fibres the latency across the ephapse remained strikingly constant from the beginning to the end of each burst (Fig. 9*C*, upper trace). The present experiments were biased towards selection of relatively tightly coupled pairs of fibres; stable latency across the ephapse is not an invariant property of ephaptically interacting fibres (see Fig. 10 in Rasminsky, 1978*a*).

*Further observations on ectopic excitation*

*Propagation of impulses away from the site of ectopic excitation.* There was usually a fixed temporal relationship between the times of arrival of successive impulses at sites respectively proximal and distal to the site of ectopic impulse generation (see Fig. 4*B–F* in Rasminsky, 1978*a*) implying that successive ectopically generated

impulses were usually propagated uniformly in both directions. This was not invariably the case. Fig. 9 illustrates simultaneous recordings made just proximal and distal to an ectopic generator of bursts of impulses. During the burst (which has an initial instantaneous frequency of > 70 Hz) successive impulses are propagated towards the distal recording site with a progressively greater delay with respect to

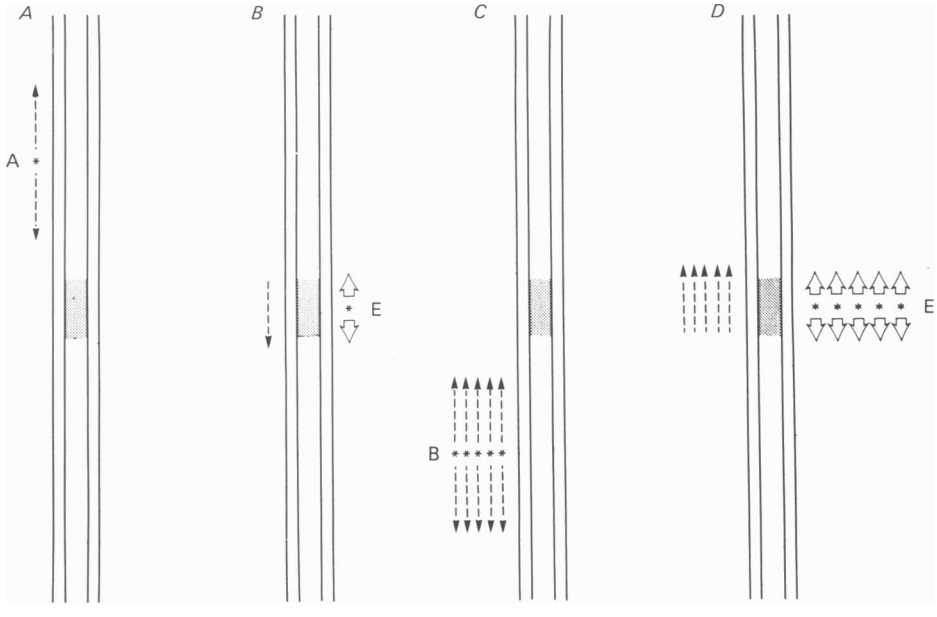


Fig. 10. Diagrammatic reconstruction of events illustrated in Fig. 8. *A*, an impulse originates ectopically at site A in the first fibre and propagates in both directions. *B*, this impulse ephaptically excites the second fibre at E giving rise to a second impulse propagating in both directions. *C*, 150 msec later a burst of impulses arises at site B in the first fibre. *D*, these impulses ephaptically excite the second fibre at E.

propagation in the proximal direction. This could reflect either a progressive delay in initiation of propagation in the distal direction or a progressive slowing of conduction in the distal direction during repetitive transmission as occurs in demyelinated nerve fibres (Rasminsky & Sears, 1972). An alternative explanation is that the progressive delay in the distal direction could reflect a progressive change in the site of origin of the impulse; this explanation seems unlikely since for Fig. 9 it would imply six separate sites of ectopic impulse initiation within a distance of less than 2 mm.

Examination of single sweeps from other pairs of fibres showed that there was sometimes a similar but much less striking lack of uniformity in the propagation of impulses away from the ephapse in the two directions in the ephaptically excited fibres.

*Interaction between sites of ectopic excitation*

Fig. 8 illustrates the interaction between two sites of ectopic generation of impulses in a single nerve fibre which ephaptically excited a second nerve fibre. The proximal ectopic site on the exciting fibre generated single impulses (Fig. 8*A*) and the distal ectopic site on the excited fibre generated bursts of four to seven impulses at an

initial instantaneous frequency of > 70 Hz (Fig. 8*B*). All impulses generated at either ectopic site in the exciting fibre caused ephaptic excitation of the excited fibre. Single impulses were generated at the proximal ectopic site of the exciting fibre at a frequency of about 2 Hz and were followed 150 msec later by a burst of impulses from the distal ectopic site in the excited fibre (Fig. 8*C*). A delay in generation of the single impulse at the proximal ectopic generator was followed by an appropriate delay in firing of the burst from the distal ectopic generator (Fig. 8*D*). This sequence of events is diagramatically illustrated in Fig. 10. Although the interval between single impulses arising at the proximal site and the burst of impulses arising at the distal site was about 150 msec, the conduction time between the proximal and distal sites was less than 5 msec (Fig. 6*A*). The effect of this impulse thus persisted for an impressive period after it had traversed the distal ectopic generator.

## DISCUSSION

In the present experiments nerve impulses in ephaptically interacting pairs of fibres in dystrophic mouse spinal roots were recorded in the immediate vicinity of the ephapse. It thus now becomes possible to draw some inferences concerning the nature of the interacting fibres in these roots.

The conduction velocities of the exciting fibres were less than 1 m/sec and the membrane current records indicated that conduction was continuous in these fibres. For a number of reasons the membrane current records were less satisfactory as an indication of the mode of conduction in the ephaptically excited fibres:

(1) In the area of interest in the immediate vicinity of the ephapse, the membrane current records from the two fibres were of necessity superimposed making it impossible to distinguish between inward current generated by one or the other fibre.

(2) Because spontaneous activity in ephaptically interacting pairs of fibres generally did not persist for long periods of time, the practice in almost all experiments was to accumulate fewer repetitions of the external longitudinal current records at each site than would have been desirable for optimal elimination of noise by averaging.

(3) The interval between the trigger spike in the ephaptically excited fibre and the impulse propagated in the opposite direction in the same fibre sometimes was subject to a jitter of as much as 60 $\mu$sec. This jitter would have had little effect on the averaged latency of the peak of the external longitudinal current but would have caused significant distortion in the shape of the averaged external longitudinal currents and the derived membrane currents.

(4) Although teased fibre studies of dystrophic mouse spinal roots have not been performed, there is morphological evidence that short internodes are common in these roots and physiological evidence that internodes of myelinated fibres in these roots are only exceptionally longer than 500 $\mu$m (Rasminsky *et al.* 1978). With an electrode separation of 150 $\mu$m for the mobile electrodes and excursions of 100 $\mu$m between successive recording sites, the spatial resolution of sites of excitable membrane was at best of the order of 250 $\mu$m, meaning that with the present technique it was impossible to make a distinction between continuous conduction and saltatory conduction across internodes of lengths of 250 $\mu$m or less.

It is thus necessary to invoke conduction velocity as a secondary criterion of the mode of conduction in the excited fibres. The maximum conduction velocity previously found for continuously conducting large diameter bare axons in dystrophic mouse roots was 1·9 m/sec at 26 °C (Rasminsky *et al.* 1978). This is comparable to the maximum velocity of 2·3 m/sec at 37 °C for conduction in bare demyelinated axons (Bostock & Sears, 1978). No definitive upper limit can be given for the anticipated conduction velocity in large diameter bare axons. The conduction velocities in the excited fibres in the immediate vicinity of the ephapse were 2–4 m/sec in two of the five pairs studied; these velocities, although not excessively slow for saltatory conduction (Rasminsky & Sears, 1972) may be within the range which is also consistent with continuous conduction and thus no firm statement can be made about the nature of the excited fibres in these cases. However, the observed velocities of 4–10 m/sec at 26–28 °C in the excited fibres in the immediate vicinity of the ephapse in the other three pairs are almost surely too high to be consistent with continuous conduction in a bare axon and suggest that for these pairs at least, ephaptic transmission was from bare axon to myelinated axon.

Since shortly after the development of the local circuit theory of propagation of the nerve impulse (Hodgkin, 1937*a*, *b*), it has been recognized that currents flowing external to active fibres can influence the excitability of neighbouring fibres (Katz & Schmitt, 1940; Arvanitaki, 1940, 1942). The current flowing external to an active fibre can flow either through extracellular space or through adjacent fibres; any increase in the resistance of extracellular space will divert current into adjacent fibres and will thus enhance the influence of an active fibre on the excitability of its neighbours (Katz & Schmitt, 1940; Arvanitaki, 1940, 1942; Ramón & Moore, 1978). In dystrophic mouse spinal roots, not only is there much less extracellular space between axons than in normal roots (Bradley & Jenkison, 1973; Bray & Aguayo, 1975; Stirling, 1975), but also there are fewer Schwann and other non-neuronal cells to act as alternative extra-axonal current pathways (Bradley & Jenkison, 1973; Bray, Perkins, Peterson & Aguayo, 1977*b*). Rosenbluth (1978) has observed septate junctions between the outermost myelin lamellae of internodes of myelinated fibres and adjacent bare axons in the spinal roots of dystrophic mice; these junctions are identical to those normally found tightly apposing Schwann cell membrane to the underlying axon at the paranodal region. Such junctions between the myelin of one fibre and an adjacent bare axon might cause increased resistance to external longitudinal current flow in the extracellular space between the two fibres and thus further enhance current flow from one fibre to the other.

Using analytical techniques, Clark & Plonsey (1970, 1971) have identified a number of factors influencing interaction between active and inactive fibres in a nerve trunk. Displacement of membrane potential of the inactive fibre in response to an action potential in the active fibre was increased by increasing the resistance of the interstitial medium, by increasing the radius and by decreasing the membrane capacitance of the inactive fibre. The distance between fibres and the membrane resistance of the inactive fibre were less critical variables. Although Clark & Plonsey's (1970, 1971) analysis dealt specifically with unmyelinated fibres, it is intuitively clear that myelinated fibres, because of their relatively low membrane capacitance, should be more susceptible to cross excitation than unmyelinated fibres; this expectation is

borne out by the experimental finding that in all cases studied, ephaptic transmission proceeded from a slowly conducting to a more rapidly conducting fibre.

An impulse arises as a result of cross-talk in the inactive fibre at a site where the stimulus of sufficient outward membrane current causes the axon membrane potential to fall to threshold for a propagated action potential. The likely paths of current flow between fibres can be visualized by considering the membrane currents generated by the advancing impulse in the active or exciting fibre. In a dystrophic mouse bare axon conducting continuously at slightly less than 1 m/sec at about 28 °C, the distance between the advancing wave of outward membrane current and the subsequent wave of inward membrane current is about 0·2 mm (Fig. 4, and see Fig. 6 in Rasminsky *et al.* 1978). Part of this current flows through the membranes of adjacent fibres as inward current preceding outward current. In a fibre adjacent to such a continuously conducting bare axon, the site of maximum passive inward current flow will thus be about 0·2 mm ahead of the site of maximal outward current flow and a current loop is completed between the two sites. The ideal situation for cross excitation between such a bare axon and an adjacent axon may be one in which membrane current flow is restricted in the intervening 0·2 mm, i.e. by an interposed myelin sheath. Such a short internode adjacent to the site of ephaptic interaction would not be identified by the physiological techniques used in the present experiments but the postulated existence of such a short internode is consistent with the experimental observations.

Although secure transmission was a feature of the ephapses which could be completely characterized, such secure transmission is not an invariant feature of ephapses in dystrophic mouse spinal roots (Rasminsky, 1978*a*) and may reflect the exceptional situation in which the geometry of the excited fibre is optimally matched to the conduction velocity of the impulse in the exciting fibre.

Ephaptic transmission in the reverse direction was not demonstrated in any of the pairs of fibres studied, although this was specifically searched for by examination of multiple single sweeps triggered by impulses in the fibre that was ordinarily the excited one of the ephaptically interacting pair. The apparent unidirectional nature of the ephapses in dystrophic mouse roots may be a feature of their inferred anatomic asymmetry. In contrast, ephaptic transmission in experimental neuromas is bidirectional (Seltzer & Devor, 1979). The ephaptically communicating fibres within neuromas are presumably regenerating sprouts, both fibres within an ephaptic pair likely having similar morphological and physiological properties.

Electrical communication between adjacent excitable cells is usually not mediated by extracellular currents as discussed above but rather via the anatomically definable intercellular low resistance pathways provided by gap junctions (Bennett, 1966). Such junctions have not been observed between adjacent dystrophic mouse axons. We have reported preliminary observations of small axonal protrusions which occasionally form interaxonal channels between bare axons in dystrophic mouse spinal roots fixed in glutaraldehyde (Bray, Cullen, Aguayo & Rasminsky, 1977*a*). Hasty & Hay (1978) have shown that the appearance of such plasmolemmal protrusions in corneal fibroblasts is dependent upon the fixation techniques used; in subsequent studies of unfixed dystrophic roots we have been unable to demonstrate axonal protrusions or interaxonal channels between bare axons.

The potassium concentration in a confined extracellular space is affected by impulse activity in nerve fibres (Frankenhaueser & Hodgkin, 1956; Kuffler & Nichols, 1966). In dystrophic mouse spinal roots bare axons are closely apposed and the possibility must be considered that interaxonal communication is mediated by changes in extracellular potassium. If depolarization were due to extracellular potassium accumulation, the effect of repeated impulses would be cumulative, and ephaptic transmission should occur at an initially progressively shorter latency for at least the first few of a train of impulses. Such a progressive shortening of latency was in fact never observed within bursts nor was ephaptic transmission ever observed to depend upon a preceding series of impulses in the exciting fibre. It thus appears unlikely that ephaptic transmission, at least for securely communicating pairs of fibres, is mediated by extracellular potassium.

The present experiments show that bursts of ectopic impulse generation in dystrophic spinal root fibres can be provoked by a preceding impulse in the same fibre propagated past the site of ectopic generation. The possibility of such auto-excitation in dystrophic mouse spinal root fibres was previously suggested by Huizar *et al.* (1975) who found that following intracellular stimulation of a dystrophic mouse lumbar ventral horn cell, a short burst of four to eight antidromically conducted impulses frequently invaded the cell some tens of msec later. Huizar *et al.* (1975) also noted that a single peripheral stimulus was frequently followed by retrograde invasion of an anterior horn cell by a burst of impulses. The present experiments do not shed any light on the mechanism of autoexcitation but do indicate that the effect of an impulse propagated past a hyperexcitable focus can be very long lasting, persisting for more than 100 msec after the initial impulse.

The sequence of events illustrated in Fig. 8 and 10 demonstrates the ability of dystrophic mouse spinal root to act as a pathological amplifier of nerve impulse activity, a single impulse in one fibre eventuating in an order of magnitude increase in the total number of impulses transmitted centrally or peripherally in only two fibres. It is possible that mechanisms similar to that illustrated may be of importance in peripheral and central sensory syndromes associated with demyelination such as trigeminal neuralgia in which minimal peripheral stimuli can give rise to a central perception of persistent and intense pain.

The continuous muscle activity in the hind limbs of dystrophic mice which is abolished by curare (Eberstein, Goodgold & Pechter, 1975) is presumably at least in part due to ectopic excitation of ventral root fibres (Rasminsky, 1978*a*). Ectopic excitation, ephaptic transmission and autoexcitation of pathological nerve fibres may also have a role in clinical motor phenomena such as myokymia which is seen in association with demyelination in both the central and peripheral nervous system (Andermann, Cosgrove, Lloyd-Smith, Gloor & McNaughton, 1961; Wallis, Van Poznack & Plum, 1970; Welch, Appenzeller & Bicknell 1972; Wasserstrom & Starr, 1977) and with peripheral nerve injury (Medina, Chokroverty & Reyes, 1976).

Supported by the Medical Research Council of Canada.

REFERENCES

ANDERMANN, F., COSGROVE, J. B., LLOYD-SMITH, D. L., GLOOR, P. & MCNAUGHTON, F. L. (1961). Facial myokymia in multiple sclerosis. *Brain* **83**, 31–44.

ARVANITAKI, A. (1940). Réactions déclenchées sur un axone au repos par l'activité d'un autre axone au niveau d'une zone de contact: conditions de la transmission de l'excitation. *C. r. Séanc. Soc. Biol.* **133**, 39–44.

ARVANITAKI, A. (1942). Effects evoked in an axon by the activity of a contiguous one. *J. Neurophysiol.* **5**, 89–108.

BENNETT, M. V. L. (1966). Physiology of electrotonic junctions. *Ann. N.Y. Acad. Sci.* **137**, 509–539.

BOSTOCK, H. & SEARS, T. A. (1976). Continuous conduction in demyelinated mammalian nerve fibers. *Nature, Lond.* **263**, 786–787.

BOSTOCK, H. & SEARS, T. A. (1978). The internodal axon membrane: electrical excitability and continuous conduction in segmental demyelination. *J. Physiol.* **280**, 273–301.

BRADLEY, W. G. & JENKISON, M. (1973). Abnormalities of peripheral nerve in murine muscular dystrophy. *J. neurol. Sci.* **18**, 227–247.

BRAY, G. M. & AGUAYO, A. J. (1975). Quantitative ultrastructural studies of the axon-Schwann cell abnormality in spinal nerve roots from dystrophic mice. *J. Neuropathol. exp. Neurol.* **34**, 517–530.

BRAY, G. M., CULLEN, M. J., AGUAYO, A. J. & RASMINSKY, M. (1977*a*). Axolemmal abnormalities in spinal roots of dystrophic mice. *Neurology, Minneap.* **27**, 362.

BRAY, G. M., PERKINS, C. S., PETERSON, A. C. & AGUAYO, A. J. (1977*b*). Schwann cell multiplication deficit in nerve roots of newborn dystrophic mice – A radioautographic and ultrastructural study. *J. neurol. Sci.* **32**, 203–212.

CLARK, J. W. & PLONSEY, R. (1970). A mathematical study of nerve fiber interaction. *Biophys. J.* **10**, 937–957.

CLARK, J. W. & PLONSEY, R. (1971). Fiber interaction in a nerve trunk. *Biophys. J.* **11**, 281–294.

EBERSTEIN, A., GOODGOLD, J. & PECHTER, B. R. (1975). Effect of curare on EMG and contractile responses in the myotonic mouse. *Expl Neurol.* **49**, 612–616.

FRANKENHAEUSER, B. & HUXLEY, A. L. (1956). The after-effects of impulses in the giant nerve fibres of Loligo. *J. Physiol.* **131**, 341–376.

GRANIT, R., LEKSELL, L. & SKOGLUND, C. R. (1944). Fibre interaction in injured or compressed regions of nerve. *Brain* **67**, 125–140.

GRANIT, R. & SKOGLUND, C. R. (1945). Facilitation, inhibition and depression at the 'artificial synapse' formed by the cut end of a mammalian nerve. *J. Physiol.* **103**, 434–448.

HASTY, D. L. & HAY, E. D. (1978). Freeze-fracture studies of the developing cell surface. II. Particle-free membrane blisters on glutaraldehyde-fixed corneal fibroblasts are artefacts. *J. cell Biol.* **78**, 756–768.

HODGKIN, A. L. (1937*a*). Evidence for electrical transmission in nerve. I. *J. Physiol.* **90**, 183–210.

HODGKIN, A. L. (1937*b*). Evidence for electrical transmission in nerve. II. *J. Physiol.* **90**, 211–232.

HUIZAR, P., KUNO, M. & MIYATA, Y. (1975). Electrophysiological properties of spinal motoneurones of normal and dystrophic mice. *J. Physiol.* **248**, 231–246.

HUXLEY, A. F. & STÄMPFLI, R. (1949). Evidence for saltatory conduction in peripheral myelinated nerve fibres. *J. Physiol.* **108**, 315–339.

KATZ, B. & SCHMITT, O. H. (1940). Electric interaction between two adjacent nerve fibres. *J. Physiol.* **97**, 471–488.

KUFFLER, S. W. & NICHOLLS, J. G. (1966). The physiology of neuroglial cells. *Ergebn. Physiol.* **57**, 1–90.

MEDINA, J. L., CHOKROVERTY, S. & REYES, M. (1976). Localized myokymia caused by peripheral nerve injury. *Archs. Neurol., Chicago* **33**, 587–588.

RAMÓN, F. & MOORE, J. W. (1978). Ephaptic transmission in squid giant axons. *Am. J. Physiol.* **234**, C162–C169.

RASMINSKY, M. (1978*a*). Ectopic generation of impulses and cross-talk in spinal nerve roots of 'dystrophic' mice. *Ann. Neurol.* **3**, 351–357.

RASMINSKY, M. (1978*b*). Cross-talk between bare and myelinated axons in spinal roots of dystrophic mice. *Neurosci. Abstr.* **4**, 592.

RASMINSKY, M. (1979). Dystrophic mouse spinal root acts as a pathological amplifier of nerve impulse activity. *Neurology, Minneap.* **29**, 588.

RASMINSKY, M., KEARNEY, R. E., AGUAYO, A. J. & BRAY, G. M. (1978). Conduction of nervous impulses in spinal roots and peripheral nerves of dystrophic mice. *Brain Res.* **143**, 71–85.

RASMINSKY, M. & SEARS, T. A. (1972). Internodal conduction in undissected demyelinated nerve fibres. *J. Physiol.* **227**, 323–350.

ROSENBLUTH, J. (1978). Septate junctions between Schwann cells and amyelinated axons in dystrophic mouse nerves. *J. cell Biol.* **79**, 101a.

SELTZER, Z. & DEVOR, M. (1979). Ephaptic transmission in chronically damaged peripheral nerves. *Neurology, Minneap.* **29**, 1061–1064.

STIRLING, C. A. (1975). Abnormalities in Schwann cell sheaths in spinal nerve roots of dystrophic mice. *J. Anat.* **119**, 169–180.

WALLIS, W. E., VAN POZNAK, A. & PLUM, F. (1970). Generalized muscular stiffness, fasciculations and myokymia of peripheral nerve origin. *Archs. Neurol., Chicago* **22**, 430–439.

WASSERSTROM, W. R. & STARR, A. (1977). Facial myokymia in the Guillain–Barré syndrome. *Archs Neurol., Chicago* **34**, 576–577.

WELCH, L. K., APPENZELLER, O. & BICKNELL, J. M. (1972). Peripheral neuropathy with myokymia, sustained muscular contraction and continuous motor unit activity. *Neurology Minneap.* **22**, 161–169.