

## Can Neuralgias Arise from Minor Demyelination? Spontaneous Firing, Mechanosensitivity, and Afterdischarge from Conducting Axons

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Mammalian peripheral axons respond to local disruption of their myelin sheath with membrane changes which support continuous conduction of the impulse through the affected region. We report here that sites of demyelination may become foci of spontaneous impulse initiation. Such sites may also generate ectopic discharges upon slow mechanical distortion. Finally, conduction of an impulse train through a demyelinated region may set off an ectopic afterdischarge that may last many seconds. Rhythmic ectopic firing in dysmyelinated but conducting axons is very similar to that observed in regenerating axons and nerve-end neuromas. Although the latter have long been recognized as sources of pathophysiologic sensations, this is the first indication that neuralgias could arise following minor demyelination in peripheral nerves without substantial conduction deficits.

### INTRODUCTION

Except for the normal site of impulse initiation near the beginning of the axon, most regions of nerve are notable for their relative inability to initiate impulses when slowly depolarized (8, 13). The conducting region

Abbreviation: LPC—lysophosphatidyl choline.

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of an axon needs to be depolarized rapidly in order to ectopically initiate an impulse. The sources of depolarization along the length of a nerve (e.g., mechanical stretch and extracellular potassium accumulations) are usually too slow to overcome the normal membrane accommodation.

Important exceptions to this generalization have been noted (3); for example, the dorsal root ganglion region will respond to slow compression with rhythmic firing which lasts many minutes (8). Nerve-end neuromas, and the leading edge of regenerating nerves, discharge spontaneously and are mechanosensitive (4, 5). We previously observed that short demyelinated regions associated with the granuloma near implanted sutures are mechanosensitive and that they may reexcite the nearby axon to send an extra impulse backward (7, 8). Rasminsky (10) observed ectopic spiking in the ventral roots of dystrophic mice with disrupted myelination. Smith and McDonald (11) recently showed that demyelinating lesions of the dorsal columns, produced by lysophosphatidyl choline (LPC, a strong detergent) injections, exhibit mechanosensitivity as well as spontaneous firing.

Here we show that LPC lesions of rat saphenous nerve exhibit mechanosensitivity, afterdischarge following priming volleys, and a characteristic pattern of spontaneous firing (which we have called "interrupted auto-rhythmicity") heretofore associated with regenerating axons.

#### METHODS

Adult white rats (Wistar males, 200 to 400 g) were initially operated under ether or pentobarbital anesthesia. A small quantity of 10 mg/ml LPC solution was ejected from the broken tip of a glass micropipet (6) placed within the perineural sheath of the saphenous nerve under visual guidance. The midhigh injection site was marked with carbon particles in adjacent tissue.

Subsequent acute recording experiments were carried out under similar anesthesia 7 to 11 days later, when there was substantial local disruption of myelin. The femoral nerve was exposed, covered with warm mineral oil, served centrally near the groin and placed on bipolar nerve hook electrodes. Muscle twitches observed upon stimulation were eliminated by severing proximal branches of the nerve, sparing only the saphenous branch, a pure sensory nerve in rats. Another set of stimulating electrodes was placed across the nerve just distal to the lesion. To avoid dissecting the nerve near the lesion, we cut fine slits in the fascia and epineurium through which two fine wires could be placed and held in position by spring tension.

Desheathed fascicles of the nerve were stripped of all connective tissue for 1 to 2 mm and fine filaments splayed out on a hard black platform

illuminated by a fiber optic light source. Specially sharpened No. 5 forceps were used to separate fine filaments from the dissected fascicle. A length of fine filament was draped over a Ag wire electrode in the oil bath. Increasing stimulation strengths centrally would provide an orderly recruitment of all-or-nothing spike waveforms. Filaments were selected that contained, at most, only a few responding axons. The waveshape differences between individual responding axons could be exaggerated by bandpass filtering. A window height discriminator was used to select a single spike waveform of interest, and these were monitored for contamination with the use of a delay line display on a discriminator-triggered oscilloscope.

Recordings were made from fine saphaneous nerve filaments dissected several centimeters distal to the demyelinating LPC lesion. The axons were studied after severing the nerve from the periphery and the femoral nerve; thus the axons were functionally isolated from both central and peripheral connections, eliminating such possibilities as dorsal root reflexes and peripheral reexcitation. Spontaneously discharging units could usually be associated with one of the stimulation-evoked units in the filament. Conduction times were determined from stimulation electrodes proximal and distal to the site of LPC injection.

## RESULTS

In pilot studies, recording central to the lesion sites, we encountered ectopically firing axons which did not conduct through the lesion; we could not determine whether they were nonetheless in anatomic continuity, or perhaps were the central stumps of inadvertently truncated axons analogous to those in neuromas (5). In the present studies, we avoided this uncertainty by recording distal to the lesion. Any axon responding to the proximal stimulating electrode must be conducting through the zone of demyelination.

Most through-conducting axons in our experiments were without ectopic firing; indeed, routine histology suggested that many fibers in the nerve were not affected by the LPC. However, we saw a number of axons with through-conduction which responded to mechanical probing of the lesion site with a train of a few spikes, often with the discharge outlasting the probing, just as in the earlier granuloma-associated demyelination study (8). Of particular interest were the units seen in three rats which exhibited spontaneous firing and prolonged afterdischarge.

The data in Figs. 1-4 are from an axon which fired spontaneously with 28-ms interspike intervals. Stepwise compression at a small zone in the region of the LPC injection, using a manipulator-held probe, caused this

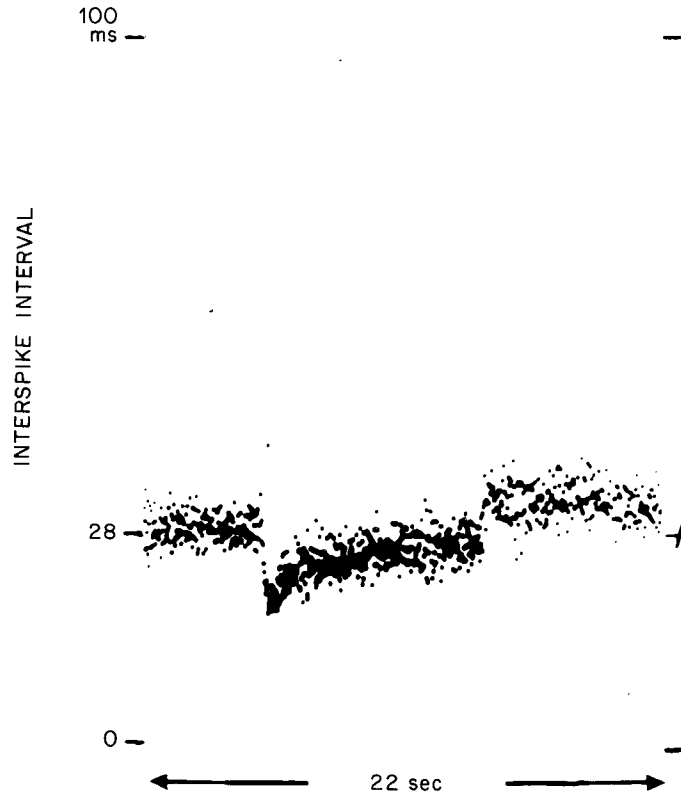


FIG. 1. Spontaneous rhythmic firing from a large axon (24 m/s conduction speed including delay at demyelinated site). The 28-ms interspike intervals were perturbed by stepwise compression of the lesion site using a manipulator-held probe, demonstrating that the LPC injection site was the ectopic pacemaker. Note the longer-than-normal intervals following release.

rhythmic firing to accelerate (Fig. 1). Longer-than-normal interspike intervals were seen for some seconds after withdrawal of the probe. Because rhythmicity, unlike more irregular discharges, implies a single site of impulse initiation or reset, the graded rhythmic response to compression serves to localize the spontaneous pacemaker at the lesion site. Axons which responded to mechanical stimulation did so only near the site of the LPC lesion.

An unusual feature of the spontaneous firing seen in Fig. 1 was the sudden pause, followed by the resumption of the discharge at virtually the same characteristic interspike interval (Fig. 2). The duration of these

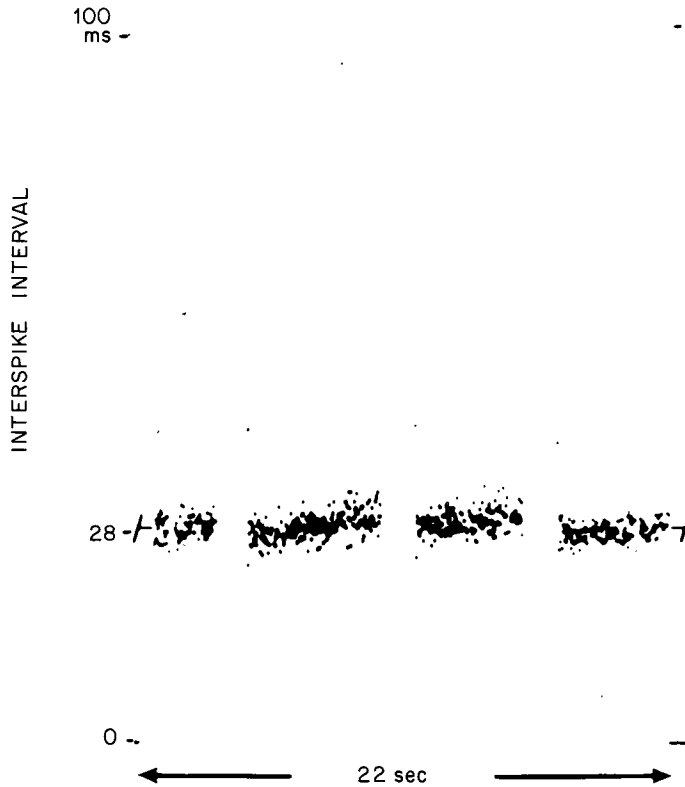


FIG. 2. Some time later, the spontaneous firing seen in Fig. 1 became intermittent, with pauses of a few seconds and then the resumption of the firing at nearly the characteristic 28-ms interval; note, however a slight drift from shorter to longer interspike intervals during each on period.

pauses tended to change with time. Similar peculiarities were seen in spontaneous rhythmic discharges of axons ending in a neuroma (5).

Although the spontaneous interspike intervals could be shortened and lengthened away from the favored 28 ms by mechanical prodding, an even more dramatic perturbation was produced by stimulating the nerve central to the lesion (Fig. 3). In the immediate aftermath of the conducted volley, the axons either became silent (in units not illustrated) or there developed an alternation of short and long interspike intervals (Fig. 3). In a manner reminiscent of successive approximation fitting, the short and long interspike intervals converged on a common value which then gradually drifted to shorter values until the characteristic 28-ms interval was reattained. In

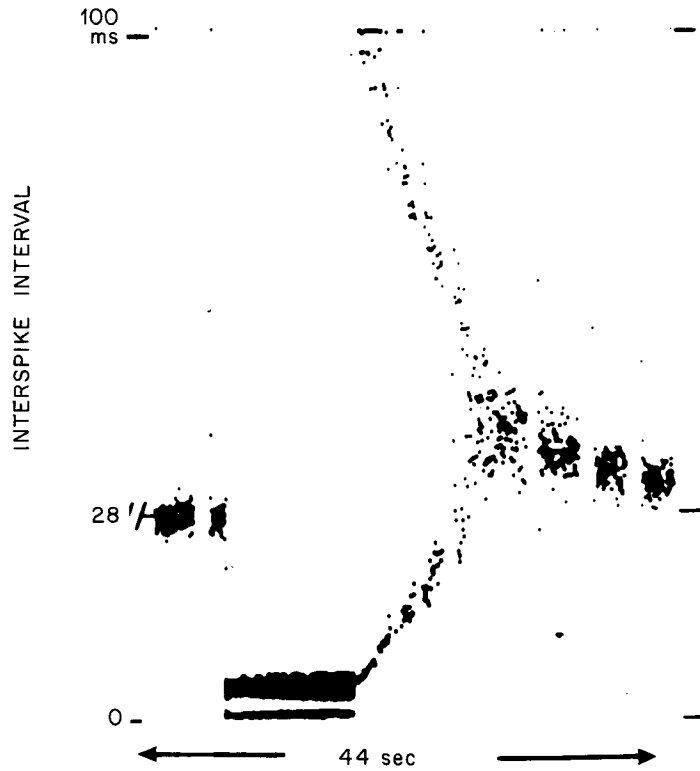


FIG. 3. Stimulating proximal to the lesion at 400 Hz propagated spikes through the lesion. At the end of the 10-s stimulus train, an afterdischarge was seen where short and long interspike intervals alternated, the mean interval slowly drifting to shorter values until the characteristic prepriming 28-ms interval was reattained (see Fig. 4). Note the pauses in the afterdischarge. Figures 1-3 are the same unit, in chronologic sequence.

units that became silent in the aftermath of the volley, the discharge resumed at intervals longer than the original and then gradually drifted back to the characteristic value. The pauses in discharge continued to be seen during both the short-long alternating phase (if present) and the later drifting phase (see Fig. 3).

### DISCUSSION

Silent pauses in an otherwise rhythmic spontaneous discharge is a feature described by Devor and Bernstein (5) in axons terminating in an end-bulb neuroma of rat sciatic nerve. DeSantis and Duckworth (4) observed a

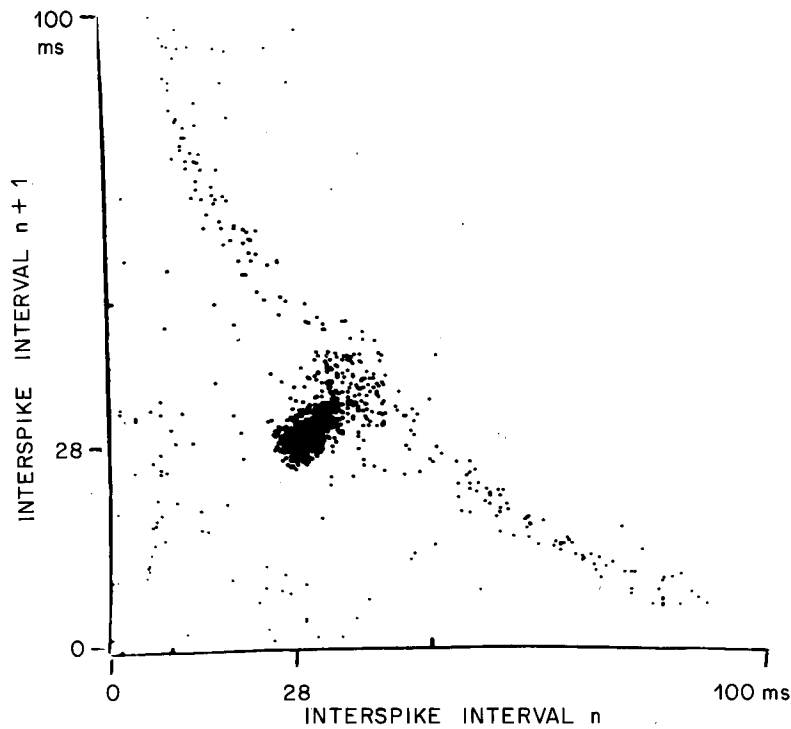


FIG. 4. Scatter plot of successive interspike intervals from the afterdischarge data of Fig. 3, immediately after the priming train. Points initially plotted at the tips of the (long, short) and (short, long) wings. They then moved centrally to the diagonal and then down the diagonal in the late phase of drifting back to the 28-ms characteristic interspike interval. The short-long alternation was also seen at the beginning of spontaneous on periods (not shown): in the spontaneous firing shown in Fig. 2, there was a shorter-than-characteristic interval after each pause, then a longer one, rapidly converging upon 28 ms.

similar discharge pattern in regenerating cat lateral gastrocnemius nerve (they note, however, that some of their spontaneous discharges seem to have originated from the dorsal root ganglion). The bursting discharges seen by Smith and McDonald (11) in cat dorsal column demyelination, and those seen by Rasminsky (10) in dystrophic mice ventral roots seem similar, though they may not share the characteristic fixed interspike intervals.

One possible explanation for the pauses in the otherwise rhythmic discharge, which we can eliminate with our data, is that the unit's firing rate could be straddling the minimum rate at which the axon will support rhythmic firing (2), i.e., that the characteristic 28 ms is the longest possible

rhythmic interspike interval. This threshold-straddling possibility, which is often responsible for on-and-off rhythmic firing in spinal motoneurons, is eliminated by the observation of longer than-characteristic interspike intervals that occurred in the aftermath of the mechanical prodding and conduction of volleys (Figs. 1, 3). The fact that our axons conducted through the lesion, and were able to follow stimulation rates of as much as 400 Hz (more than half normal), suggests that intermittent conduction failure due to fatigue at 36 Hz (1/28 ms) is also not a viable explanation of the on-off pattern.

The membrane changes that support ectopic rhythmic firing in the chronically injured axon, including this unusual spontaneous firing pattern ("interrupted autorhythmicity") common to dysmyelinated and regenerating axons, remains to be described. It seems clear, however, that ectopic repetitive firing could account for many neuralgias and dysesthesias where the nerve appears to be in continuity with the periphery. Indeed, segmental demyelination is a common feature of many peripheral nerve disorders (9, 12) in which clinical symptoms suggest ectopic firing.

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