
Silicone rubber cuff and patch electrodes with multiple contacts were implanted along the sciatic-tibial-plantar nerves in cat for repeated studies of conduction properties of normal peripheral nerve over periods of time. The contacts were used in various combinations for precise localization of changes in conduction velocities and excitability along the extent of normal nerves. In this paper the particular characteristics and limitations associated with cuff-electrode recordings of neural activity are discussed. The nerve action potential was recorded using a tripolar configuration with a central lead flanked by two shunted leads at symmetrical distances. This configuration records the spatial derivative of the action potential and rejects potentials from sources outside the cuff. The voltage changes are restricted by the silicone cuff, and the dynamic range is therefore very high, allowing detection from single myelinated fibers to whole nerve responses. The electrodes are well suited for following the development of regeneration and degeneration following experimental lesions.

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CONDUCTION STUDIES IN PERIPHERAL CAT NERVE USING IMPLANTED ELECTRODES: I. METHODS AND FINDINGS IN CONTROLS

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Determination of physiological changes in peripheral nerve provides a valuable tool to evaluate deterioration or recovery of function following experimental lesions in animal models. Due to the ease of recording the electrically evoked compound muscle action potential (CMAP), longitudinal studies are often confined to motor fibers. This has the methodological advantages that large and reasonably reproducible responses can be obtained and that the recording conditions remain constant with different sites of stimulation. In addition, it has the biological significance that the

presence of a MAP from a distal muscle proves continuity of one or more motor fibers (see, for example, Baba et al.²). The method has the limitations that a diminution in amplitude due to interruption of axonal continuity cannot be localized along the extent of the fiber, and that information is obtained only for fibers which remain in continuity. Recognizing this limitation, investigators have used the compound nerve action potential (CNAP) to localize the site of loss of axonal continuity.⁹ However, the shape and amplitude of the CNAP recorded over periods of time by repeated insertions of stimulus and recording probes are critically dependent on the volume conduction properties and the temporal relationship of the components from individual fiber groups.

The aim of this study was to develop a method to record the CNAP at multiple sites over periods of time in order to be able to (1) localize the spatial and temporal development of pathophysiological changes caused by nerve lesions, (2) follow and localize the growth of nerve fibers during regeneration, and (3) characterize the diameters of nerve fibers giving rise to response components obtained from well-defined sites.¹³ We have used chronically implanted stimulating and recording

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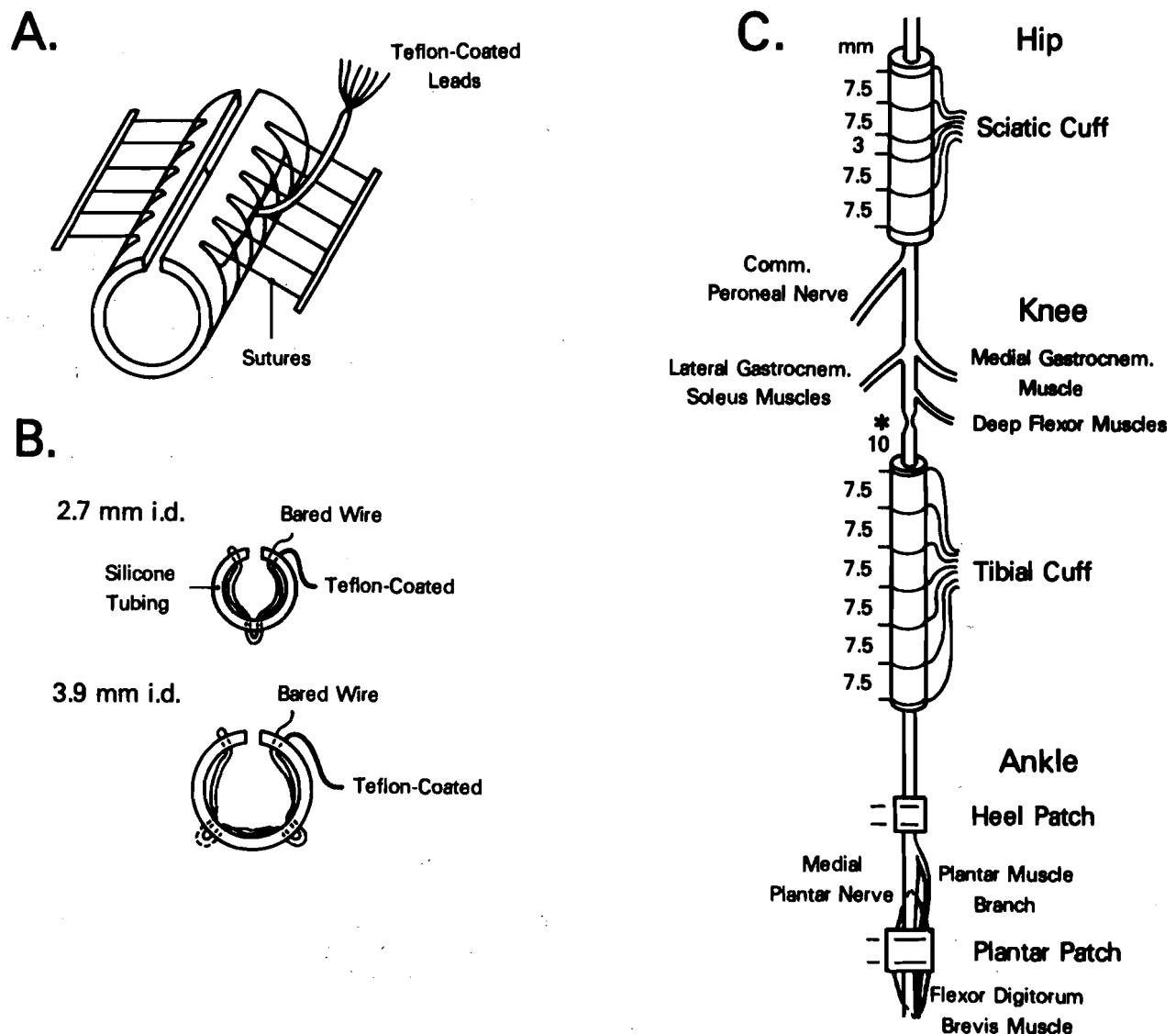


FIGURE 1. Schematic representation of cuff and patch electrodes implanted in the hindlimb of cat. (A) Silicone cuff electrode with external closing sutures (2-0, Mersilene, Ethicon, Somerville, NJ) and Teflon-coated cables connecting the leads to the back-pack socket. (B) The bared leads along the inside circumference of the cuffs were anchored through the wall at three of four points; the bared loops on the outside of the cuff were insulated with silicone adhesive (Silastic Medical Adhesive A, Dow Corning Corp. Midland, MI). The internal diameter (id) of the tibial cuff was 2.7 mm and that of the sciatic cuff 3.9 mm. (C) Shows full complement of cuff and patch electrodes at hindlimb nerves and muscle. The distances between contacts are indicated. Patch electrodes had interlead distances of 5–7 mm. The bared leads were placed on Dacron-reinforced silicone sheeting (Dow Corning Corp.). The heel patch was placed facing the plantar nerve just distal to the calcaneus bone and the plantar patch facing the flexor digitorum brevis muscle and the medial branch of the plantar nerve. *Position of crush lesion distal to the leg branches of the tibial nerve.¹³

electrodes to establish constant and quantifiable anatomical and functional relationships with the nerve. Multicontact cuff¹⁹ and patch electrodes were implanted along the extent of the sciatic-tibial-plantar nerves in cat hindlimb. The method provided recordings with reproducible temporal features over a wide dynamic range of activity from whole nerve compound action potentials to single unit responses. In this paper we describe

methodological considerations and findings in normal nerve.

MATERIALS AND METHODS

Experimental Animals. Seven hindlimbs from adult cats (2.4–5.3 kg) had multicontact electrodes implanted around hindlimb nerves (Fig. 1). Following the chronic experiments all nerves were examined microscopically, and morphometric

analysis was performed at multiple levels in some nerves. One nerve from an animal without implanted electrodes was examined for comparison. Implantation was performed under aseptic conditions during deep anesthesia induced by intraperitoneal pentobarbital (40 mg/kg body weight) and maintained by intravenous pentobarbital (16 mg/ml). After surgery the cats resumed normal locomotion within 2–3 days. Cephalosporin was given preoperatively and for 7 days postoperatively to prevent infection which nevertheless occurred in the distal leg in two small (2.4 kg) cats due to devitalization of the skin over the cuff electrode.

Implanted Electrodes. Two types of electrodes (MicroProbe Inc. Clarksburg, MD) were implanted in the hindlimb (Fig. 1C). (1) One *cuff electrode* made from silicone rubber tubing (Sil-Med Corp. Taunton, MA) was placed around the sciatic and one around the tibial nerve. They had internal circumferential contacts (Fig. 1B) and were closed after implantation using external sutures (Fig. 1A). The internal diameter of the cuff electrodes was 30–40% larger than the nerve⁸ to avoid compression (Fig. 1B). (2) One *patch electrode* was placed at the heel (heel patch, Fig. 1C) with contacts facing the plantar nerve and used for stimulation. Another more distal patch electrode (plantar patch, Fig. 1C) was placed on the plantaris fascia to record from the flexor digitorum brevis muscle or stimulate the medial plantar nerve.

The leads of the cuff electrodes were braided stainless steel wire (type 316 alloy, 9 strands of 25 μm diameter, AS 631, Cooner Sales Co. Chatsworth, CA), 100 μm in diameter, and insulated with Teflon with the exception of the bared part inside the cuff. The leads of the patch electrodes were made from sturdier stranded stainless steel wire (type 302 alloy with Teflon jacket; BWR 3.48, Bergen Wire Lodi, NJ). The insulation of electrodes was tested by applying a dc potential with the electrodes in saline. The leads from the electrodes were passed subcutaneously to the back, resurfaced through small skin incisions, and soldered on connections on a printed circuit board anchored to the spine. To avoid pulling on the nerve, care was taken to leave loose loops of wire that allowed full range of movement of the hindlimb. The electrical stability of the contacts was tested *in situ* before each recording session by measuring the impedance between contact and ground (implanted subcutaneously over the hip) using a 1 kHz, 10 nA, sinusoidal signal. The first

day after implantation the impedances ranged between 0.8 and 3.2 k Ω with a mean \pm standard error of the mean (SEM) of 1.6 ± 0.03 k Ω ($n = 216$). Over the next week the impedances decreased by 25% and then increased slightly but remained stable from about 3 weeks after implantation as previously described.²⁰ Breakage of leads was rare and could be recognized by a >10-fold increase in impedance. Owing to the rapid formation of mesothelial scar tissue, the position of the cuffs carrying the electrode changed by at most 10% early in the course of the study.

Electrophysiological Studies. Serial observations began the day after implantation of the electrodes and were repeated every 5–8 days for 6–8 weeks and then every 2–3 weeks for 2–3 months. The animals were followed for 27–119 days. The cats were anesthetized with subcutaneous Ketamine (70 mg/kg) and Xylazine (3 mg/kg), repeated as necessary. To keep the temperature of the legs and feet constant, the cat was placed on a water-heated rubber pad and covered with cotton or another heating pad during the study. The rectal temperature remained at 37–38°C.

In two animals, a terminal laminectomy was performed under pentobarbital anesthesia. Ventral and dorsal roots from L₇ to S₂ were each tied separately with a ligature and cut proximally. The cut roots were placed on platinum hooks in mineral oil (37°C) for stimulation or for recording.

Stimulation of Nerve and Recording of Action Potentials. The connections between the electrodes and the external circuit board were designed to allow the individual leads to be used in various combinations for stimulation and recording. Figure 2A illustrates an example where the plantar nerve was activated distally in the foot, and the ascending compound nerve action potentials (CNAP) was recorded at five different sites along the nerve. In Fig. 2B the recording electrodes were then connected to the stimulator, and the compound muscle action potential was recorded through the electrode previously used for stimulation. Changes in conduction properties and excitability along the nerve were delineated by applying the stimulus to the different fixed sites.

Stimulation. To reduce the stimulus artifact, the nerve was activated by a biphasic^{10,14} negative-positive pulse (zero interphase delay), with a duration of each phase of 0.05 or 0.1 msec and a rise time less than 10 μsec , from a photoisolated, battery powered, constant current stimulator, with an out-

put between 0.01 and 10 mA. The threshold of normal nerve within the cuff electrode was 0.1–0.5 mA and slightly lower immediately after implantation than later in the course of the experiment. A maximal amplitude response was obtained at 1.5 times threshold, and the usual test current was 2–3 times threshold, ensuring activation of slower conducting A fibers.¹⁰

Recording of Nerve and Muscle Action Potentials. The CNAP was recorded with leads in the cuff electrodes connected in a tripolar configuration. The shape was triphasic with an initial upward (because the action current as well as the action potential was measured) deflecting positive phase (Fig. 2A). The center lead was connected to the positive input, and the two flanking leads at symmetrical distances of 7.5 mm from the center lead were connected together to the negative input of the high-impedance differential amplifier (200–5000 Hz bandwidth). Action potentials were digitized (12 bits) in a Nicolet 1174 averager allowing responses from single myelinated fibers to be

distinguished from noise when 512–2048 responses were summated (Fig. 3B).

The CMAP from the flexor digitorum brevis muscle was recorded between one lead in the plantar patch electrode (Fig. 1C) and a remote electrode implanted on the dorsum of the foot.

The Influence of the Electrode Configuration on the Nerve Response. The tripolar leading-off configuration records the spatial derivative of the action potential and rejects potentials from sources outside the space enclosed by the cuff.^{15,19,21} This characteristic was very effective in focusing the site of recording and reducing the duration of the stimulus artefact compared with bipolar or unipolar recording (Fig. 3A). This was particularly useful when recording close to stimulating electrodes, from single fibers, or during high current stimulation of regenerating fibers.¹³ However, the amplitude of the action potential recorded by the tripolar electrode was smaller than that recorded by the bipolar or unipolar configuration (Fig. 3A), and both the amplitude and the duration were de-

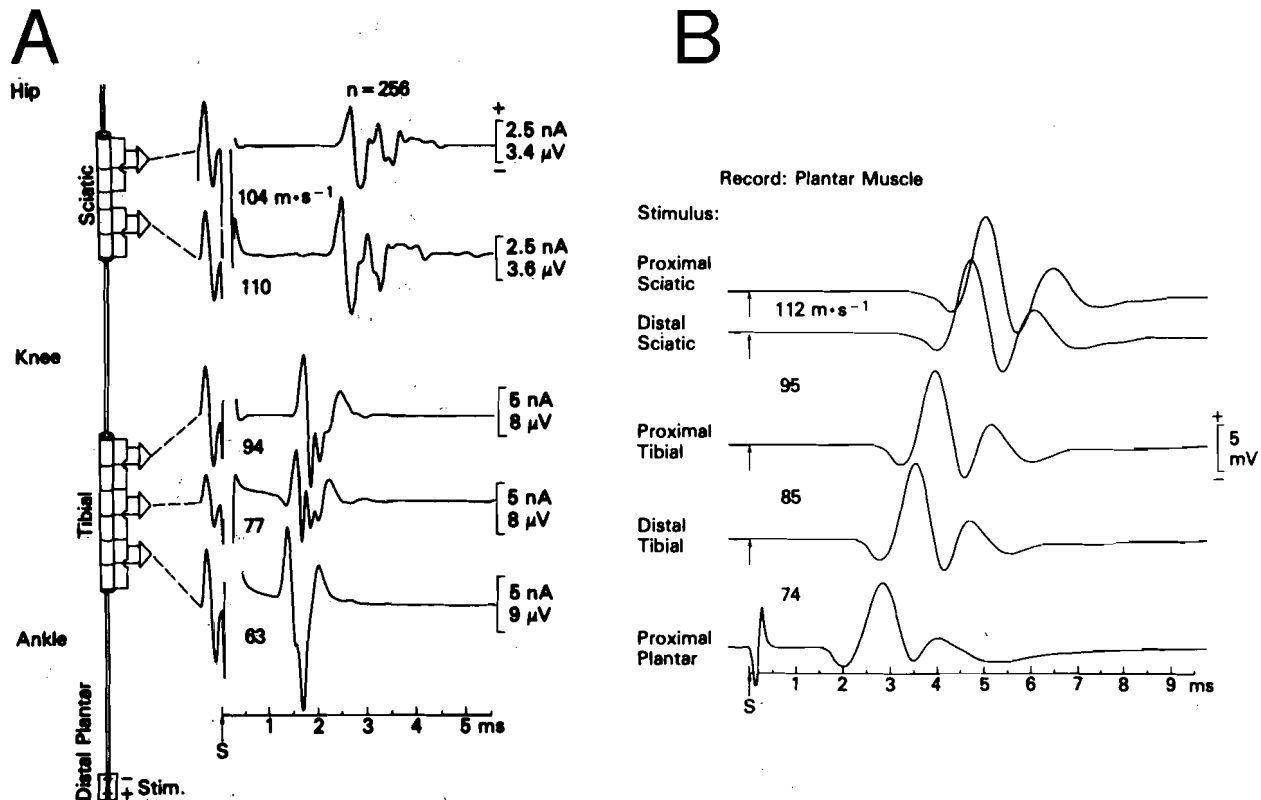


FIGURE 2. Recordings of compound action potentials from control nerve and muscle. **(A)** The compound nerve action potential was evoked by a stimulus to the distal plantar nerve (left) and recorded at three sites in the tibial and at two sites in the sciatic cuff electrodes. The number of sweeps averaged is indicated above top trace (n), and the conduction velocities are indicated below traces. In this and subsequent figures, the amplitude was calibrated in both voltage and current units (see text). The current calibration was recorded during the 0.5 msec before the stimulus (S). **(B)** The compound muscle action potential recorded at the plantar patch electrode was evoked by stimulation at the different electrode sites (N17, left hindlimb).

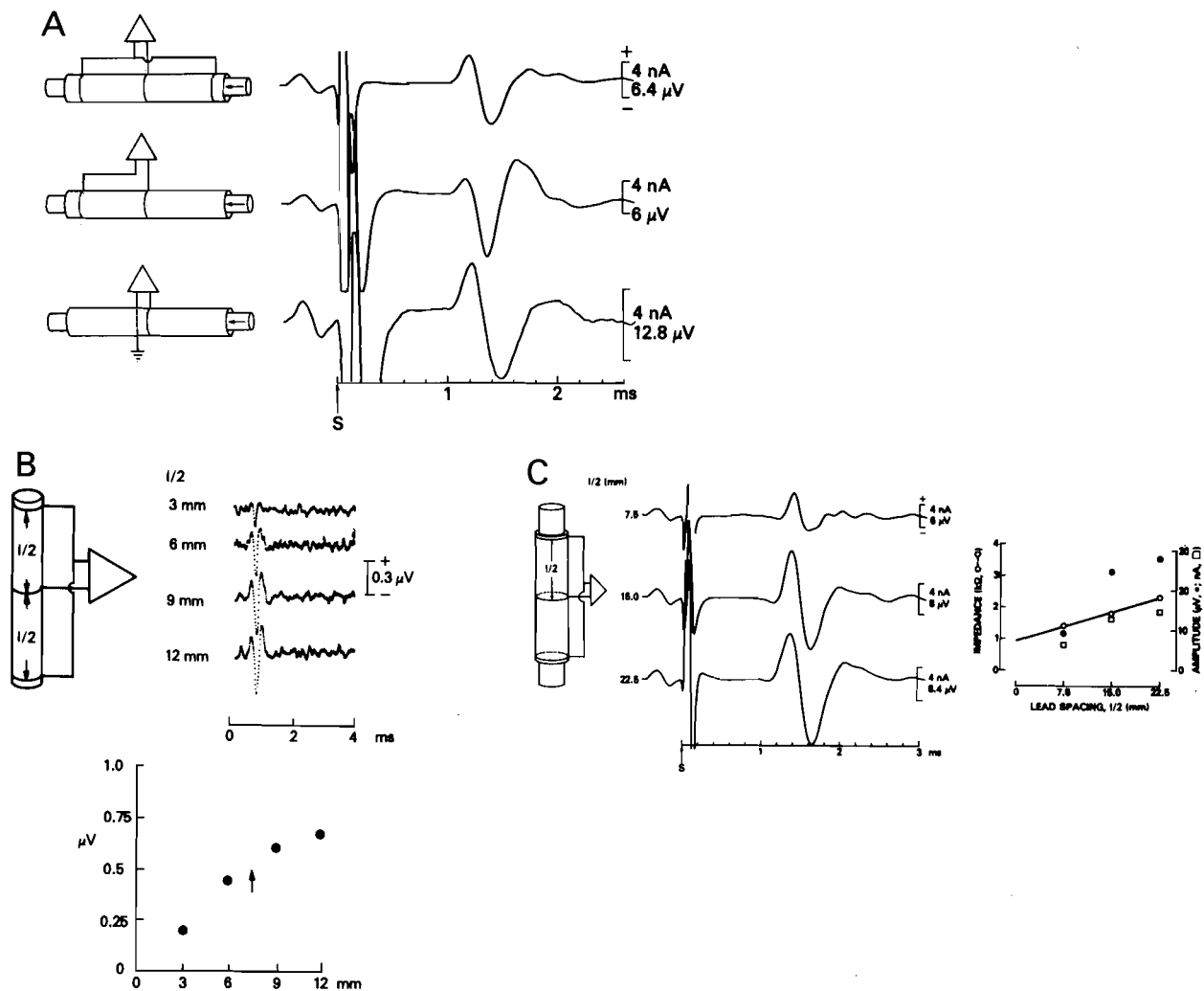


FIGURE 3. Influence of electrode configuration and dimensions on single-fiber and whole nerve action potentials. **(A)** Whole nerve action potentials recorded by tripolar lead configuration (top trace) compared with bipolar (middle trace) and unipolar configuration (bottom trace). In all instances, the same lead was used for the positive input to the amplifier. Note the reduction of the stimulus artifact in the tripolar configuration. (N9, left leg). **(B)** Single-fiber potential to show the effect of different lead spacing ($l/2$, mm) on the amplitude and shape of the potential. Above: Traces of action potentials in the tibial nerve conducting at 67 m/sec from a touch receptor in the foot pad. The potential was recorded by spike-triggered averaging of 512 responses. The potential used to trigger the averager³ was recorded from the dorsal root ganglion via a conventional metal microelectrode. The amplitude and the duration of the response increased at longer lead spacing. There was little effect on the latency to the first positive peak. Below: The amplitude of the potential as function of $l/2$ (mm); the arrow indicates the spacing of 7.5 mm used in the chronic experiments. **(C)** Whole nerve action potential to show the effect of different lead spacing ($l/2$, mm) on the amplitude and shape of the response. Left: Traces of action potentials from the tibial nerve conducting at 81 m/sec. The response was evoked by electrical stimulation of the plantar nerve. The amplitude and duration increased with longer spacing, and the latency was slightly shortened. Right: Amplitude of the action potential voltage (right ordinate, μV ; ●), action current (right ordinate, nA; □), and lead impedance (left ordinate, $\text{k}\Omega$; ○) as functions of the interlead spacing (abscissa, mm). The tissue impedance changed directly with the distance between leads (regression line). The curve was extrapolated to zero distance to measure the contact impedance. For a circuit consisting of two parallel impedances (the flanking contacts) in series with one similar impedance (the center contact) the impedance of each contact was about 600Ω (N9, left leg).

pendent on the distance between leads in the electrode as shown in recordings from a single nerve fiber (Fig. 3B) and whole nerve (Fig. 3C). At longer lead spacing the action potential voltage increased more than the action current (Fig. 3C, right). The increase in action current resulted

from spanning of more active current generating regions of the nerve and less cancellation of common-mode potentials by the differential recording.¹⁵ The voltage of the potential was in addition dependent on the impedance of the tissue column captured between the recording electrodes which

increased linearly with the distance between leads (Fig. 3C, right). As the tissue impedances changed somewhat over time (see above) due to changes in interstitial tissue composition, the voltage of the action potential might change independently of the number or caliber of nerve fibers at the electrode site. To correct for this possibility, the amplitude of the action current as well as the voltage was measured (see, for example, Fig. 2A). The latency of the compound action potential was slightly shorter at longer lead spacing (Fig. 3C). To allow comparison between different parts of the nerves in different cuffs, the interlead spacing was the same (7.5 mm), and the internal diameter was 30–40% larger than the nerve both at the tibial and sciatic nerves.

The voltage of the action potential was calibrated by a 20 μ V, 3 kHz sinusoidal test pulse applied to a 1 k Ω resistive load in place of the electrode configuration. To calibrate the action current, a 3 kHz sinusoidal current pulse of known amplitude was injected into the tripolar recording system (Fig. 2A) during the predelay before the stimulus. The calibration current and the generated action current passed through the same fluid-tissue column within the tubular electrode. However, the calibration current produced an additional voltage drop across the electrode contact impedance, resulting in an underestimate of the absolute action current of 50–60% and therefore useful only in following relative changes in longitudinal studies.

Parameters. The amplitudes of the CNAP and CMAP were measured from peak to peak. Latencies were measured to the first positive peak of the CNAP which reflects the conduction velocity of fast fibers.⁷ Conduction velocities were calculated from stimulus-response latencies when the stimulus was delivered through one set of electrodes and the action potential recorded through another set of electrodes and from increments in conduction time when the responses were recorded at different electrodes (Fig. 2A). Conduction velocities along motor fibers were calculated from differences in latencies between stimulus sites (Fig. 2B). The latency of the CMAP was measured to the first deflection from baseline. The conduction distances were measured between center leads in the tripolar electrodes²¹ and between implanted patch and cuff electrodes along the exposed nerve after termination of the experiment.

The safety of transmission was studied by measuring the minimal interval during double stimulation which allowed transmission (refractory pe-

riod of transmission, RPT) of a second pulse.¹⁸ The recovery of conduction was followed by gradually increasing the interval between the conditioning and the test pulse. The stimulus strength during this test was increased by a factor of 2 or 3 to improve excitation during the refractory period when the threshold was increased.

Histological Studies. The plantar, tibial, and in some instances the sciatic nerves were fixed by immersion in glutaraldehyde 2% cacodylate-buffer or by perfusion with glutaraldehyde 2% phosphate buffer *via* the abdominal aorta. After further fixation for 24 hours (4°C), the nerves were kept in a cacodylate-buffered isotonic sucrose solution (0.2 M) for 24 hours, postfixed in 1% buffered osmium tetroxide, dehydrated in graded alcohol, cleared in propylene oxide, and embedded in plastic.

Light Microscopy. For morphometric analysis, semithin transverse sections (1 μ m) were digitized in a computerized *camera lucida* by tracing the perimeter of 500–600 fibers and axons at a final magnification of 1000 \times . In a given view area all myelinated fibers were traced and the fiber diameters were calculated from the area measurements. The fiber density was calculated from the number of myelinated fibers in several view areas of known size, and the total number of fibers in the nerve was calculated from the density and the total fascicular area. The *g* ratio¹⁷ was calculated from the ratio of axon diameter: fiber diameter, and the index of circularity of fibers¹ was calculated from the ratio of fiber diameter from area: fiber diameter from perimeter.

RESULTS

Long-term Effects of Implanted Electrodes. Followed over time there was no consistent change in the relative amplitude or conduction velocity of the evoked CNAP even when different portions of the nerve within or between cuff electrodes were considered individually (Fig. 4). However, when each measurement in the seven control nerves was related to the first obtained value, the conduction velocity of the CNAP's decreased by $3 \pm 0.9\%$ ($n = 213$ observations), and the action current decreased by $9 \pm 3\%$. These changes were statistically significant ($P < 0.001$) and suggested that the presence of the cuff electrodes caused loss of some large fibers. A mild fiber loss was confirmed in transverse sections showing 1–2% of fibers undergoing Wallerian degeneration. The main finding in control nerves with implanted electrodes

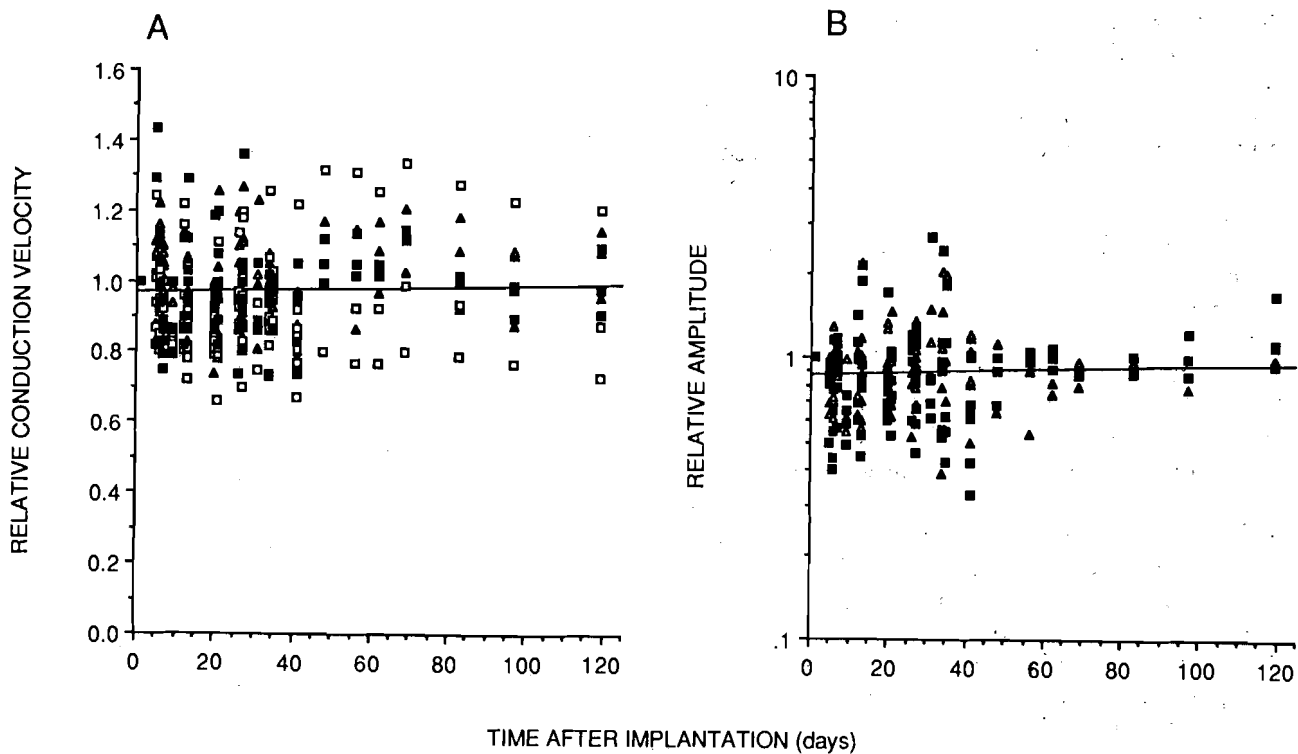


FIGURE 4. Relative changes in conduction velocity (**A**) and amplitude (**B**) of the compound nerve action potential followed over time in seven control legs. (**A**) The conduction velocity along different segments of the hindlimb nerves are shown: (▲) plantar nerve, (△) tibial nerve, (□) tibial sciatic nerve, (■) sciatic nerve. (**B**) The amplitude of the CNAP recorded at the tibial (△) and the sciatic (■) nerve cuff electrodes. The relative amplitude is drawn to log scale. The regression lines obtained from the least square method did not deviate significantly from zero.

was a 30% lower fiber density due to diffuse edema, not associated with a cellular response.

In one tibial nerve without implanted electrodes the total number of fibers was 7776, the diameters of the largest fibers were 14–15 μm , the average *g* ratio was 0.60, and the index of circularity of fibers was 0.90. In two nerves with im-

planted electrodes the total number of fibers was 6977 and 8653. The diameters of the largest fibers (16–18 μm), the index of circularity (0.95 and 0.94), and the *g* ratio (0.55 and 0.58) differed slightly from the nerve without implanted electrodes but probably not more than could be explained by variability between animals.

Table 1. Maximal conduction velocities (m/sec) in control nerve.

	Nerve segment				
	Root-sciatic nerve	Sciatic nerve	Sciatic-tibial nerve	Tibial nerve	Plantar nerve
Motor nerve*	105 ± 3 (14)†	101 ± 3 (37)†	91 ± 2 (36)‡	86 ± 2 (37)‡	2.15 ± 0.04 (37)§
<i>t</i> -test		NS¶	$P < 0.005$	$P < 0.05$	
Mixed nerve	108 ± 4 (9)#	102 ± 1 (121)†	105 ± 1 (146)‡	92 ± 1 (109)‡	64 ± 1 (55)
95% confidence limits		75–129	81–129	72–112	49–80
<i>t</i> -test		NS	NS	$P < 0.001$	$P < 0.001$

*Mean ± standard error of the mean (*n* = number of observations in seven control nerves).

†No significant difference (*t*-test) between motor and sensory conduction velocities.

‡The motor conduction velocity was significantly ($P < 0.001$ – 0.005) slower than the mixed nerve conduction velocity.

§Distal motor latency from the ankle to the flexor digitorum brevis muscle (msec).

|| Paired *t*-test between different nerve segments.

¶Not significant.

#Recording from dorsal root.

Conduction Properties in Control Nerve. In addition to the slight decline, there was a larger random variation of $\pm 25\%$ in conduction velocity, and random reductions of up to about 50% of the amplitude of the CNAP (Fig. 4) were observed without evidence of fiber loss. A similar large variability was present in the CMAP, indicating that only large and consistent changes were of significance.

From proximal towards distal there was a general decrease in the velocity of conduction along both mixed nerve (Fig. 2A) and motor fibers (Fig. 2B). The values from all observations in the seven control nerves have been pooled in Table 1: the mixed plantar nerve conduction velocity was 30% slower ($P < 0.001$) than that of the tibial nerve, which in turn was 10% slower ($P < 0.001$) than the velocity between the tibial and sciatic nerve cuffs, but there was no consistent increase in velocity proximal to the knee. Motor fibers (Table 1) showed a slightly different distribution of velocity changes: the conduction velocity along the segment between the tibial and sciatic cuff electrodes was 5% faster ($P < 0.05$) than that along the tibial nerve, and there was a further 10% increase ($P < 0.005$) from the segment across the knee to the sciatic nerve. As in mixed nerve, there was no further increase in velocity proximal to the sciatic nerve.

The diameters of large fibers of the control proximal plantar nerve were 2–4 μm thinner than in the midtibial nerve (Fig. 5). This difference was smaller than expected considering a proportionality factor of $5.7 \text{ m/sec}/\mu\text{m}^6$ and an observed difference in conduction velocity of $27 \pm 1 \text{ m/sec}$ (mean \pm SE, $n = 55$) between the two nerve segments. Although the whole hindlimb was heated, the larger than expected slowing in the plantar nerve may be due to a slight temperature gradient. The distribution of fiber diameters was trimodal as earlier described⁵ with a minor reduction in the number of fibers at 3–4 μm and the major reduction at 6–9 μm . The diameter of the large fiber was slightly smaller at the midtibial level within the cuff electrode than at the proximal tibial level proximal to the cuff (Fig. 5) corresponding to a $12 \pm 1 \text{ m/sec}$ ($n = 109$) slower conduction velocity.

Distal to the knee, conduction velocities in motor fibers were slower ($P < 0.005$) than in mixed nerve (Table 1). However, at and proximal to the sciatic nerve the CMAP and the CNAP had similar conduction velocities. In accordance with this, latencies of potentials conducted between the sciatic

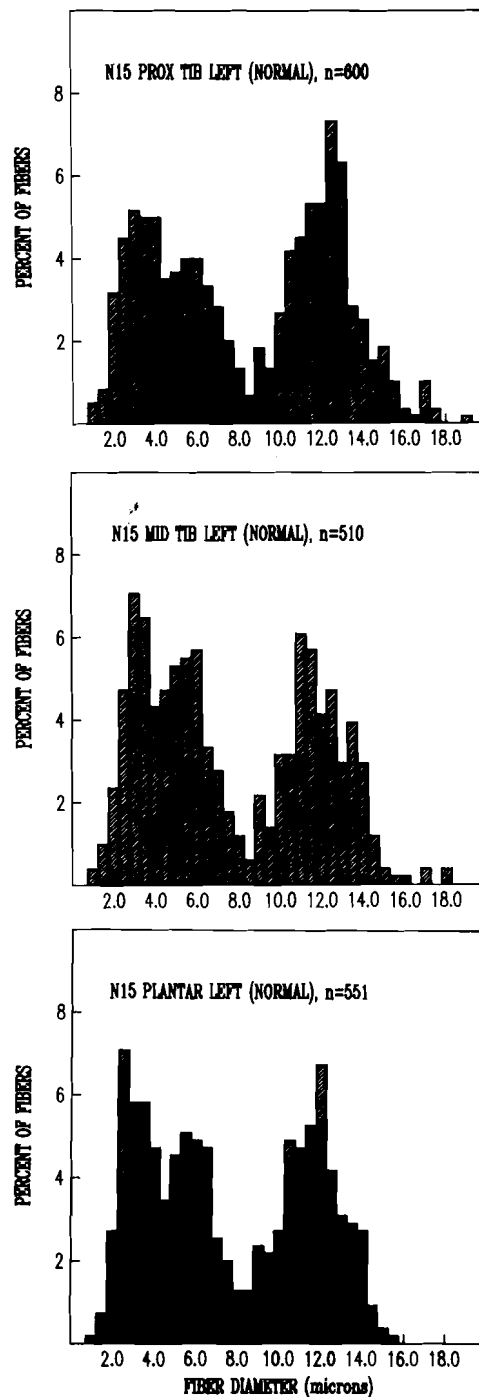


FIGURE 5. Relative distribution of fiber diameters at the tibial nerve proximal to the cuff electrode (top), within the cuff-electrode (middle), and at the plantar nerve (below). The number of fibers measured at each level are indicated.

nerve and ventral or dorsal roots (Fig. 6) were similar for motor and sensory fibers. The more pronounced distal slowing of conduction of motor fibers than mixed nerve was confirmed in ventral and dorsal root responses since the latencies of di-

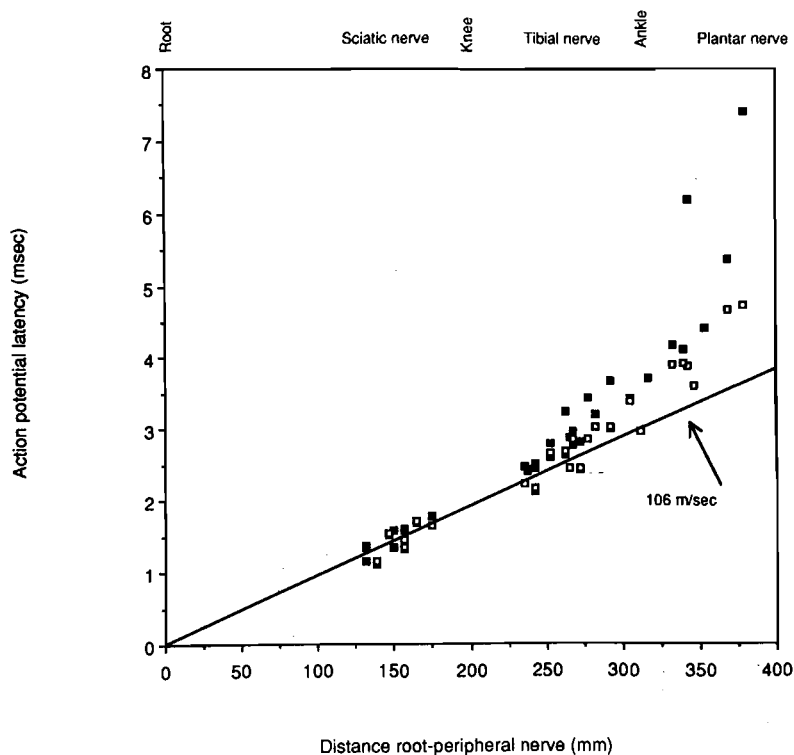


FIGURE 6. Relationship between action potential latencies (ordinate) and distances from the spinal cord (abscissa) in two control nerves. Action potentials between L₇, S₁, and S₂ ventral (■), L₇ and S₁ dorsal (□) roots, and different distal electrode sites were obtained separately. The motor and sensory fiber conduction velocities between the sciatic nerve and the roots were similar (average 106 m/sec, solid line). The latencies deviated from the extrapolated latency distal to the knee, indicating progressive slowing along distal segments more pronounced in motor than in sensory fibers.

stal responses were more prolonged, and this prolongation occurred at more proximal levels in motor than in sensory fibers (Fig. 6). The predominantly distal slowing is consistent with measurements from single nerve fibers¹⁶ and with the histological findings that the distal diminution in fiber diameter occurs at branching sites rather than being due to gradual tapering of fibers (see Berthold⁴).

Refractory Period of Transmission. The absolute refractory period (RPT) using a stimulus strength 2–3 times supramaximal was 0.3–0.4 msec (Fig. 7). The amplitude of the CNAP gradually increased to normal at an interstimulus interval of 2.0–2.5 msec. During this relative refractory period, the latency of the CNAP shortened to normal at a stimulus interval of 1.5–2.0 msec (Fig. 7).

DISCUSSION

Implanted cuff electrodes have previously been used in a simpler configuration mainly to monitor sources of neural activity in freely moving cats^{11,12} and to evaluate retrograde atrophy in nerve with peripheral degeneration.⁸ By using electrodes at

different sites of hindlimb nerves from the sciatic to the plantar nerve with fixed, well-defined sites of stimulation and recording of nerve and muscle action potentials, it was our aim to develop a setup which would permit detection and characterization of changes in nerve fiber conduction both proximal and distal to experimental nerve lesions. Repeated observations of conduction properties along different segments of nerve were considered an advantage to localize not only the pathological changes but also to identify the diameter of predominantly affected fibers.

The recording conditions allowed resolution of averaged action potentials from single myelinated fibers, which was of particular advantage when recording during early regeneration.¹³ This high signal-to-noise ratio was due to the tripolar differential recording arrangement,^{15,21} the containment of the action current along the resistive pathway within the cuff, and to the low impedance and noise of the electrode contacts. This approach also ensured extremely effective stimulation of the nerve placed within the cuff electrode, allowing activation of newly regenerated nerve fi-

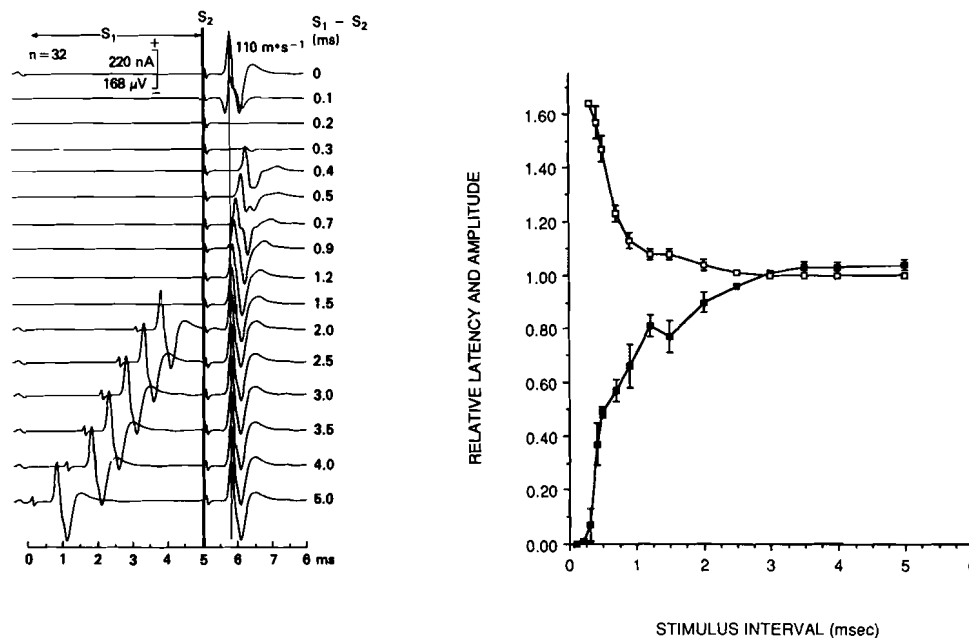


FIGURE 7. The refractory period of transmission of the ascending CNAP evoked by stimulation of the tibial and recorded from the sciatic nerve. *Left:* Traces recorded from a normal nerve one day after implantation of cuff electrodes. The first trace in the raster was obtained by giving the test stimulus (S₂) alone 5 msec after the start of the sweep (bold vertical line). The conditioning stimulus (S₁) was applied at variable intervals before the test stimulus as indicated to the right of each trace. The response to the conditioning stimulus at intervals of less than 2 msec was subtracted electronically from the double response to avoid interference with the test response (at the interstimulus interval of 0.1 msec the subtraction was imperfect due to interference between stimuli). The shortest interval allowing propagation of a response was 0.3 msec. During the increasing interstimulus intervals, the latency recovered to normal (light vertical line). *Right:* Relative change in latency (□) and amplitude (■) of the test response as a function of the interstimulus interval. The value of 1.00 was obtained by S₂ alone. The vertical bars denote the standard error of the mean.

bers with conduction velocities of 1–3 m/sec.¹³ Similarly the absolute refractory period in normal nerve, using a relatively slight increase in stimulus current to avoid damage to the nerve, was 0.3–0.4 msec and the same as that found by Smith using a 20 times higher than maximal stimulus strength. Due to the restricted anatomical conditions, cuff-electrodes could not be implanted around the plantar nerve. The geometrical relationship between the patch-electrode and the nerve is less optimal than the cuff-electrode and required larger currents for stimulation, and hence the site of activation was less certain.

Electrodes implanted around peripheral nerve may themselves cause damage which would interfere with the interpretation of the effects of nerve lesions. Over periods of up to 4 months in control nerve and in experiments on regenerating nerve followed for 10 months,¹³ there was only slight evidence of fiber loss. The most pronounced change seen in normal nerve was a reduction in fiber density. This was related to diffuse edema of the nerve and made it necessary to use electrode cuffs 30–40% larger than the largest diameter of the nerve to prevent compression damage. Infection

may occur with implantation of any device, in particular when percutaneous contacts are used. This complication was seen in two small animals due to devitalization of the skin over the cuff electrodes in the leg.

The variability of the peak-to-peak amplitude CNAP was large, even though the position of the electrodes became stable rapidly after implantation. The amplitude depends on the summation and cancellation of action current phases from individual nerve fibers with a range of conduction velocities. Small changes in these factors would lead to large changes in the peak-to-peak response, particularly in tripolar recording arrangements having relatively short spacing between leads (Fig. 3), which record the spatial derivative (gradient) or voltage changes.^{15,21} Measurements of the CNAP area would reduce some but not all of this variability since the changes caused by phase cancellation would be reflected also in area measurements. The variability in the amplitude of the nerve action potential in long-term observations indicated that only large and consistent changes were relevant.

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